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ABSTRACT BOOK

GSA

1

Microtubule-dependent endosomal mRNA transport

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Mitochondria receive the vast majority of proteins by cytoplasmic translation of nuclear encoded mRNAs. Evidence is accumulating that these mRNAs are actively transported throughout the cytoplasm. This appears to be particularly important in highly polarised cells such as hyphae, in which mitochondria far away from the nucleus need to be served with proteins. However, information on the mechanistic link between long-distance mRNA transport, mitochondrial physiology and mitochondrial protein import is scarce. Here, we demonstrate that loss of endosomal mRNA transport causes defects in mitochondrial metabolism in infectious hyphae of *Ustilago maydis*. Using the subunit Atp3 of respiratory chain complex V as an example, we demonstrate that endosomal mRNA transport is needed to govern efficient protein entry in mitochondria distant from the nucleus. Thus, endosomal transport of mRNAs encoding mitochondrial proteins is crucial to determine the subcellular mitochondrial homeostasis, a process conserved from fungal to neuronal cells.

2

Control of conidiation in *Aspergillus nidulans*: On the centrality and specificity of the master regulator BrlA

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Asexual spores are the main vehicle for dissemination of fungi. *Aspergillus nidulans* is one of the most commonly used reference systems for the study of the genetic and molecular control of asexual development. According to the generally accepted model, in *A. nidulans*, BrlA is a master transcription factor of conidiophore development and conidia production linking upstream regulators of the UDA pathway with downstream regulators of the central developmental pathway. This talk will discuss specific features of this model, and whether it is extrapolable or not to other clades of the fungal tree. It will also present recent results on the study of the role that BrlA and other transcriptional regulators have in conidiation and introduce recently identified genes necessary for asexual development. Future approaches to the topic will also be suggested, as stimulating elements contributing to the understanding of how BrlA, in coordination with other regulators of growth and development, controls conidiation.

3

Circadian clock control of mRNA translation, translation fidelity, and aging

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The circadian clock is a fundamental regulator of human health and drug metabolism, coordinating daily rhythms in protein production that affect cellular function and metabolism. Many proteins that cycle robustly are produced from non-rhythmic mRNAs, pointing to translational control as a key mechanism of rhythmic protein levels. Using the model eukaryote *Neurospora crassa*, we discovered that the clock exerts this regulation through rhythmic control of a conserved translation initiation factor (eIF2α) and by remodeling ribosome composition. Even more unexpectedly, we discovered that the circadian system governs the fidelity of protein synthesis by modulating ribosome makeup and tRNA synthetase activity. Both translational fidelity and circadian amplitude decline with age. We identified compounds that restore clock amplitude in old *N. crassa* cells, leading to improved translation accuracy and extended lifespan. These findings reveal how the circadian clock programs daily changes in the proteome beyond genomic instructions and highlight a novel link between circadian regulation, proteome integrity, and aging.

4

Emergent behaviour of mycelial networks

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Mycelial networks are the predominant multicellular growth form across the fungal kingdom and allow exploration and exploitation of resources, location of potential mates, or colonisation of host organisms. The extent that the external network architecture is mirrored in the level of internal connectivity varies depending on the species and environment, and different species may exploit different mechanisms for movement over different length scales. Our work over the last 25 years has focused on systems where mass flow appears to make a significant contribution to long-distance transport. We infer from both measurement and modelling that hydraulic coupling though contiguous compartments facilitate long-distance translocation of resources through the network from sources to sinks. We also propose that hydraulic coupling provide an alternative or additional mechanism to conventional biochemical signal transduction pathways for information transfer. This can lead to biophysical emergence of co-ordinated colony-wide behaviour, and may even provide evidence for the evolutionary advantage of the hyphal life-style. We are slowly developing approaches to characterise mycelial traits from these networks that may provide insights into life-history strategy and ecological role. However, fungal network trait-based ecology lags well behind trait-based ecology in other fields, and it is less clear the best way to link measurements that have to be made in lab microcosms, with real communities in the field.

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Structural insights into mechanisms of zinc scavenging by the *Candida albicans* zincophore 1

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Candida albicans causes over 400,000 life-threatening, and an additional half a billion of mucosal infections annually. In response to infection, the host limits essential micronutrient availability, including zinc, to restrict growth of the invading pathogen. As assimilation of zinc is essential for *C. albicans* pathogenicity, limitation induces secretion of the zincophore protein Pra1 to scavenge zinc from the host. Pra1 also plays a number of important roles in host-pathogen interactions and is conserved in most fungi. However, the structure of fungal zincophores is unknown. Here, we present cryo-EM structures of *C. albicans* Pra1 in apo- and zinc-bound states, at 2.8 and 2.5 Å resolution respectively. Our work reveals a hexameric ring with multiple zinc binding sites. Through genetic studies, we show that these sites are essential for *C. albicans* growth under zinc restriction but do not affect the inflammatory properties of Pra1. These data create a foundation for future work to explore the structural basis of Pra1-mediated host-pathogen interactions, *C. albicans* zinc uptake, as well as therapeutics development.

6

Impact of global change drivers on dryland fungi: Implications for public health and biodiversity

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Climate change in the Southwest United States is impacting ecosystems and may be contributing to the emergence of infectious diseases. Indeed, increasing evidence suggests that enhanced stress tolerance can promote pathogenicity potential in fungi, particularly in facultative pathotrophs that can switch between saprotrophic and pathogenic lifestyles. Yet, little is known about how global change drivers affect the ability of fungi to tolerate stress. To address this gap, we investigated how soil fungal communities respond to global change drivers over time. We collected topsoil from the Jornada Basin in the northern extent of the Chihuahuan Desert and established microcosms exposed to three treatments, heat, drought, and their interaction (i.e., hxd), and one control group. Microcosms were harvested at 3, 6, 9, and 12 months after the onset of treatments. We extracted DNA from all samples and conducted ITS metabarcoding for taxonomical identification. We hypothesized that there would be a greater abundance of pathogenic and facultative pathogenic fungi in treatment groups as well as decreased biodiversity compared to the control. We found that heat reduced alpha diversity and beta diversity analysis revealed that heat strongly structured community composition, with heated communities becoming more similar to one another and more distinct from those not exposed to heat. We also found an increased abundance of pathogens in the fungal communities exposed to heat, and this was especially noticeable over time. Our results show that heat is a major driver shaping the structure and function of fungal communities, raising concerns that continued warming could not only alter ecosystem functioning and reduce biodiversity but also increase the risk of emerging infectious diseases that threaten public health.

7

Global patterns of diversity and distribution in *Aspergillus* fungi are driven by human and environmental influences

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Aspergillus fungi are key producers of pharmaceuticals, enzymes, and food products and exhibit diverse lifestyles, from saprophytes to opportunistic pathogens. The genus is divided into 28 taxonomic sections sharing traits (e.g., natural products) and lifestyles (e.g., xerophily, pathogenicity). Understanding global patterns of *Aspergillus* occurrence and diversity would deepen our ecological understanding and help monitor pathogens and producers of mycotoxins and diverse natural products. To identify key environmental factors influencing their geographic distributions and estimate the impact of future climate change, we trained a random forest machine learning classifier on 19,105 terrestrial occurrence records for 27 taxonomic sections and 96 environmental variables to predict distributions of each section. We found that regions with high section diversity are concentrated in temperate forests, suggesting that areas with mild seasonal variation serve as diversity hotspots. Section range estimates revealed extensive variability, and low range overlap suggests distinct niches. The top predictors of average section richness were the index of cumulative human impact, ecosystem productivity, and forested vs non-forested ecoregions. Our future climate analyses revealed considerable variation in section range estimates in response to changing climates; suitable habitats for some sections are predicted to expand (e.g., section *Restricti*), and others to contract (e.g., section *Nigri*) or remain stable (e.g., section *Fumigati*) in the next few decades. Our findings reveal that both natural and human factors influence the macroecology of *Aspergillus* fungi and highlight their ecological diversity, including the diversity of their responses to changing climates, which is of relevance to pathogen and mycotoxin risk assessment.

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Annual grass invasion is associated with differences in the community structure and abundance of biocrusts, arbuscular mycorrhizal fungi, and general fungi

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Interactions between biocrusts, arbuscular mycorrhizal (AM) fungi, and other soil fungi, may increase plant resilience to environmental stress in native grasslands. However, when non-native plants invade, they can have cascading effects on soil biota and their interactions, destabilizing grassland ecosystems. In a field survey we assessed the percent cover and composition of biocrusts in plots varying in percent cover of the invasive annual grass, *Ventenata dubia*. We used field-collected soil as inocula in a glasshouse experiment to test whether the abundance of viable AM fungal propagules differs under biocrusts compared to bare soil, or across invasion levels and used amplicon sequencing to test whether the structure of the AM fungal and general fungal community differs under biocrusts versus bare soil across invasion levels. We found that invasion was associated with lower biocrust cover, distinct biocrust community structure, and lower AM fungal abundance below biocrusts. AM fungal and general fungal community structure also differed

between invaded and non-invaded plots. Finally, preliminary analysis of fungal guilds suggests that the abundance of symbiotrophic fungi was lower in invaded compared to non-invaded plots. A better understanding of the interactions among plants, biocrusts, and different guilds of fungi is important for mitigating the impacts of invasive species and informing the restoration of invaded grasslands.

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Metatranscriptomics of natural communities reveals functional differentiation and seasonal nutrient transport in the Populus soil microbiome

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The plant–soil microbiome is a critical interface where roots, fungi, and other microbes exchange carbon (C) and nitrogen (N), yet we lack a community-level view of how these exchanges are coordinated over time in natural systems. Using a seasonally resolved metatranscriptomic dataset of Populus tremuloides fine roots collected across an elevational gradient, we characterized the functional landscape of the aspen soil microbiome through time. By annotating fungal and host transcripts with CAZyme families, transporters, and core C–N metabolism pathways, we resolve clear functional differentiation among fungal guilds. Specifically, we find pronounced functional differences between ectomycorrhizal (ECM) basidiomycetes and ascomycetes, and a distinct behavioral profile of cryptic rhizosphere taxa such as dark septate endophytes. Integrating plant and fungal transporter expression, we infer seasonal trajectories of C and N movement between Populus and its fungal partners. C and ammonium transport appear to be linked and both peak late in the growing season, whereas nitrate transport exhibits inverse patterns. Notably, inferred N transfer to the host is decoupled from seasonal patterns of soil N availability, which we hypothesize reflects elevated fungal energetic demands and intensified C competition during fall mushroom production. Our work demonstrates how genome-resolved metatranscriptomics can link guild-specific functional traits to seasonal C and N flows in a natural forest rhizosphere, providing a mechanistic framework for predicting when cooperative nutrient exchange gives way to belowground carbon competition.

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A 150-Year Record of Foliar Fungal Endophyte Dynamics in Norway Spruce (*Picea abies*) Across Finland

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Herbarium specimens offer valuable insights into plant history, yet their potential to reveal past plant-associated microbiomes remains underexplored. Here, we reconstructed the temporal and spatial dynamics of foliar fungal endophytes in Norway spruce (*Picea abies*) by sequencing the ITS region from herbarium needles collected in Finland (1861–2023). Fungal communities were analyzed across 50- and 20-year intervals and between southern and northern regions, considering temperature and precipitation effects. Ascomycota dominated the community, with *Lophodermium, Ceratocystis, Yarrowia, Saccharomyces*, and *Heterobasidion* as the most abundant basidiomycete genera. *H. parviporum* appeared in one 50-year interval, while *Lophodermium piceae* increased significantly during 2001–2023 ($P < 0.05$). Community composition differed significantly between 1951–2000 and both 1851–1900 and 1901–1950 ($P < 0.05$). The southern region showed distinct composition and lower alpha diversity than the north ($P < 0.05$). Functional predictions indicated a rise in Saprotroph, Symbiotroph, and mixed guilds during 1901–1950 ($P < 0.05$). Although temporal and spatial factors strongly structured communities, no direct correlations with historical temperature or precipitation were found. These findings demonstrate that herbarium specimens are powerful tools for reconstructing long-term microbial dynamics and reveal time and geography as key drivers of foliar fungal community structure.

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Tissue specificity, ecological function, and salinity tolerance of fungi associated with the pneumatophores and leaves of black mangrove (*Avicennia germinans*)

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Mangroves are woody-plants that have been identified as keystone species in their ecosystems due to their providing invaluable ecosystem services including habitat, structure, and nutrients. Not phylogenetically related, mangrove species have each adapted to saline conditions through convergent evolution of one of two major mechanisms: removal of salt from water at the root vs. at the leaf. Whereas microbes associated with mangroves adapted to remove salt at the root may tolerate salinity due to cohabitation of saline environments with mangroves, we predict that microbes living within stem and leaf tissue are most likely to have novel adaptations due to having to tolerate salt water taken up by the plant for photosynthesis. We also predict that such microbes may mitigate salinity stress of their hosts. To begin to understand the functional ecology of mangrove-associated microbes in this context, we profiled small metabolites and fungi from the pneumatophores and leaves of *Avicennia germinans* (black mangrove; removes salt at the leaves). We surveyed three adult trees from each of three distinct sites at Elmer's Island Wildlife Refuge in Grand Isle, Louisiana, generating metabolome (i.e., volatile small molecule) and mycobiome (i.e., fungal) profiles. For the mycobiome, we generated a collection of cultivable endophytes isolated from pneumatophores and apparently healthy leaves, as well as a database of full-length sequences from the fungal internal transcribed spacer generated directly from plant DNA extracts from the same tissues. We cut pneumatophores into sections to additionally examine differences in fungi based on degree of submersion in tides. We hypothesize the alpha-diversity of halotolerant fungi to be greatest in lower sections of pneumatophores more often submerged in tides, as well as leaves from which this species excretes salt. Here, we present results from a community analysis of small molecules and fungal communities from these samples, including analyses of tissue specificity, co-occurrence between metabolites and fungi, and salinity tolerance of fungal endophyte cultures. Our results provide important baseline information regarding fungi associated with a keystone species supporting ecosystems threatened by global climate change, and pave the way for much needed follow-up studies including additional mangrove species and a broader geographic range.

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Direct and indirect effects of the keystone fungal endophyte *Epichloë amarillans* under anthropogenic global change

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Microbes living within hosts can exert direct and indirect effects on microbial communities via changes to the host anatomy, chemistry, and physiology. Thus, in host associated microbiomes, understanding the determinants of microbial community structure and function requires assessing how microbes act to change their host. Here, we used a long-term field experiment to investigate changes to host associated microbial communities as well as to plant anatomy and chemistry induced by the inoculation of a key fungal endophyte *Epichloë amarillans* to its host, *Ammophila breviligulata*. To understand how these effects were dependent on ecological context we additionally challenged plants grown with and without *Epichloë* (E+/E-) to three levels of nitrogen amendment representing projected anthropogenic change.

Across above and belowground plant compartments, preliminary results indicate that plant physical traits, chemistry, as well as microbiome structure were affected by both *Epichloë* inoculation and nitrogen. In terms of plant traits, we found *Epichloë* infection reduced growth though this effect was dependent on nitrogen treatment. Belowground, we found that nitrogen played larger roles, generally shifting root traits toward less conservative growth patterns. Both nitrogen amendment and *Epichloë* infection induced metabolomic changes in Ammophila. Interestingly, *Epichloë* infection had more upregulatory impacts on roots metabolites and down regulatory impacts on leaf metabolites. Lastly, aboveground microbial communities in *Ammophila* were affected by *Epichloë* but this was dependent increased nitrogen whereas belowground microbial communities were less sensitive to the effects of *Epichloë* infection. Overall, our results indicate that a key fungal microbe within *Ammophila* acts to structure the rest of the microbiome in part via changes to plant physical and chemical traits. Understanding how plants and their associated microbes will respond to global change remains an outstanding challenge. Future work that similarly measures multi-omics changes and host responses via plant traits may advance community ecology to become more predictive in the face of global changes.

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Assessing the Ecological and Genomic Determinants of Facultative Pathogenicity Through *Colletotrichum siamense* on Strawberry

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Facultative plant pathogens are microbes capable of endophytic colonization that only transition to disease under specific host or environmental contexts. These pathogens represent a major, yet underappreciated, dimension of plant–fungal interactions, as their cryptic presence in healthy tissue complicates disease forecasting and challenges assumptions about host resistance. Currently, few systems enable controlled study of this transition. To address this gap, the pathogenicity of *Colletotrichum siamense*, a causal agent of anthracnose crown rot (ACR), was evaluated across a panel of differentially susceptible, field-grown strawberry cultivars. Despite striking differences in symptom severity, the frequency of C. *siamense* isolation was constant across genotypes, including those with apparent tolerance. This system isolates the host-genotype component of facultative pathogenicity and supports a genotype-by-environment model in which virulence emerges opportunistically rather than constitutively. Further, field evaluations indicate that nutrient stress and physical damage play substantial roles in triggering disease. Given this pattern of endophytism paired with variable pathogenicity, subsequent analyses sought to determine whether recent increases in ACR incidence and severity might be driven by genomic changes. Historically, *C. siamense* has been a relatively minor pathogen of strawberry, but its virulence and geographic range have increased markedly in recent years. To investigate genomic mechanisms underlying this shift, a hybrid assembly of the highly virulent isolate CT6-1 was generated. Comparisons of conspecific genomes identified an accessory chromosome (AC) present in all sequenced Rosaceae-infecting isolates. This AC is maintained at an elevated copy number (5–8x the core genome), representing the first documented case of extreme aneuploidy affecting fungal ACs and presenting a previously unrecognized form of genomic plasticity in *Colletotrichum*. Genomic evaluation identified predicted pathogenicity factors, suggesting that copy number escalation may potentiate virulence. Together, these findings position *C. siamense* on strawberry as a tractable system for dissecting facultative pathogenicity and highlight the possibility that extreme AC aneuploidy catalyzes the transition between endophytism and disease. The ecological and epidemiological implications of such symbioses suggest a previously unrecognized biological ubiquity.

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Candida auris can acquire echinocandin tolerance via HOG-MAPK pathway modulation and cell-wall remodeling that escapes routine AFST, reduces in vivo treatment efficiency, and facilitates resistance evolution

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Candidozyma (Candida) auris is a rapidly emerging fungal pathogen and a significant challenge to global public health, partly due to its resistance to antifungal treatments. *In vitro* experimental evolution in 180 independent lineages exposed to three different echinocandins revealed that besides classical resistance, cells can acquire echinocandin tolerance; slow growth in supra-MIC drug concentrations, without a change in the traditional MIC readout.

Genome sequencing revealed that this acquired tolerance involves disruption of the HOG-MAPK signalling pathway via single mutations in effectors like *SKK1, SSK2, PBS2, NIK1,* or *HOG1.* The deletion of HOG1, specific mutations in NIK1, or knocking out upstream effectors of HOG1 like *SLN1, SSK1, SSK2*, or *PBS2* showed that the loss of HOG-MAPK activity results in tolerance via *MKC1* cross-talk, since *mkc1*Δ confer hypersensitivity to echinocandins. We confirmed the conservation of this efect, without major fitness trade-offs, in strains from three clades of *C. auris*, but not in other species like *Candida albicans*. In an immunocompromised murine systemic infection model treated with micafungin, we found that the tolerant strains cause significantly higher fungal burden compared to the non-tolerant strain, despite identical MICs, showing that tolerance can jeopardize treatment success. Genome database mining revealed many high-impact mutations in the HOG pathway effectors in clinical isolates of *C. auris* and other *Candida* species, suggesting that this mechanism of acquired tolerance might influence treatment success in the clinic. To further investigate the mechanism of action, we performed comparative proteomics, which revealed changes in several biological processes involved in cell wall homeostasis. Cell wall analyses using HPLC, solid-state MAS NMR, TEM, flow cytometry and fluorescence microscopy demonstrate that tolerant cells show significantly different cell wall modulation in the form of a dampened response to echinocandin exposure. Finally, through mathematical modelling validated via large-scale parallel experimental evolution and deep sequencing of mutational hotspots, we show that tolerance can facilitate drug resistance development via the acquisition of *FKS1* hotspot mutations. In conclusion, our data show that in *C. auris*, echinocandin tolerance can be genetically acquired, and, although it is not detectable via standard antifungal susceptibility tests (AFST) in the clinic, may play a critical role in treatment failure and resistance development.

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Functional Genomic Mapping Illuminates the Network Architecture of Drug Resistance in *Candida glabrata*

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Candida glabrata (*Nakaseomyces glabratus*) is the second most common cause of invasive candidiasis, with a mortality rate of ≥49%. It exhibits intrinsic low-level azole resistance and rapidly acquires high-level resistance. Resistance is primarily attributed to gain-of-function mutations in *CgPDR1*, leading to the upregulation of efflux pumps and reduced intracellular accumulation of azoles. Due to this, echinocandins are now the recommended as first-line treatment. However, echinocandin resistance is increasingly documented in *C. glabrata* primarily due to hotspot mutations within *CgFKS1* and *CgFKS2*.

To identify novel therapeutic targets, we employed synthetic genetic array (SGA) screening in *Saccharomyces cerevisiae* to explore the genetic interaction networks driving drug resistance in *C. glabrata*. We have generated genome-wide double mutant strains which express either a *CgPDR1*, *CgFKS1* or *CgFKS2* allele and a single gene deletion. These combinations reveal synthetic sick or lethal interactions, offering insights into resistance mechanisms. Screens using both wild-type *CgPDR1* and the clinically derived $CgPDR1^{R592S}$ allele highlighted the role of chromatin remodelling and transcription pathways in resistance, identifying *CgGCN5* a component of the SAGA complex, as a promising therapeutic target. In silico screening identified the Methotrexate as a potential inhibitor of *CgGCN5. In vitro* testing demonstrated synergistic effects between Methotrexate and Fluconazole against *C. glabrata* and other *Candida* species. Additionally, this study is the first to map the genetic interaction networks associated with *CgFKS1* and *CgFKS2* mediated echinocandin resistance using SGA. Collectively, these findings demonstrate the utility of unbiased SGA screening for uncovering novel antifungal targets.

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Proteomic profiling of antifungal drug responses of *Aspergillus fumigatus* indicates drug-specific signatures and modes of action

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The human fungal pathogen *Aspergillus fumigatus* has become increasingly resistant to clinically used antifungals agents, in particular to azole-based drugs. This has led to high mortality rates in patients with azole-resistant invasive aspergillosis. The discovery of new antifungal agents, especially those with novel modes of action (MoA), is therefore urgently needed. However, due to the closer evolutionary relationship between cells of fungi and humans in comparison to bacteria, the number of fungal-specific targets for drug development is rather limited. This often leads to the rediscovery of drugs with known MoA. To address this challenge and to learn more about how antifungal agents affect fungal cells, we employed expression-based proteomics to construct a comprehensive drug-specific proteomic response library based on the profiling of 14 anti-*Aspergillus fumigatus* compounds. Central to this effort was the development of a robust, standardized workflow from cultivation to LC-MS/MS data evaluation. Our analysis revealed that the strongest proteomic responses were linked to processes involved in ‘detoxification’ and ‘defence’ against environmental insults. Our analysis allows to distinguish between synthetic drugs and natural products. Based on our response library, we successfully identified MoA-specific signature proteins, enabling the prediction of similarities between MoAs of compounds. Our strategy also uncovered drug-specific induction patterns of biosynthetic gene clusters (BGCs). We also validated our approach by using three additional drugs, including azalomycin F, a natural product with a predicted new MoA. Notably, we identified the drug-specific increase of poorly characterised transcription factors, which suggests complex regulatory circuits of antifungal drug resistance.

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An *Aspergillus fumigatus* homeobox transcription factor provides resistance to copper stress and alters lipid metabolism through oxylipin signaling

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Fungal pathogens like *Aspergillus fumigatus* must maintain copper homeostasis during infection, balancing copper's essential role as an enzyme cofactor against its toxicity when weaponized by host immune cells. While copper transporters have been well characterized, the regulatory mechanisms coordinating copper stress responses with broader metabolic adaptations remain poorly understood. We identified HbxH, a previously uncharacterized homeobox transcription factor, which when overexpressed (OE::*hbxH*) increases both germination and biomass in copper starvation and toxicity up to fourfold. To investigate the mechanisms underlying this phenotype, we performed untargeted proteomics under copper starvation, identifying 522 differentially expressed proteins between the deletion (Δ*hbxH*) and OE::*hbxH*. Multiple predicted lipid biosynthetic and regulatory proteins were significantly altered in OE::*hbxH*, prompting lipid visualization using Nile Red fluorescence microscopy. OE::*hbxH* displayed pronounced lipid accumulation and increased lipid droplet abundance across all tested conditions. Additionally, metabolomics revealed the oxylipin 5,8-diHODE was increased by approximately 30-fold in the overexpression. Strikingly, exogenous addition of 5,8-diHODE to wild type and Δ*hbxH* phenocopies the increased growth and lipid accumulation of OE::*hbxH* in copper stress. These findings reveal an unexpected link between copper homeostasis and lipid metabolism mediated by oxylipin signaling. Future investigation aims to determine if lipid accumulation itself confers copper stress resistance and understand these effects using *in vivo* and *in vitro* host-pathogen interaction models.

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Digging into organic waste: Environmental selection of azole-resistant *Aspergillus fumigatus.*

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Aspergillus fumigatus is a ubiquitous, opportunistic fungal pathogen, of which part of the population is resistant to the first-line treatment; triazole antifungals. Organic waste piles can contain triazole fungicides which have the same mode of action as their medical counterparts, which selects for cross-resistant *A. fumigatus*. As we are unable to source-trace *A. fumigatus* spores, improving our understanding of the ecology of triazole-resistant *A. fumigatus* is essential to overcome disease burden. Research has focused on global sampling of *A. fumigatus* to capture the wider genetic diversity, a scale that misses the level of niche differentiation. To study the role of niche differentiation and spread of resistance, we sampled an organic waste pile without and with fungicide (65 mg/kg, 5-8% triazoles) compounds and cultured *A. fumigatus* (thousands to millions ofCFU/gram). To understand the genetic diversity within a single pile, we sampled in five locations within one pile, and subcultured isolates from 3 gram per sample. To measure the genetic diversity, we whole genome sequenced 270 isolates of the fungicide-free pile, and 180 isolates of the fungicide-containing pile. We found a total of ±82,000 variants among the isolates from the fungicide-free pile. This number is consistent with inoculation from the environment, rather than clonal outgrowth. This also indicates that a single pile contains a ±70% of the diversity that was previously found in ±1,600 global isolates. It is expected that the selective pressure of fungicides will result in lower variation in the high-fungicide pile, due to lower genetic diversity within the triazole-resistant cluster. This will provide an initial indication of the strength of selection exerted by triazole fungicides on the population diversity of *A. fumigatus.* Understanding how specific environmental niches contribute to the propagation and spread of resistant isolates is crucial for enabling informed, multi-stakeholder decision-making to address triazole-resistant *A. fumigatus.*

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Rapid Emergence of Highly Fungicide-Persistent Variants During Cryptococcal Infections

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Drug persistence significantly impairs the efficacy of fungicidal treatments, yet its evolutionary dynamics in clinical settings remain poorly understood. Through a nationwide collection of more than 1,000 *Cryptococcus* clinical isolates from cryptococcosis patients in China, we developed a high-throughput computer vision-based antifungal persistence assay (ComPer-TEST) to quantitatively assess their persistence level to amphotericin B (AMB). Our results revealed that while AMB resistance remains rare (MIC ≤ 0.5 μg/mL across all isolates), persistence levels vary by up to four orders of magnitude among strains, indicating extensive heterogeneity in persistence. Genomic and phylogenetic analyses showed no significant correlation between persistence level and genetic background, and even closely related isolates from the same patient exhibited divergent persistence phenotypes without changes in resistance. Longitudinal patient samples and a murine infection model confirmed that persistence variants emerge rapidly during infection, even in the absence of drug pressure. By functional genomics approach, we revealed that persistence-specific genes—distinct from those conferring resistance—impose minimal fitness costs *in vitro* and *in vivo.* Notably, we identified the Qsp1 quorum-sensing system and ergothioneine biosynthesis as key contributors to persistence. Disruption of these pathways suppressed the emergence of highly fungicide-persistent variants during cryptococcal infections. Our findings demonstrate that persistence evolves readily during infection with minimal fitness trade-offs, highlighting a critical and overlooked dimension of antifungal treatment failure.

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Evolutionary adaptation of *Candidozyma auris* during human infection

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Candidozyma [*Candida*] *auris* is an emerging fungal pathogen recognized by both the Centers for Disease Control and Prevention and the World Health Organization as an urgent threat to public health. Unique among fungal pathogens, *C. auris* poses challenges to clinicians reminiscent of healthcare-associated bacterial pathogens, frequently being associated with both clinical outbreaks of infection and multi-drug resistance. Unlike other agents of invasive candidiasis, *C. auris* is not considered part of the human normal flora and is not known to be widely distributed in environmental reservoirs. Instead, *C. auris* infections are currently thought to be acquired predominantly through dissemination of recently emergent drug-resistant, and possibly infection-adapted, lineages within medical environments. In addition to notably elevated rates of intrinsic antifungal resistance, *C. auris* has repeatedly been reported to acquire additional antifungal resistance during treatment of human infections. In this study, we performed molecular and genetic interrogation of closely related *C. auris* isolates cultured from patients being treated for invasive candidiasis. Included isolates were found to exhibit both elevated and diminished resistance to agents representing multiple classes of antifungals. Comprehensive sterol profiling and cell wall constituent characterization revealed variations associated with altered drug susceptibility. Differences in tolerance to a variety of cell stressors was also observed. Whole genome sequencing analysis identified underlying genetic variants between related isolates, including single nucleotide polymorphisms, frameshift mutations, and large scale insertions or deletions. Interrogation of these variations using the *C. auris*-optimized Episomal Plasmid Induced Cas9 gene editing system confirmed the contribution of identified mutations to drug resistance. Taken together, the results of this study demonstrate the genetic plasticity of *C. auris*, and highlight the ability of this emerging fungal pathogen to rapidly adapt to antifungal induced stress during therapy.

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ColonyPy: A python-based image analysis pipeline to quantify tolerance and heteroresistance in *Cryptococcus neoformans*

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Clinical outcomes in cryptococcal meningitis patients infected with fluconazole-susceptible *Cryptococcus neoformans* isolates vary widely and can include treatment failure, relapse, prolonged therapy, and high mortality. These discrepancies suggest that conventional drug susceptibility testing may overlook subtle phenotypes that survive above the Minimum Inhibitory Concentration(MIC) in *C.neoformans*. Two phenomena are increasingly recognized in susceptible isolates of pathogenic yeasts. Tolerance, defined as slow-growing subpopulations that survive above the MIC, and heteroresistance, where ~1% of cells grow at concentrations above the MIC. These phenomena are frequently observed with fluconazole (FLC), a fungistatic drug central to cryptococcal meningitis therapy. Importantly, tolerance and heteroresistance differ from resistance. In *Candida albicans* tolerance is common whereas *C.neoformans* exhibits both tolerance and heteroresistance. To simultaneously measure tolerance and heteroresistance, we developed ColonyPy, a Python-based image analysis pipeline using open-source libraries. ColonyPy detects and quantifies colony number and size within the zone of inhibition (ZOI) in disk diffusion assays, and above the MIC in agar dilution plates. Using ColonyPy, we quantified tolerance and heteroresistance in 40 *C.neoformans* clinical isolates using disk diffusion with FLC 25ug disks. Culture plates were photographed at 48, 72, 96 and 120 hours post-inoculation on Sabouraud dextrose agar (SDA), Yeast peptone dextrose agar (YPD) and Müller-Hinton agar (MHA) with 2% glucose incubated at 37°C. The number and size of colonies within the zone exhibiting ≥80% inhibition (ZOI 80) were analyzed using the ColonyPy program. This analysis revealed that: (1) clinical isolates of *C. neoformans* varied in their levels of tolerance and heteroresistance; (2) tolerance and heteroresistance levels differed significantly across media types; (3) YPD showed the highest levels of both tolerance and heteroresistance; and (4) both tolerance and heteroresistance stabilized after 96hrs, defining a reliable measurement time point. Taken together, our results demonstrate that tolerance and heteroresistance are distinct, stable phenotypes in *C.neoformans* that are influenced by nutrient source. Our robust assay that differentiates between tolerance and heteroresistance will facilitate future studies to elucidate underlying mechanisms of these phenomena and their role in treatment outcomes.

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Quantitative imaging of fungal life cycle transitions through generative deep learning

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The life cycle of biomedical and agriculturally relevant filamentous fungal pathogens involves complex transitions between proliferative and non-proliferative states. However, few experimental setups allow the quantitative study of living filamentous fungi through stages such as germination, mating, sporulation, and filamentous growth. Here, we provide a solution to quantify and track life cycle transitions in fungal organisms using a combination of computer vision algorithms and microfluidics. We used high aspect ratio microfluidic devices to induce transitions from dormant into proliferative states in representative ascomycetes and basidiomycetes. To detect and track specific fungal structures or life cycle stages, we created a deep learning fungal tracking algorithm based on generative image synthesis and life cycle stage-specific segmentation of spores, hyphal tips, branching points, mating events, septa, and fungal biomass on phase contrast images. Using this experimental framework, we quantitatively imaged life cycle transitions in the pathogens Verticillium dahliae, Colletotrichum acutatum, Ustilago maydis, and Neopestalotiopsis spp, and the ectomycorrhizal fungus Hebeloma cylindrosporum, including spore germination, filamentous growth with branching and septation, and alternative life cycle paths, such as microcyclic conidiation. We found that germination follows species-specific kinetics that change in response to environmental conditions, whereas apical tip growth accelerated over time during proliferation, with changes in tip orientation being inversely correlated to the speed of hyphal growth. The quantitative characterization of individual life cycle stages allowed us to track complete asexual life cycles using cyclic nutritional stress. Our experimental framework could be applied to quantitatively investigate other fungal life cycle landmarks such as anastomosis, minichromosome maintenance, microcyclic conidiation, mating, and sporulation. Our code will be available as a free pip-installable user interface “GenFungAI.”

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AI-driven protein design to understand effector function within the *Magnaporthe oryzae* rice pathosystem

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Artificial intelligence (AI)-based protein design tools have great potential to advance our understanding of molecular interactions between pathogenic fungi and their hosts. This study illustrates how AI-designed binders can be deployed to characterise enigmatic fungal effectors and reveal conserved virulence mechanisms, complementing traditional molecular approaches. The blast fungus *Magnaporthe oryzae* infects agronomically significant crops such as rice and wheat, but also subsistence crops such as fingermillet. Globally, rice blast disease has an economic impact of $66 billion each year. Like many phytopathogens, *M. oryzae* utilises an arsenal of secreted effectors to facilitate plant infection. Through a structure-based phylogenetic analysis of the *M. oryzae* effector repertoire, we have discovered a previously uncharacterised effector, *Magnaporthe* effector protein 26 (Mep26). Structural predictions suggest that Mep26 functions as an enzymatic effector with phytate-degrading activity. Furthermore, we have identified three structural orthologues of Mep26 that exhibit low sequence similarity yet share a conserved structural fold. To probe the function of Mep26, we utilised AI-based protein design to generate *de novo* protein binders for Mep26. Designed binders were filtered *in silico* for cross-reactivity across structural orthologues to dissect the mechanism by which the Mep26 effector family contributes to fungal virulence. By targeting the binders to the putative active site, we hope to disrupt ligand binding and consequentially phytate degradation. Enzymatic assays can then to be used to test whether activity is reduced in the presence of the binders. Furthermore, by customising the binders with various tags, such as localisation signals or degradation motifs, we aim to assess whether we can phenocopy mutants lacking all members of the Mep26 family. This proof-of-concept study of the Mep26 family will allow for these techniques to be applied to other challenging research areas, such as targeting highly redundant structural families in genetically intractable organisms where multiple knockouts could prove more difficult. Finally, we provide an overview of AI-driven protein design and outline how *de novo* protein design strategies can be broadly applied in other areas of fungal protein-protein interaction research. Through this work, we aim to stimulate discussion on how emerging technologies can address fundamental questions in fungal biology.

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Allopolyploidization as a driver of structural, functional, and phenotypic diversification in *Brettanomyces bruxellensis*

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Polyploidization, particularly allopolyploidization, plays a major role in shaping genome evolution. Using, long-read sequencing and subgenome-aware analyses, we have revealed that *Brettanomyces bruxellensis* has undergone several independent polyploidization trajectories, giving rise to genetically and ecologically distinct subpopulations. These polyploid genomes contain either closely related or highly diverged chromosomal copies, demonstrating the coexistence of auto- and allopolyploidization mechanisms, with additional restructuring through loss-of-heterozygosity events that further remodel genome architecture.

Using subgenome-resolved assemblies, a graph-based pangenome, and population-scale genomic datasets, we uncovered extensive structural variation (SV), with 212,177 SVs detected across 1,060 *Brettanomyces bruxellensis* isolates. Allopolyploid genomes harbor markedly more SVs than diploids, and a subset of these variants, those fixed between parental lineages, form hotspots of structural diversity. Notably, 27.5% of SVs exert local regulatory effects, with hybridization-associated SVs displaying disproportionately strong impacts on gene expression, underscoring allopolyploidization as a driver of both structural and functional diversification.

Population-level sequencing and phenotyping of 1,060 isolates revealed that allopolyploidization also profoundly shapes phenotypic landscapes. Allotriploid isolates exhibit enriched extreme phenotypes, and genome-wide association studies demonstrate that variants located on the acquired subgenomes consistently show greater effect sizes than those on the primary genome. Acquired genomes remain more conserved across isolates than primary genomes, suggesting different evolutionary tempos for the two genomes.

Together, these findings illustrate how allopolyploidization establishes long-lasting genomic, structural, and phenotypic consequences across an entire species.

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Deep Learning and Generative AI for Fungal Genomics

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Pathogenic and toxin-producing fungi pose a major risk to food security through yield loss and mycotoxin contamination, yet many genomic and proteomic features underlying virulence, toxin production, and host specificity remain poorly characterized. To address this, we are developing an AI-native methodological framework for plant pathogens that spans a wide range of multi-omic datasets, including genomes, transcriptomes, proteomes, and host–pathogen interactomes. We deployed resources for *Fusarium* and *Aspergillus* that leverage state-of-the-art tools to analyze these multi-omic datasets. First, we apply transfer learning with a convolutional neural network-based gene prediction framework to refine and harmonize gene model annotations across multiple fungal genomes, providing improved coding and regulatory annotations as browsable genome browser tracks. We then use AlphaFold and ESMFold to generate predicted 3D structures and provide structure-based search to enable hypothesis generation about novel effector virulence mechanisms and detoxification enzyme mechanisms. To quantify the impact of genetic variation, we used DNA language models (e.g., DNABERT) to score regulatory and coding variants, and protein language models (e.g., ESM) to score missense mutations likely to alter protein stability or function. Building on these foundations, we use multimodal methods (integrating structural and small-molecule features) to model the binding of different toxins, such as deoxynivalenol (DON), to putative detoxification or resistance proteins, including FHB7-like candidates, to identify alleles that could improve predicted affinity. We further employ protein diffusion models (e.g., RFDiffusion) to generate de novo binders targeting key effectors and toxins. Finally, we leverage multimer versions of protein prediction software (e.g., AlphaFold-Multimer) to screen potential host-pathogen protein-protein interactions between predicted effectors and crop targets, integrating these predictions with variant and structural scores in web-accessible tools. These AI-driven

resources create a reusable, computable framework for exploring fungal biology and prioritizing host and pathogen targets for breeding, protein engineering, and functional validation to effectively reduce disease and mycotoxin contamination.

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Interrogating yeast biodiversity by analyzing "silent" synonymous codon bias with machine learning

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Why are some Saccharomcyotina yeasts human pathogens while others make great cheese? Genetic differences contribute to the vast biodiversity in yeast ecology, metabolism, and other growth phenotypes in our sample of 1,154 yeasts. Some of this diversity is associated with gene gain and loss, which is relatively straightforward to detect in genomes. Much of this diversity, however, is due to altered gene expression, which is difficult to assess across more than one thousand non-model yeasts. Despite the moniker "silent," synonymous genetic changes that do not alter the protein sequence can impact both transcription and translation rates. We are leveraging machine learning methods to interrogate the role of codons and their associated tRNAs in yeast evolution, ecology, and phenotypes. We find that codon usage is highly informative for classifying yeasts into thermotolerance phenotypes using a random forest algorithm. Within species, genes belonging to the same metabolic pathway exhibit classifiable differences in codon usage, opening the door for the classification of genes of unknown function. We also identified tRNA sequence variation that is highly predictive of yeast taxonomic order. Altogether, this work is building our understanding of how cells tune their codon usage and tRNA expression over cellular and evolutionary time.

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Characterization of *de novo* miniproteins targeting *Candida albicans* virulence traits

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Fungal infections affect more than a billion people annually, yet the number of licensed antifungals remains low, and drug resistance is emerging in many species, demanding new therapeutic interventions. An alternative strategy to combat mycotic disease is to target virulence traits. Such an approach can potentially expand the therapeutic target space while minimizing disruption to the host microbiota and reducing selective pressure on the pathogen to develop resistance. To explore this strategy, we designed *de novo* miniprotein inhibitors, peptides of three to four alpha helices, using a machine learning-based protein structure and binding prediction software called BindCraft. These miniproteins were designed to target virulence factors of the fungal pathogen *Candida albicans*, including the adhesin Als3 and the cytolytic peptide candidalysin. To confirm binding between the designed miniproteins and the target protein, we are employing genetic and biochemical approaches. Specifically, we tested a library of miniproteins designed against the N-terminal region of Als3 for target protein interaction through a yeast two-hybrid assay. Amplicon sequencing of the yeast two-hybrid strains isolated on medium that selected for interactions between bait (Als3) and prey (miniprotein) revealed three structurally similar miniproteins that interacted with the adhesin. Functional assays will be performed to assess the impact of these miniproteins on *C. albicans* adherence to plastic surfaces as well as mammalian cells in culture. Furthermore, a library of miniproteins designed against the candidalysin monomer were screened against synthetic candidalysin in a toxin neutralization assay. Three structurally dissimilar miniproteins with low peptide identity significantly reduced mammalian cell death in the presence of candidalysin, suggesting toxin binding and neutralization. Future work will be performed to define the mechanism by which the miniproteins interact with candidalysin directly as well as their therapeutic potential against *C. albicans* itself. Overall, this project exemplifies an innovative approach to address the need for novel therapeutics to combat serious fungal infections.

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AI-Driven *Fusarium* Databases: identify key genetic variation and target to combat agricultural threats and aid development of crops resistant to disease and mycotoxin contamination

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Fusarium is a major threat to agriculture because collectively its species cause disease and mycotoxin contamination of diverse crops. In the U.S., mycotoxins are estimated to cause annual losses of over one billion dollars to the agricultural economy. In addition, new epidemics and species of *Fusarium* with novel pathogenic and/or toxigenic abilities continue to emerge, causing additional economic threats. Understanding interactions of *Fusarium* and plants at the protein level, including impacts of single nucleotide polymorphisms (SNPs), has tremendous potential to improve resistance of crops to disease and mycotoxin contamination. Advances in Artificial Intelligence (AI)-driven computational approaches are accelerating development of disease-resistant crops by enhancing the ability to analyze large-scale datasets to more rapidly identify protein-protein interactions (PPIs) that impact plant disease. However, progress has been limited by fragmented data and inadequate tools to visualize and assess how structural and missense variation can impact the outcome of PPIs and in turn affect crop disease. To address these limitations, we developed the *Fusarium* Protein Toolkit (https://fusarium.maizegdb.org/), an interactive, AI-driven database that contains models of all predicted proteins from 22 *Fusarium* species. The Toolkit also includes tools to explore predicted 3D protein structures, functions, and effects of missense mutations on protein structure. Currently, we are developing two additional interactive databases that will allow users i) to query, visualize, and explore SNP variation at a population genomic level (https://fusariumsnps.maizegdb.org/) and ii) to determine which pairs of *Fusarium* and crop proteins are most likely to interact (https://fusarium.maizegdb.org/multimer). These databases use AI language models and computational biology to predict PPIs that impact disease. We anticipate that the three databases can be used to accelerate development of crop varieties with enhanced resistance to *Fusarium*-incited disease and mycotoxin contamination by identifying targets for plant breeding and genetic engineering programs.

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AI-Driven Phenotyping Uncovers Predominantly Antagonistic Dynamics in Poplar Root Bacterial–Fungal Interactions

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Understanding bacterial–fungal interactions remains a major challenge in systems biology and is critical for designing synthetic microbial communities for bioinoculant development. Among model organisms, *Bacillus* and *Pseudomonas* bacteria and the ectomycorrhizal fungus *Pisolithus* have been extensively studied in pairwise systems. However, the rhizosphere represents a far more complex environment. Leveraging our extensive microbial collection from poplar trees, we investigated how 10 commonly isolated endophytic bacteria and fungi from poplar roots interact over a three-week period using an AI-enabled phenotyping platform to image and quantify pairwise interactions on different nutrient medias. The workflow integrates open-source vision–language models for microbial localization, time-series segmentation, and feature extraction, enabling automated and consistent assessment of growth dynamics. This approach addresses a key limitation of traditional assays, namely, their labor-intensive nature and susceptibility to missing critical time points such as inflection moments in growth trajectories. While many pairings remained neutral, others showed distinct changes in growth dynamics revealing both colony morphology and interaction events shift with nutrient composition. These results underscore how media choice, often discipline-specific, can influence microbial behavior and interpretation of interspecies relationships. Collectively, the findings suggest that poplar-associated bacteria and fungi interact primarily in neutral or antagonistic ways *in vitro*, and that apparent plant benefits *in vivo* may emerge from host mediation or higher-order community effects rather than direct mutualism between individual partners. Representative interaction patterns and implications for designing synthetic microbial communities will be presented.

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Heat adaptation in the pathogenic yeast *Cryptococcus deneoformans*

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Invasive fungal infections are a leading cause of mortality in populations with weakened immunity. Human body temperature (37°C) serves as a key barrier to fungal infections, as most environmental fungi grow optimally between 25–30°C. Rising global temperatures and extreme heat events exert selective pressure on fungi to adapt to growth at higher temperatures, which may increase their pathogenic potential. The genetic mechanisms by which fungi evolve greater heat tolerance remain poorly understood. We use *Cryptococcus deneoformans* as a model to study heat adaptation in fungi because it is less thermotolerant and less virulent than other disease-causing *Cryptococcus* species, primarily associated with skin infections and, more rarely, fungal meningitis. In a 30°C passaging experiment, we found that spontaneous insertion of a transposable element into a single gene (*PP4R1*) conferred increased thermotolerance in *C. deneoformans* (growth up to 39°C). *PP4R1* encodes a predicted scaffold of the conserved protein phosphatase 4 (PP4) complex, found to regulate DNA repair, chromatin remodeling, and cell cycle progression in other systems. We compared growth of a *PP4R1* deletion mutant with wildtype cells at different temperatures using spot dilution assays and microscopy. Additionally, we characterized the fitness of the *PP4R1* mutant by assessing virulence traits and survival under different stress conditions. Finally, we assessed fitness in a murine model of infection to compare infection outcomes among wildtype, mutant, and complemented strains. These studies will help to uncover evolutionary mechanisms that underlie fungal heat adaptation and test their impact on pathogenesis.

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Evolutionary Analysis of Pmk1 MAPK-dependent fungal pathogenesis across the Fungal Tree of Life

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Fungal pathogens are a significant threat to global agricultural, causing devastating plant diseases and yield losses. Some pathogens develop specialized infection structures to penetrate the plant cuticle while others have evolved mechanisms that exploit natural openings or wounds to enter plants. Among the evolutionarily conserved Mitogen-Activated Protein Kinases (MAPKs) in eukaryotes, the Pmk1-related MAPKs have been shown to play important roles in fungal pathogenesis, but is also functionally related to the well-characterised Fus3/Kss1 pathway in yeast, which regulates mating and filamentous growth. In the plant pathogenic fungus *Magnaporthe oryzae*, Pmk1 regulates appressorium formation, invasive growth and virulence. Pmk1 homologues have been identified in more than 33 fungal pathogens and shown to play a central role in regulating pathogenicity towards different host plant species, including major agricultural crops. To understand how phosphorylation-mediated regulation has evolved throughout the fungal kingdom, we used phosphoproteomics data that identified over 8000 phosphopeptides from ~2000 *Magnaporthe oryzae* proteins and performed comprehensive phosphosite conservation analysis across 1,143 fungal genomes spanning the entire kingdom Fungi. Our analysis shows striking patterns of phosphosite conservation, correlating with ecological niche and lifestyle adaptation in fungi. Most interestingly, we found a specific cluster of phosphosites conserved in plant pathogenic fungi but largely absent from saprophytes, animal pathogens, and species with other fungal lifestyles. Gene function enrichment provides evidence that these conserved phosphosites are overrepresented in genes within key regulatory hubs involved in signal transduction, transcriptional regulation, vesicular trafficking, cytoskeletal organization, and protein degradation, representing cellular processes that require synchronized regulation throughout host invasion. Our analyses suggest that the infection-related phosphorylation landscape is therefore evolutionarily conserved in plant pathogens, but has diversified according to fungal lifestyle to orchestrate the complex cellular reprogramming required for successful host tissue invasion. Kingdom-wide analysis therefore provides a comprehensive illustration of how post-translational mechanisms have evolved in plant pathogenic fungi and identifies conserved regulatory mechanisms that could be targeted for disease control strategies.

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Investigating the repeated evolution of pathogenesis in *Aspergillus* section *Fumigati*

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Opportunistic fungal pathogens repeatedly evolve across all major clades of fungi. The ability of these fungi to cause disease is the culmination of multiple exaptations resulting in the convergent trait, pathogenicity. In clades like *Aspergillus* section *Fumigati*, pathogenicity evolved independently over relatively short evolutionary time scales. We utilized 36 closely related species of pathogens and non-pathogens from within *Aspergillus* section *Fumigati* to ask whether the independent evolution of pathogenicity is species-specific or conserved through a combination of growth and immunological assays and high-quality *de novo* assemblies. Growth at four different temperatures (30°C, 37°C, 40°C, 45°C) underlines the section's thermotolerance, with many species growing better at 37°C than 30°C. Conidiation and conidia germination, important ecological factors, display large outliers and no clear signal for pathogenic species. Similarly, survival with murine macrophages and the production of pro and anti-inflammatory cytokines support the independent evolution of pathogenesis across the section. The genomic data tells a similar story. Genome size, gene presence-absence, TE cargo, gene functional conservation, and gene gain/ loss rate all support the species-specific model of evolution. Finally, we consider potential ecological roles using predicted species occupation ranges and overlaps that may influence phenotypic and genotypic differentiation while still resulting in pathogenic potential

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Single-cell genomics of Freshwater Zoosporic Fungi

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Fungi are critical research systems as they impact global carbon cycling and have remarkable potential for sustainable biofuel production processes. Current estimates suggest that under 10% of total fungal species worldwide have been described, and the overwhelming majority of those fall within the Dikarya. The true extent of the phylogenetic diversity among the non-Dikarya lineages is poorly explored. Thus, "Dark Matter Fungi" (DMF) is used to describe this understudied fungal diversity. The difficulties inherent in exploring the genetics of "fungal dark matter", a reflection of their absence from sequence databases, can be overcome using single-cell sequencing. Reconstructing the genomes of uncultivated organisms directly isolated from the environment allows for meaningful comparative analyses and lifestyle predictions. In this study, samples were collected from freshwater environments around Michigan, USA, which included fungi associated with algae, micro-invertebrates, and protists. Coupling phenotypic characterization through direct microscopy with single-cell sequencing produced nearly 100 genomes representing previously and newly described lineages within the Chytridiomycota, Blastocladiomycota, Aphelidomycota, and Rozellomycota. We observe genome sizes ranging from 2.2 to 37.9 Mb (median 14.3 Mb), with completeness estimates of 44.98% to 98.91% CEGMA (median 92.79%). Phylogenomic analyses of these unsampled lineages support placements previously established using rDNA sequences. Additionally we explored functional gene family patterns, such as CAZYme profiles as well as primary and secondary metabolism, to establish correlation with biotrophic lifestyles of these organisms. Finally we utilized these genomes to improve gene annotations in published metatranscriptomic datasets. This work represents the first large scale genomic investigation of environmental fungi using single-cell and microscopy approaches. These genomes, which include novel phylum level lineages, will help further refine the phylogeny and biological understanding of undescribed zoosporic fungi.

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When Preparedness Meets Opportunity — A Genomic Perspective on the Ecological Plasticity of *Trichoderma*

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As most fungi dwell out of sight, our knowledge of their natural behaviour remains remarkably limited. We still know little about the *in situ* biology of many common species that we routinely use in agronomy and biotechnology. A striking example is *Trichoderma* (*Hypocreales*, *Ascomycota*) — a mycoparasitic genus central to industrial cellulase production and eco-sustainable agriculture through its roles in biocontrol and plant growth promotion. Yet its ecology is paradoxical: the main cellulase producer, *T. reesei*, carries only a modest repertoire of cellulolytic genes; species traditionally viewed as soil fungi prove to be negligible members of soil communities; most lineages inhabit wood but cannot degrade lignin; and despite being primarily mycoparasitic and arboreal, many *Trichoderma* species are reported from marine habitats. *Trichoderma* also causes green mould and includes opportunistic human pathogens. The recent emergence of maize disease caused by the biocontrol strain *T. afroharzianum* T22 underscores the need to assess the opportunistic potential of these fungi.

In this talk, we present first results from the *Trichoderma* Whole-Genus Genomic Study undertaken by the international *Tricho*Cosm Community. Using machine learning to analyse genomes of 37 ecologically diverse strains and over 150 phenotypic traits, we generated a genus-wide map of *Trichoderma* fitness. The analysis identifies convergent genomic determinants — including regulatory genes, stress-response modules, transporters, and small secreted proteins — that underpin ecological plasticity and resilience.

Our follow-up studies reveal how *Trichoderma* exploits ecological "windows." For example, we detected a distinct pulse of *Trichoderma* abundance in a postfire Tibetan pine forest occurring immediately after pyrophilous fungi, capturing a short-lived niche before wood-inhabiting taxa arrived. Moreover, we will present results of the molecular ecological and gene-engineering study of *Trichoderma* halotolerance, revealing regulatory adaptation of the glycerol biosynthesis pathway in *T. asperelloides*, confirmed by comprehensive gene deletion, promoter-swap, and microcosm experiments with synthetic communities.

Together, these findings show how genomic preparedness intersects with ecological opportunity to produce *Trichoderma*'s remarkable plasticity and provide a foundation for informed biosecurity evaluation of *Trichoderma* bioeffectors in agriculture.

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High-throughput genetics in the *Cryptococcus* genus

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Pathogenesis has emerged repeatedly and independently across the fungal tree of life. In the *Cryptococcus* genus, the pathogenic species split from a group of saprophytic nonpathogens around 65 million years ago. Our group is interested in studying this transition within the *Cryptococcus* genus to understand general principles underlying the evolution of virulence in fungi. To do so, we have developed a high-throughput quantitative genetics approach in *Cryptococcus neoformans* using massively parallel insertional mutagenesis coupled with targeted sequencing (TN-seq) to identify and quantify transposon insertions across a pool of cells. We can apply selections to these pools of cells and measure transposon insertion frequencies before and after selection to determine the suite of genes that plays a role in growth under any given condition. We have previously used TN-seq to map genes that affect susceptibility to fluconazole in *C. neoformans*. We are now taking advantage of the portability of the TN-seq system to enable genome-wide screens outside of the reference H99 strain of *C. neoformans* and explore the evolution of virulence and virulence relevant traits, such as growth at human body temperature and drug resistance.

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The Evolution of Host Preference Across the *Botrytis* Genus

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The genus *Botrytis* consists of over 35 necrotrophic fungal plant-pathogens that cause great economic impact to both the agricultural and floricultural industries. Previous work has established two major clades: a clade of eudicot generalist pathogens and another of monocot specialist pathogens. However, little is known about the evolutionary underpinnings of this clade division and the genetic differences underlying host preference within the genus. Primary metabolic genes are associated with growth and reproduction, while specialized metabolic genes mediate host-pathogen interactions. Both have the potential to influence the spread and virulence of a fungal pathogen. We compared the evolutionary histories of metabolic genes to identify correlations between host range diversification and the evolution of metabolic pathways in *Botrytis*. To make this comparison, we constructed a phylogeny of *Botrytis* using a multispecies coalescence model and whole-proteome sequence data for 20 species within the genus.

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Recent lineage diversification and gene flow shape climate adaptation in the Swiss needle cast pathogen of Douglas-fir

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Recurrent epidemics of Swiss Needle Cast (SNC) on *Pseudotsuga menziesii* var. *menziesii* in the Pacific Northwest have intensified alongside climate change, spreading from the Oregon coast into British Columbia. Using whole genome sequencing of the pathogen *Nothophaeocryptopus gaeumannii* (~200 SNC genomes), we identified highly divergent genetic lineages with different levels of suitability to climates. We resolved additional population structure within lineage 1, revealing sublineages 1c (coastal) and 1i (inland). Lineage 2 occurs sympatrically with lineage 1c on the coast, while lineage 1i is primarily associated with *P. menziesii* var. *glauca*, the Rocky Mountain Douglas-fir. Modeling of the demographic and evolutionary history indicated that lineages 1c and 1i diverged from a common ancestor about 20,000 years ago. This coincides with the divergence between the hosts of these lineages after the last glacial maximum. The hypothesis of complete reproductive isolation among these lineages was rejected: we find strong evidence of gene-flow between lineage 1c and 1i, with hybridization events likely happening on trees in the hybrid zone of Coastal and Rocky Mountain Douglas-firs. Similarly, introgressive hybridization from lineage 2 to lineage 1c suggests that genes with adaptive functions are likely transferred between these two sympatric lineages. Our genotype-by-environment association analysis finds that a few climatic variables explain a significant part of the genetic. Lineage 2 is adapted to cooler temperatures in cold months, while lineage 1c contains genomic signatures of adaptation to lower precipitations during summer months and the larger range of temperatures observed inland. These predictions were corroborated by phenotypic data and distribution modeling predicting that lineage 1c has a higher adaptive plasticity to temperatures than lineage 2 and that the current environmental tolerance range of lineage 1c will keep exceeding that of lineage 2. Our results demonstrate that recent diversification, hybridization, and adaptive introgression enhance climate adaptation in *N. gaeumannii* lineages.

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A ceramide synthase is important for filamentous fungal biofilm morphology and antifungal drug resistance

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The complex structure of fungal biofilms generates microenvironments that impact the fitness of cells within the biofilm community. Contributions to fitness include the development of emergent properties resulting in the tolerance or resistance to external stressors such as rapid environmental changes and in the context of an infection, antifungal drug exposure. The biofilm developed by the filamentous fungal pathogen *Aspergillus fumigatus* develops regions of low oxygen that contribute to a reduction in antifungal drug susceptibility, however the genes and mechanisms involved in driving this emergent property of the biofilm are ill-defined. In this study, we first utilized a transcriptomics approach to probe the biofilm gene expression landscape in comparison to drug susceptible planktonic (batch) cultures to identify transcriptional patterns and genes unique to the *A. fumigatus* biofilm. Importantly we utilized two phenotypically diverse strains that allowed us to identify biofilm specific gene co-expression networks. One of these networks was highlighted by a gene encoding a ceramide synthase, designated *barA*, with a striking increase in *barA* transcript abundance specifically in the biofilm. Null mutants for *barA* in two strain backgrounds display altered biofilm morphology with strain specific differences. Importantly, *barA* has a role in regulating biofilm susceptibility to ergosterol targeting antifungal drugs. These data identify biofilm specific genes in *A. fumigatus* for further study and highlight the importance of fungal ceramide synthases and lipid metabolism in mediating antifungal drug susceptibility in infection relevant biofilms.

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Genomic and transcriptomic profiling of *Hanseniaspora guilliermondii*'s ethanol stress response

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Hanseniaspora guilliermondii (*Hg*) is a non-conventional yeast with recognized potential as a bioflavorant, particularly in winemaking. Nonetheless, its low ethanol tolerance, a trait in the *Hanseniaspora* genus, is a recognized "Achilles-heel" that reduces their competitiveness during fermentation and limits their aromatic contribution to the final product. Leveraging the previous disclosure of the first *Hg* genomic sequence (of the UTAD222 strain) (Seixas et al., 2019), we explored transcriptomics, chemogenomics and comparative genomics to disclose the molecular mechanisms by which this species responds to and tolerates ethanol stress. RNA sequencing performed on ethanol-stressed *Hg*UTAD222 cells (exposed to 6% v/v for 1 hour) uncovered massive alterations in the transcriptome of this yeast, resulting in 305 up- and 392 down-regulated genes, compared to transcript levels observed in non-stressed cells. A substantial portion of the differently transcribed genes under ethanol stress play a role in lipid metabolism, pointing to a substantial membrane restructuring in response to ethanol toxicity. Results from phenotyping of a mutant library obtained by chemical mutagenesis of *Hg*UTAD222 with EMS (ethyl methanesulfonate) corroborated this idea, being identified at least one mutant strain hyper-susceptible to ethanol that was found to harbor disruptive modifications in UFD2, a gene encoding a putative ubiquitin ligase predicted to regulate unsaturated fatty acid biosynthesis. This represents the effective first ethanol-resistance gene identified in the *Hg* species.
This is the first reported study so far to examine stress tolerance in *Hg*, which remains poorly characterized especially from a molecular point-of-view. The results elucidate essential aspects of this yeast's physiology and stress response mechanisms, unlocking *Hg*'s potential in the industry as a bioflavorant and as a cell factory for production of add-value molecules where low ethanol tolerance can be a bottleneck.

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Tolerance to the antifungal drug fluconazole is mediated by tuning cytoplasmic fluidity

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The efficacy in treating infections with the most widely used antifungal drug fluconazole, which is fungistatic, is impacted by the emergence of tolerance, *i.e.* the ability of strains to grow at drug concentrations above the minimal inhibitory concentration (MIC) [1]. Drug tolerance is thought to be a manifestation of phenotypic heterogeneity within a population of cells. A range of critical cellular functions take place in the cytoplasm, including protein folding, enzymatic catalysis, intracellular signaling, intracellular transport ***etc.*** In this dense, crowded and heterogenous milieu, diffusion is reduced,- in contrast to an aqueous phase where molecules diffuse freely-, which can negatively affect diffusion-limited reactions. The physical properties of the cytoplasm have been shown to change in response to external perturbation; hence we have been using a genetically encoded micro-rheological probe [2,3] to investigate how prolonged exposure to high antifungal concentration affects the cytoplasm in *Candida albicans*. Our results reveal a dramatic decrease in cytoplasmic fluidity associated with fluconazole tolerance. This striking reduction in diffusivity at the mesoscale was observed upon inhibition of the sterol biosynthesis pathway, either using antifungal drugs or specific mutants, and was reversible upon antifungal drug removal. Reducing ribosome concentration decreased drug tolerance and restored cytoplasmic fluidity, highlighting a link between tolerance and cytoplasmic crowding and/or viscosity. However, growth in fluconazole concentrations above the MIC did not increase ribosome concentrations [3,4]; rather the cytoplasmic protein concentration, viscosity and condensate formation increased. Specifically, growth in these conditions resulted in a substantial increase in processing bodies (P-bodies), whose presence correlated with azole tolerance. Together, our results reveal that changes in the physical properties of the cytoplasm occur in response to antifungal drug and suggest that these changes, as well as increased cell-cell heterogeneity, are crucial for survival in fungistatic drugs.

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Lipid homeostasis in antifungal resistance of *Cryptococcus neoformans*

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The fungal pathogen *Cryptococcus neoformans* exhibits innate resistance to the echinocandin class of antifungal drugs, through mechanisms that are poorly understood. Previous work has identified Cdc50, the regulatory subunit of lipid flippase, as a key contributor of resistance to the echinocandin drug caspofungin. The *cdc50*D mutant has impaired flippase function, resulting in increased phosphatidylserine (PS) levels at the outer leaflet of the plasma membrane. We hypothesize that phospholipid distribution in at the plasma membrane alters the interaction between caspofungin and its target, b-1,3-D-glucan synthase (Fks1). To test this hypothesis, we are using a cell-free system, in which Fks1 is expressed in liposomes with varying concentrations of PS, to examine interactions between caspofungin and Fks1. We are also examining how PS levels affect the Fks1-caspofungin interaction by altering levels of the PS synthase enzyme Cho1. As *CHO1* is an essential gene, we have generated a CTR4 promoter-based system to conditionally reduce Cho1 expression, in order to test if altering PS synthesis affects drug sensitivity. These studies provide mechanistic insight that can be used towards drug discovery, which will focus developing inhibitors of lipid flippase and PS synthase.

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Powdery Mildew Fungi Manipulate Plant Lipid Metabolism for their Growth & Reproduction

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Powdery mildew fungi are obligate biotrophic pathogens of diverse plant species. Their dominant mode of reproduction is asexual resulting in the production of spores replete with storage lipids. These storage lipids are utilized during fungal germination and early colonization before the fungal feeding structure, the haustorium, develops. We performed integrated molecular genetic, biochemical, and metabolic analyses coupled with confocal microscopy and RNAi silencing of powdery mildew genes to elucidate how powdery mildew manipulate plant lipid metabolism to promote asexual reproduction. We found that powdery mildews induce a localized shift in primary plant metabolism, bypassing the mitochondrial pyruvate dehydrogenase complex pathway, to direct plant resources to lipid accumulation. These lipids accumulate primarily as triacylgycerols (TGs) in cells underlying the haustoria-containing cells and are incorporated into spore TGs. The induced plant TGs that support spore formation are made in the chloroplast via the atypical diacylglyceroltransferase (DGAT) DGAT3 at the expense of thylakoid lipids. Furthermore, DGAT3 is localized to the plastoglobule (PG), a plastidic lipoprotein particle that acts as a hub for lipid metabolism and storage. A powdery mildew effector also localizes to the PG and interacts with the PG core protein fibrillin 2 (FBN2). This interaction may facilitate fungal acquisition of PG or PG composition. Silencing this powdery mildew effector or genes involved in lipid catabolism reduces powdery mildew asexual reproduction. Finally, field trials spraying RNAi biopesticides that target powdery mildew genes in lipid catabolism show the efficacy of this approach for powdery mildew control.

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Evolution of short amino-acid insertions in Gcn4 rewires metabolic gene regulation in the *Candida* lineage

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This study seeks to understand how expression patterns change over evolutionary time, specifically how new genes come under the control of a transcription factor (TF). Using the well-characterized regulator of amino acid starvation Gcn4 as a test case, we found that Gcn4 brought genes involved in central and fatty-acid metabolism under its control in the *Candida albicans* clade. We hypothesized that this occurred through protein-coding changes within Gcn4's unstructured region as such regions are not under strong purifying selection, tend to evolve rapidly, and can become fixed over short evolutionary timescales. To test this hypothesis, we replaced native *C. albicans* Gcn4 with Gcn4 from the distantly related yeast *Saccharomyces cerevisiae*, whose unstructured region has diverged substantially over ~200 million years of evolution. This experiment revealed that the expression of recently added Gcn4-activated genes is attenuated through protein-coding changes that arose during this evolutionary window. Using a novel computational tool that scans fungal genomes from the Y1000+ project, we identified multiple 5–10 amino-acid stretches that recently emerged in the *Candida* clade. We determined that one of these stretches is necessary and sufficient to attenuate the expression of the recently gained gene orf19.2607 (a paralog of a glycolysis-pathway gene). We further found that attenuation of this gene is driven by the DNA-binding cofactor CaPho2 (Grf10), which interacts with Gcn4 by binding a site 11 bp upstream of the Gcn4 site. Together, these results suggest that (1) new genes can come under a TF's control through protein-coding changes in its unstructured region, and (2) such changes, that appear as fixed peptide sequences over short evolutionary timescales, can encode interactions with other transcription factors. Overall, this study provides new insights into transcription-circuit rewiring and how gene-expression patterns evolve.

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Membrane fluidity control by the *Magnaporthe oryzae* acyl-CoA binding protein sets the thermal range for host rice cell colonization

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During early infection, following leaf cuticle penetration, the devastating rice blast fungus *Magnaporthe oryzae* grows biotrophically as invasive hyphae (IH) in living host plant cells. IH are separated from host cytoplasm by plant-derived membranes forming an apoplastic compartment. A punctate biotrophic interfacial complex (BIC) also forms to mediate the molecular host-pathogen interaction by functioning as a platform to deploy cytoplasmic effectors. What molecular and cellular processes determine the temperature range for this biotrophic growth stage is an unanswered question pertinent to a broader understanding of how phytopathogens may cope with environmental stresses arising under climate change. Here, we shed light on thermal adaptation in *M. oryzae* by disrupting the *ACB1* gene encoding the single acyl-CoA-binding protein, an intracellular transporter of long-chain acyl-CoA esters. Loss of *ACB1* affected fatty acid desaturation levels and abolished pathogenicity at optimal (26ºC) and low (22ºC) but not elevated (29ºC) infection temperatures (the latter following post-penetration shifts from 26ºC). Relative to wild type, the Δ*acb1* mutant strain exhibited poor vegetative growth and impaired membrane trafficking at 22ºC and 26ºC, but not at 29ºC. *In planta*, Δ*acb1* biotrophic growth was inhibited at 26ºC – which was accompanied by a multi-BIC phenotype - but not at 29ºC, where BIC formation was normal. Impaired membrane fluidity at 22ºC and 26ºC but not at elevated temperatures underpinned the ∆*acb1* phenotype, indicating Acb1 suppresses membrane rigidity at optimal- and suboptimal- but not at supraoptimal temperatures. Taken together, by deducing a temperature-dependent role for Acb1 in maintaining membrane fluidity homeostasis, we reveal how the thermal range for rice blast disease is both mechanistically determined and wider than hitherto appreciated – findings that may be applicable to understanding a wide range of current and emerging fungal plant diseases under climate change conditions.

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Elucidation of fluconazole resistance in the emerging fungal pathogen *Candida auris*

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Fungal infection diseases are estimated to cause approximately 3.8 million deaths worldwide each year. Developing antifungal agents is particularly challenging because fungal cells share many components with human cells. In addition to this difficulty, there is a growing global concern about antimicrobial resistance (AMR). To prevent the emergence of AMR and to inform the development of a new drug, elucidating the mechanisms of drug resistance is crucial.

Since fungal infections and drug resistance are social issues, we focused on a yeast pathogen, *C. auris*, which is persistent, causes severe diseases such as bloodstream infections, and contains a high proportion of isolates resistant to fungicides. We focused specifically on fluconazole resistance in this study, as it is a first-line drug. Fluconazole is an azole fungicide that inhibits *ERG11*, a key enzyme involved in ergosterol synthesis, an essential component of the fungal cell membrane. Thus, the canonical mechanism of fluconazole resistance relates to mutations in *ERG11*. In addition to this gene, overexpressed transporters play a role in drug resistance through their efflux activities.

Our institute, Medical Mycology Research Center at Chiba University, has deposited eight clinical isolates of *C. auris*. Although the Caur-R1 strain is resistant to fluconazole, no *ERG11* mutations were detected; therefore, an unknown mechanism of fluconazole resistance remains. This study aimed to uncover novel genetic and molecular mechanisms of non-canonical fluconazole resistance in *C. auris* Caur-R1.

Whole-genome sequencing was conducted on *C. auris* strains, and phylogenetic relationships were inferred from single nucleotide variants (SNVs). This analysis suggested that Caur-R1 was closest to a sensitive isolate Caur-S6, having 79 SNVs and 65 in/dels. The comparative genomic analysis between these two strains suggested that Caur-R1 harbors three SNVs and two in/dels, possibly causing loss of function due to protein truncation. We chose these mutations for the first candidates, as they can be verified by knockout of the genes. We conducted knockouts of the respective genes in Caur-S2 to investigate their functions and involvement in drug susceptibility.

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Large-scale transcriptomics reveal a kexin protease required for the formation of the multicellular host morphology of the fungal pathogen *Coccidioides*

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Coccidioides, a dimorphic fungus endemic to the Southwest United States, Central America, and South America, causes infections in people with or without immunocompromise. The most serious manifestation of infection, meningitis, still causes 30% mortality despite current treatments and survivors often have irreversible neurologic damage. In recent years, the incidence of coccidioidomycosis has continued to rise and its endemic region is spreading. The ability of *Coccidioides* to cause infection depends on an elaborate developmental transition: hyphae found in the soil produce spores (arthroconidia) that are inhaled by mammals and then transform into spherules, a multicellular host morphology unique to *Coccidioides*. Once mature, spherules are filled with hundreds of endospores. The spherules then rupture, releasing endospores to disseminate to other sites within the host, where each can go on to form another spherule in a cycle called spherulation. The molecular determinants of spherulation remain poorly understood, likely explaining our lack of effective treatment options. Candidate effectors involved in spherulation are a family of secreted proteases, subtilases, that have undergone evolutionary expansion in the *Coccidioides* lineage compared to other fungal pathogens that do not form spherules. Using a class-wide serine protease inhibitor targeting the subtilases, we have inhibited both spherulation and endospore release in vitro. Guided by the first large-scale transcriptomic atlas of *Coccidioides* development we recently created, we prioritized subtilases demonstrating dynamic expression over spherulation for deletion. One such subtilase, Sub6, whose transcript abundance peaks during early spherule formation, is required for normal spherule morphology and efficient endospore release. Mutants lacking *SUB6* are more sensitive to cell wall stressors during spherulation. Upon *Coccidioides* co-culture with bone-marrow derived macrophages, *SUB6* influences the size of spherules formed. Using an established intranasal mouse model of infection, we found that *SUB6* is required for virulence. By linking subtilase activity to multiple steps of spherulation, we have demonstrated the importance of further dissecting the role of this protease family in spherule biology and discovered a promising drug target, Sub6, whose inhibition could cure coccidioidomycosis.

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Transcriptional regulation during vegetative reproduction in the Mucoral fungus *Phycomyces blakesleeanus*

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Phycomyces blakesleeanus is a member of the Mucoromycota that grows vegetatively as a mycelium with branched, non-septate hyphae. During asexual reproduction, vegetative hyphae develop fruiting bodies, called sporangiophores, that are long aerial and unbranched hyphae with an apical sporangium filled with spores. Its development is regulated by environmental signals such as light, temperature, or the concentration of nitrogen or CO_2. *P. blakesleeanus* uses light as an environmental signal to activate the biosynthesis of the pigment beta-carotene, for the development of sporangiophores and to guide sporangiophore growth (phototropism). The main photoreceptor is the Mad complex, a light-sensor and transcription factor complex composed of the zinc-finger proteins MadA and MadB, homologs of WC-1 and WC-2 of *N. crassa,* respectively. One of the primary roles of the Mad complex is the regulation of transcription upon light reception. While the transcriptional response to light in vegetative mycelia has been characterized, little is known about how light influences gene expression during sporangiophore development.

We have observed that the transcriptional response to light in vegetative mycelia is different to that of sporangiophores, in particular regarding the number of repressed genes (189 genes repressed in mycelia and 23 genes repressed in sporangiophores, FC>2). However, the transcriptional changes during sporangiophore development are far more extensive than those induced by light (516 regulated by light in mycelia compared to 4,321 regulated during

development), and indication of the relevance of this developmental transition in the life cycle of *P. blakesleeanus*. During the development of sporangiophores, *P. blakesleeanus* activates preferentially genes for protein synthesis, while it reduces the expression of metabolic genes. We have identified 214 genes that are induced both in response to light and during sporangiophore development, which reveals an interplay of regulatory networks in this fungus. In addition, we have observed that the Mad complex participates in transcriptional regulation in the dark in mycelia and sporangiophores supporting an additional role for this photoreceptor complex.

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Cellularization in chytrid fungi uses distinct mechanisms from conventional cytokinesis and cellularization in animals and yeast

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Chytrid fungi provide a model for studying foam-like cellularization, where nuclei that are dispersed throughout the cytoplasm are synchronously compartmentalized into daughter cells. This organization poses geometric challenges not faced by cells undergoing conventional cytokinesis or *Drosophila* monolayer cellularization, where nuclei are organized in linear or planar arrangements with ready-access to the plasma membrane. We use the chytrid *Spizellomyces punctatus* to show that chytrid cellularization begins with migration of nuclei and their attached centrosomes to the plasma membrane, where centrosome-associated vesicles mark sites of membrane invagination. These vesicles then extend inwards, resulting in tubular furrows that branch and merge to create a honeycomb of polyhedral membrane compartments–a cellularization foam–each with a nucleus and cilium. Using inhibitors and laser ablation, we show that tensile forces produced by actomyosin networks drive aphrogenesis (foam-generation), while microtubules are important for foam patterning and ciliogenesis but are not essential for cellularization. Finally, we suggest that chytrids may have incorporated ancestral mechanisms associated with ciliogenesis to coordinate the association of internal nuclei with membrane furrows to solve the unique geometric challenges associated with aphrogenic cellularization.

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Cell polarity and the making of a trap for nematode predation

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The ability to reprogram cell morphogenesis in response to environmental cues is fundamental for microbial adaptation and survival. Across the fungal kingdom, multiple lineages have independently evolved carnivory under nutrient starvation, converging on the formation of specialized trapping structures to capture nematode prey. In Ascomycetes, nematode-trapping fungi such as *Arthrobotrys oligospora* develop three-dimensional adhesive networks composed of hyphal loops that ensnare nematodes. In Basidiomycetes, the oyster mushroom *Pleurotus ostreatus* captures nematodes using toxocysts—spherical structures that store the volatile toxin 3-octanone, which rapidly paralyzes and kills prey. In both systems, trap morphogenesis requires reorganization of cell polarity and remodeling of the cytoskeleton, yet the mechanisms coordinating these events remain unclear. Here, using live-cell imaging, genetics, and functional assays, we show that polarity reprogramming and the assembly of actin and septins are essential for trap and toxocyst development in carnivorous fungi. Together, these systems provide powerful models for uncovering how the evolution of novel cellular traits enables lifestyle transitions in fungi.

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A cytokinetic contractile-ring-associated short kinesin-3 motor is required for sexual development in *Podospora anserina*

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Sexual development in multicellular fungi is a complex morphogenetic process, where progression through the nuclear phases of the sexual cycle is accompanied by the formation of multiple cell types occurring within complex multicellular fruiting bodies. This process involves major changes in cell structure and subcellular organization, which are critical for the cell division and differentiation events that conduct this process. Sexual development in the model fungus *Podospora anserina* involves precise spatiotemporal regulation of the dynamics of distinct organelles, including mitochondria, peroxisomes and the endoplasmic reticulum (ER). In filamentous fungi, kinesin-3 motor proteins are implicated in the transport of peroxisomes and the ER, which depends on an early endosome-driven hitchhiking co-transport system. Here we analyzed the role of the two kinesin-3 motors of *P. anserina*. KIN2 consists of long protein possessing the conserved domain structure of kinesin-3 motors, while KIN3 is a short motor that lacks PH, FHA, and cargo-binding domains. We show that KIN2 is required for early endosome and peroxisome transport and regulates mitochondrial and ER dynamics. In contrast, KIN3 is dispensable for these processes. Likewise, we found that optimal vegetative hyphal growth depends on KIN2 but not on KIN3. We found that KIN3 dynamically localizes to septa, where it associates to their leading edge along their closure, consistent with association with the contractile ring. In addition, we found that KIN3 is strictly required for sexual development, where its elimination affects meiotic spindle positioning and function, as well as ascospore differentiation, producing sterility. Our findings disclose a novel essential role for kinesin-3 motors in meiotic development in fungi. This research was supported by grants CONACYT-DFG 277869 and PAPIIT, DGAPA, UNAM IN227823.

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Environmental drivers and cellular mechanisms of development in the fungal pathogen *Coccidioides*

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The fungal pathogen *Coccidioides* causes the human respiratory disease Valley fever when a patient inhales airborne spores from an environmental reservoir. An estimated 40% of infected individuals develop symptoms, which range from pneumonia to fatal multiorgan failure. Since antifungals against this pathogen are minimally effective, prevention remains the first line of defense. However, without understanding the mechanisms and ecological factors

that trigger *Coccidioides* to produce its infectious spores, public health models of Valley fever transmission risk remain limited in accuracy. In one wing of the project, we aim to elucidate the specific environmental conditions that promote sporulation, as well as the mechanistic basis underlying spore development. We present data showing that dry and saline conditions promote sporulation in *Coccidioides*, as well as in closely-related species; this indicates that adaptation to environmental conditions characteristic of their native desert habitat is a conserved trait within the Onygenaceae family. In another wing of the project, we aim to understand the genetic regulators of hyphal development. The transcription factor NRG1 has been implicated in morphological transitions in other fungi; here we show that nrg1 deletion in *Coccidioides* delays spores from developing into hyphae without affecting their development into spherules, the parasitic form of the fungus in mammalian hosts. This supports the hypothesis that in *Coccidioides*, nrg1 expression specifically promotes the spore-to-hyphae transition. Our work in sum sheds light on the environmental drivers of and cellular mechanisms underlying production of *Coccidioides*' infectious spores, as well as the regulators that govern their development, informing future efforts to prevent the spread of Valley fever.

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Phosphoproteomic analysis of the Target of Rapamycin (TOR) signalling pathway in the rice blast fungus *Magnaporthe oryzae*

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Eukaryotic growth and development are regulated by the Target of Rapamycin (TOR) signalling pathway, which integrates nutritional cues with anabolic and catabolic processes via the TORC1 and TORC2 complexes. In filamentous plant pathogens, such as the rice blast fungus *Magnaporthe oryzae*, the contribution of TOR signalling to infection-related development remains poorly understood. While treatment of *M. oryzae* conidia with TOR activators inhibits appressorium initiation, TOR inhibitors induce constitutive autophagy and suppresses pathogenicity. Using phosphorylation of the ribosomal protein Rps6 as a biochemical readout, TORC1 activity increased between 1 and 8 hpi during appressorium development. Acute inhibition of TOR signalling with Torin1 abolished Rps6 phosphorylation but led only to a marked delay, rather than complete arrest, of appressorium development. This disconnect between loss of TORC1 activity and continued, albeit slower, development prompted us to map the downstream phosphorylation events that might buffer or relay TOR signals.

We therefore profiled the phosphoproteome of conidia undergoing early appressorium development on a hydrophobic surface in the presence of Torin1 or DMSO. Phosphopeptides enriched through sequential TiO_2 and Fe-NTA purification were analysed by data-dependent LC-MS/MS generating a high-confidence catalogue of 2,973 phosphosites across 1,098 proteins. Among these 698 phosphosites across 439 proteins were significantly regulated by TOR inhibition. Clustering of dynamic phosphosites, coupled with gene ontology overrepresentation analysis, identified discrete modules enriched for lipid-binding factors, regulators of the actin cytoskeleton, and proteins controlling cell-cycle progression. Comparative analyses further identified phosphosites co-regulated with the Pmk1 MAPK pathway as well as sites restricted to appressorium-forming fungi, highlighting putative lineage-specific innovations in TOR signalling.

Together, these data identify candidate signalling nodes through which TOR may coordinate appressorium morphogenesis, including phosphoproteins that were also recovered in TORC1 and TORC2 immunoprecipitates, and help explain why perturbing TOR signalling slows, but does not abolish, infection-related development.

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From dormancy to disease: a life cycle proteome atlas of *Cryptococcus neoformans*

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Cryptococcus neoformans spores are the infectious propagules that initiate disease, yet the proteins that control their germination and transition to host infection are poorly defined. To place spore biology in a full life cycle context, we used data-independent acquisition mass spectrometry to quantify protein expression in *C. neoformans* across the sexual cycle, multiple in vitro stress conditions, and mammalian infection, profiling more than 90 biological samples and about 6,200 proteins, roughly 90% of the genome-inferred proteome. A spore germination time course revealed rapid proteome reprogramming as spores exit dormancy and highlighted numerous secreted proteins and quorum sensing peptides as early responders, with deletion of several candidates compromising efficient germination. We define stage specific protein programs, including spore enriched sets that mark infectious propagules, and identify 43 proteins that are enriched in vivo, 14 of which were detected only during infection. Ongoing work is testing how these factors influence virulence. Together these data deliver the most complete fungal proteome to date and will be accessible through an interactive atlas website.

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Ubiquinone promotes nutrient sensing in the fungal pathogen *Candida albicans*

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Ubiquinone (CoQ) is a key component of the mitochondrial electron transport chain. Interestingly, ubiquinone has other important functions in fungi that are independent of its role in respiration. For example, studies in our lab have shown that ubiquinone acts as an important antioxidant to protect the plasma membrane from oxidative damage. To better define the roles of ubiquinone in *C. albicans*, we analyzed *abc1*Δ and *coq3*Δ mutant strains that are unable to synthesize ubiquinone. As expected, these strains could grow fermentatively on glucose medium but were unable to grow on non-fermentable carbon sources such as glycerol. Interestingly, they were also defective in growing on a range of different sugars including sucrose, maltose, galactose and N-acetylglucosamine. Some other mitochondrial respiratory mutants were defective in growing on a subset of these sugars. However, *abc1*Δ and *coq3*Δ mutant strains were unable to grow on a broader range of sugars, indicating a special role for ubiquinone in nutrient sensing. The lack of ubiquinone did not appear to cause a defect in the nutrient sensing protein kinase Snf1, as an *abc1*Δ mutant produced Snf1, and Snf1 was phosphorylated on the key regulatory residue Thr-208 that is important for its activity. Similarly, the defect did not appear to be due to a failure to derepress Mig1, which represses genes needed for the catabolism of alternative sugars in the presence of glucose, since an *abc1*Δ *mig1*Δ mutant still failed to grow on other sugars including sucrose, maltose and N-acetylglucosamine. The a*bc1*Δ mutant also died rapidly after growth in rich medium, potentially because of a defect in

coordinating nutrient availability and metabolism. These results identify a novel role for ubiquinone in the nutrient sensing in *C. albicans* and highlight that ubiquinone has key functions beyond its role in the electron transport chain. These studies also indicate ubiquinone plays critical roles in the ability of *C. albicans* to colonize diverse environments in the host.

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Metabolic crosstalk between the opportunistic pathogen *Candida albicans* and the gastrointestinal tumor microenvironment

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C. albicans infections are highly prevalent in cancer patients, largely due to gastrointestinal (GI) dysbiosis triggered either by broad-spectrum antibiotics or chemotherapeutic agents. Current epidemiological and experimental evidence supports *C. albicans* involvement in GI cancer, including an association of these pathogens with poor prognosis. While the ability of *C. albicans* to colonize tumors is well established, it remains unclear whether these fungi act as oncomicrobes promoting cancer progression or instead exploit the tumor microenvironment to thrive and evade immune surveillance. To address this question, we are employing *in vivo* models of GI cancer to profile the metabolic responses of multiple *C. albicans* clinical isolates to the tumor environment. Using genetic approaches, we have identified specific *C. albicans* nutrient transporters and receptors that confer physiological advantage under this niche. Our findings suggest that tumor secreted metabolites support fungal proliferation, pointing to a potential metabolic crosstalk between tumour cells and *C. albicans*. By dissecting the molecular mechanisms that enable some environmental fungi to thrive in the tumor niche, our goal is to clarify whether these organisms act as passive bystanders or active participants in GI carcinogenesis.

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Copper homeostasis links plant colonization and trap formation in the nematode-trapping fungus *Arthrobotrys flagrans*

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Plant-parasitic nematodes are major agricultural pests responsible for over US$150 billion in annual crop losses. Their management depends largely on synthetic nematicides, whose environmental impact and increasing regulatory restrictions highlight the need for sustainable alternatives. Nematode-trapping fungi (NTF) offer a promising solution. These soilborne fungi grow saprophytically, but can switch to a predatory lifestyle by forming specialized traps. Species such as *Arthrobotrys flagrans* and *A. oligospora* show strong biocontrol potential against root-knot nematodes such as *Meloidogyne incognita* or *M. javanica*, and have been linked to enhanced nutrient uptake and growth in tomato plants. In our group, we are investigating the ability of *A. flagrans* as a fungal endophyte to control the plant parasitic nematode *M. javanica*. RNA-seq analyses revealed that interaction with *Arabidopsis thaliana* or *Solanum lycopersicum* induced *A. flagrans* genes associated with copper starvation. We investigated fungal growth and transcriptional response under varying copper conditions and our results interestingly revealed a strong induction of copper-deficiency genes not only during plant colonization but also during trap formation. In particular, we found a putative small cluster of three genes, containing two X325 LPMO-like proteins and the copper transporter CtrC, which is specially upregulated during both, plant colonization and trap formation. On top of that, one of the X325 proteins, named CblB, whose putative orthologue in *Cryptococcus neoformans* (Cbi1) had been found to be involved in copper uptake and virulence, was strongly induced in all conditions analyzyed. We are currently molecularly dissecting the copper-dependent mechanisms underlying root colonization and trap development, with a particular focus on this gene cluster during the interaction with *M. javanica*. Our results suggest that copper limitation is a shared feature of plant colonization and nematode predation and that copper homeostasis could be central to the NTF biology and thus, to the fight against plant parasitic nematodes.

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Fungal Foundries: Differential Nutrient Uptake Across the Fungal Kingdom

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Within the fungal kingdom, differential lifestyles, genetics, morphotypes, and environmental conditions confer different nutrient uptake strategies for survival. While much has been studied on fungal nutrient exchange for applications in agriculture, little has been done to understand the overall differential elemental uptake across the fungal kingdom. To help address this knowledge gap, we assessed elemental uptake in more than thirty fungi from across the fungal kingdom in three common growth media formulations, one defined and two undefined, representing a gradient of growth conditions. To investigate the fungal elemental uptake, laser-induced breakdown spectroscopy (LIBS) was employed to determine the elemental profiles of these fungi, for comparison across media, species, lifestyle, morphotype, and taxonomy. A “core ionome” was found to be composed of Na, P, K, Fe, H, and C based on PCA biplot analysis, however, these elements had little ability to provide clustering or classification of traits. While there is no defining characteristic to explain all fungal nutrient profiles, certain fungal lifestyles (mutualist, pathogen, saprotrophs) take up some elements in higher relative quantities. This data provides evidence for different nutrient affinities by different lifestyles that cannot be easily explained by traditional evolutionary classifications, and no one fungal trait has high predictive power of elemental patterns. Future experiments will be needed to further investigate the roles of the elemental partitioning patterns may play within their traditional ecological or evolutionary niches as well as how different growth conditions may affect their patterns. Our results show mutualist fungi having a lower elemental uptake trend when compared to pathogens and saprotrophs. More research is needed to further investigate the role that different nutrients play within fungal systems, as well as how different conditions may influence these profiles. Finally, with the crucial symbioses that fungi form with plants, understanding how differential nutrient uptake and accumulation strategies may provide some insights into the exchange of nutrients between fungi and their host plants.

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Fungi at the buffet: The regulatory landscape of nutrient sensing

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Sensing available nutrients and efficiently utilizing them is a challenge common to all organisms. Many fungal species can catabolize a variety of carbohydrates: from simple sugars to the complex carbohydrates found in plant cell walls. To ensure efficient growth in a mixture of nutrients, fungi evolved mechanisms to preferentially utilize carbon sources that require less energy to utilize over those that require more energy to utilize. The genetic mechanisms through which fungi accomplish this nutrient prioritization are varied and many have not been identified. We performed high-throughput screens in the oleaginous basidiomycete yeast *Rhodosporidium toruloides* and the ascomycete filamentous fungus *Neurospora crassa* to identify conserved and divergent genes involved in regulating nutrient choice. A transcription factor in *R. toruloides* is required not only for regulating carbon source choice but also utilization of cellobiose and tricarboxylic acid cycle intermediates. In Ascomycetes, regulation of nutrient choice is typically accomplished through broad acting transcription factors, while carbohydrate utilization is typically regulated by transcription factors specific to particular classes of carbohydrates. Our data suggest that regulation of nutrient choice and nutrient utilization may be explicitly linked in some Basidiomycete fungi.

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The High Cost of Assimilation: How Wood-Decaying Fungi Balance Transcriptional Investment Between Extracellular Deconstruction and Intracellular Metabolism

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Wood degradation by basidiomycete fungi requires coordination between extracellular enzymes and intracellular metabolism. Yet, how these systems integrate and how transcriptional resources are globally allocated during lignocellulose decomposition remain unclear. This study applies a standardized comparative transcriptomics framework to five Agaricomycete fungi—three white-rot (WRF: *Pleurotus ostreatus*, *Phanerochaete chrysosporium*, *Heterobasidion irregulare*) and two brown-rot (BRF: *Fomitopsis schrenkii*, *Rhodonia placenta*)—grown on glucose or washed poplar wood. The goal was to compare transcriptomic effort and differential expression of carbohydrate-active enzymes (CAZymes) between WRF and BRF and to contextualize these investments using central carbon metabolism (CCM), a conserved metabolic reference.

The transcriptomes showed a highly uneven distribution of expression, with a small set of genes dominating in all species, especially in BRF. Within this high-priority set, core CCM enzymes such as GAPDH and enolase matched or exceeded the expression levels of the most induced CAZymes, indicating that intracellular carbon processing demands remain high even during intense extracellular degradation. CCM profiles revealed a metabolic continuum: WRF displayed coordinated upregulation of oxidative pathways (glycolysis, PPP, TCA cycle), whereas BRF exhibited constrained oxidative metabolism and a stronger transcriptional bias toward fermentative NAD^+ regeneration via alcohol dehydrogenase.

CAZyme comparisons highlighted contrasting decomposition strategies. WRF deployed a broad and diverse enzymatic arsenal (e.g., GH7, AA9, PLs), while BRF invested in a narrower set of activities. Despite these differences, four enzyme classes—expansins, AA3_3, GH16, and GH128—were consistently prioritized across all fungi. The exceptionally high expression of central glycolytic enzymes across species suggests possible moonlighting roles beyond metabolism, potentially involving redox sensing or transcriptional coordination.

Overall, the study shows that fungal adaptation to lignocellulose involves an integrated transcriptional strategy: species-specific investment in extracellular enzymatic repertoires is coupled with a conserved, high-priority rewiring of central carbon metabolism. These findings refine our understanding of ecological niche differentiation and support the development of fungal platforms for biorefinery applications.

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Fire-adapted fungi: specialists, generalists, and charcoal chompers

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Fire dramatically alters the chemical properties of soil and reshapes fungal communities. Pyrophilous (“fire loving”) fungi thrive within days-to-months following fire, growing rapidly and exploiting various nutrient niches in their freshly flambéed habitat. Many of these fungi are easily cultured in the lab and grow well on media with charcoal as the sole nutrient-source. We used C^{13}-labeled charcoal to demonstrate that these fungi can metabolize charcoal to CO_2. To identify the metabolic pathway(s) involved, we did an RNAseq experiment with an isolated pyrophile that consistently dominates post-fire ecosystems, *Pyronema domesticum.* These data illuminated a charcoal metabolism pathway, including a suite of largely uncharacterized Cytochrome P450 monooxygenases. Beyond carbon, we used comparative genomics and BioLog metabolic screening plates to characterize the metabolic potential for a set of core pyrophilous fungi. These data provide a possible explanation for observed fierce competition within fire-following fungal communities while also illuminating species that employ generalist or specialists strategies. Taken together, we illustrate how nutrient availability and competition for nutrients shapes fire-responsive fungal communities.

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A candidate effector modulates virulence dependent on temperature and host phosphate status by regulating root colonization

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The genus *Colletotrichum* includes both devastating pathogens and beneficial endophytes. The plant-associated species *C. tofieldiae* (Ct) has diverse lifestyles, ranging from pathogenic to mutualistic, depending on the strain and/or environment. Strain Ct3 is a pathogen at 22°C, but at 26°C, it instead promotes plant growth in the model plant *Arabidopsis thaliana*. To investigate the genetic basis of this lifestyle transition, we performed *in planta* transcriptome profiling of Ct3 at 22°C and 26°C. We found that effectors were enriched in the set of down-regulated genes, and in particular, a predicted effector gene, which we here call *Necessary for Full Colonization 1* (*NFC1*), was expressed at 22°C under high phosphate conditions, but not under low phosphate conditions or at 26°C. We next performed a time-course inoculation with Ct3 and found that *NFC1* was expressed especially strongly in the early infection stage. We therefore generated a targeted knock-out of *NFC1* in the Ct3 background, and we found that the *nfc1* mutant had reduced virulence and root fungal biomass compared to wild-type Ct3, potentially indicating an important role in colonization. Given the expression profile of *NFC1*, we hypothesized that its expression, and thus contribution to virulence, might depend on the plant phosphate starvation response (PSR). Surprisingly, we found that the phenotype of *nfc1* in *phr1phl1* plants disrupted in the PSR regulators mimicked wild-type plants, indicating that the PSR regulators do not play a role in regulating *NFC1*. However, in the constitutively phosphate-starved phosphate transport facilitator mutant *phf1*, *NFC1* expression was dramatically reduced even under high environmental phosphate conditions, indicating that the plant phosphate status is an important determinant of *NFC1* expression level. Interestingly, in *pho1* mutants–which exhibit shoot phosphate deficiency despite root sufficiency–the *nfc1* mutation resulted in root colonization and a plant growth phenotype similar to that of wild-type Ct3. Considering that *NFC1* expression is attenuated under low Pi conditions, this finding suggests the existence of a shoot-to-root signal transduced independently of the PSR regulators PHR1/PHL1. Together, these data identify *NFC1* as a candidate effector gene that functions in root colonization, with expression level dependent both on temperature and on host phosphate status. Next, we intend to apply yeast two-hybrid to identify the host target of NFC1 and *in planta* RNA-sequencing of the *nfc1* mutant and a complementation line, in addition to wild-type Ct3, in wild-type and *phf1* plants to identify the host response to *NFC1*.

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Life in the Burrow: Vertebrate Hosts and the Maintenance of Coccidioides in Desert Ecosystems

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Coccidioides immitis and *C. posadasii* are dimorphic fungal pathogens responsible for coccidioidomycosis (Valley Fever) in humans and other animals. These species are endemic to arid and semi-arid regions of the Americas, where they persist in soil and are associated with animal habitats. While many vertebrates are susceptible to infection, desert rodents have long been hypothesized as key hosts influencing the ecology and persistence of these fungi. Burrowing mammals create microenvironments enriched with keratin, fecal material, and decomposing carcasses, which may provide ideal conditions for fungal growth and survival. Such habitats could serve as reservoirs that facilitate the maintenance and transmission of *Coccidioides* spp. in nature.

We propose the endozoan hypothesis as a life history strategy for *Coccidioides* spp., suggesting that these fungi exist within vertebrate hosts as commensals in the spherule (parasitic) stage. Upon host death, the fungus transitions back to a saprobic phase, forming mycelia and producing infectious arthroconidia that can disperse into the surrounding soil. This dual lifestyle may confer ecological advantages by allowing persistence during periods of environmental stress and promoting long-term survival in desert ecosystems. Evidence supporting this hypothesis includes the frequent recovery of *Coccidioides* from animal burrows in endemic regions and high infection rates observed in captured rodents, despite the absence of clinical disease. These findings indicate that rodents and other burrowing animals may act as asymptomatic carriers, contributing to fungal maintenance without significant morbidity.

Furthermore, *Coccidioides* has been documented infecting a wide range of vertebrate species, including wild and domestic animals, suggesting that it may function as a generalist rather than a strict rodent specialist. Understanding this host–pathogen relationship has important implications for disease ecology, environmental persistence, and the prediction of outbreak dynamics under changing climatic conditions. Future research should focus on experimental validation of the endozoan hypothesis, host range characterization, and the role of animal-associated microhabitats in shaping the evolutionary trajectory of these pathogenic fungi.

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Deciphering the effector code of a gall-inducing fungus

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Smut fungi form a large group among the basidiomycetes and are biotrophic specialists in infecting a diverse set of mainly grasses, among them important crops like sorghum, millet, barley and maize. The maize smut fungus *Ustilago maydis* serves as an important model for smuts fungi and induces prominent galls on all aerial parts of its host, reflecting a metabolic and developmental reprogramming of the plant. This massive manipulation of the host is achieved with the help of fungal secreted molecules, so called effectors. In a systematic approach we screened in the past decade hundreds of putative effector proteins to identify their specific place of action and their functions on the plant side. Here I will present our current molecular understanding of the fungal effectome and the biotrophic interaction between the fungus and its host plant maize.

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Dissecting Wheat Tandem Kinase–Mediated Immunity Using a Rice Blast Model

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Plants detect pathogens through cell-surface and intracellular receptors that activate defense responses. Tandem Kinase Proteins (TKPs) have emerged as key regulators of plant immunity, yet their activation mechanisms remain poorly defined. Here, we characterize the wheat TKP RWT4, a resistance gene effective against the wheat blast pathogen *Magnaporthe oryzae* pathotype Triticum (MoT).

We show that RWT4 specifically recognizes the fungal effector AvrPWT4, leading to strong defense gene induction and programmed cell death. Biochemical and *in planta* interaction assays demonstrate that RWT4 is an immune sensor and directly binds AvrPWT4. Sequence analysis and structural modeling reveal that RWT4 contains an N-terminal partial kinase duplication (KDup) that functions as an integrated effector-recognition domain. Mutations within conserved KDup residues abolish AvrPWT4 binding and immune activation, establishing the KDup as a core determinant of TKP-effector specificity.

To dissect TKP-mediated immunity in a genetically tractable system, we reconstructed the RWT4–AvrPWT4 interaction in rice. Transgenic rice expressing Rwt4 displayed specific resistance when challenged with *M. oryzae* Oryza (MoO) strains carrying AvrPWT4, mirroring RWT4-dependent resistance in wheat. This heterologous rice blast system enables precise visualization of infection structures and provides a powerful platform for mapping RWT4-mediated signaling events and immune outputs.

Together, these findings define a clear molecular model for TKP-mediated effector perception and establish a versatile pathosystem for studying cereal immune signaling. This work advances fundamental understanding of TKP biology and provides a foundation for the rational engineering of TKPs to achieve durable blast resistance in major crops.

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The Spore Factory: Evolutionary experiments in the Anthropocene

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Modern agribusiness has inadvertently become one of the most powerful engines driving the evolution, amplification, and global dispersal of pathogenic fungi. In this lecture, I will explore how contemporary crop protection and production systems have together created a vast, decentralised “spore factory”—one that shapes fungal population genetics at a planetary scale. Drawing on population genomic data from *Aspergillus fumigatus*, barcoded lineage competitions, environmental surveillance and modelling I will show how agricultural substrates contaminated with fungicides enrich mutator backgrounds, driving the emergence of environmental antifungal resistance. I will discuss how scientists, agribusiness and society can collectively redesign agri-environmental systems to interrupt these evolutionary pathways. This includes deploying AI-supported genomic surveillance, developing spore-aware waste-treatment strategies, and informing policy transitions aligned with One Health principles. Understanding—and ultimately reimagining—the spore factory is now central to reducing fungal disease burden and protecting the efficacy of frontline antifungal drugs.

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Deletion of septin AspB unlocks caspofungin fungicidal potential in the human pathogen *Aspergillus fumigatus*

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Invasive aspergillosis is a pulmonary disease that primarily affects immunocompromised individuals and is caused by the fungus *Aspergillus fumigatus*. Invasive aspergillosis has a high mortality rate, ranging from 50-90%. This is partially due to the limited available drugs with fungicidal activity and growing drug resistance. Treatment typically begins with triazoles, which disrupt the plasma membrane. A second class of drugs that targets cell wall synthesis, echinocandin, is used as a salvage therapy. However, the echinocandins are fungistatic against *A. fumigatus*., leading to a high therapeutic failure rate. Previous studies determined that the Δ*aspB* strain was more sensitive to anti-cell wall drugs, especially the echinocandin caspofungin. However, the mechanism behind this is unknown. Here, we aim to understand if targeting AspB leads to a fungicidal response to caspofungin and how AspB contributes to the fungal response to this antifungal. Cell viability staining of the deletion strains post-caspofungin exposure showed that the *ΔaspA*, *ΔaspB*, and *ΔaspC* strains have significantly lower cell viability. Of note is that Δ*aspB* showed the lowest viability, resulting in a fungicidal effect. This effect is specific to caspofungin, as micafungin remains fungistatic against the septin deletion strains. These strains also showed extensive hyphal damage after 2-hours of exposure to Caspofungin. Animals infected with the *ΔaspB* strain and treated with caspofungin showed improved survival (70%) compared to the animals infected with *akuB*^{KU80}wild-type or complemented strains (~30%). Histological analysis showed reduced fungal burden and inflammation in the *ΔaspB*infected, caspofungin-treated group. Quantitative proteomics identified proteins involved in the septin-dependent response to caspofungin, including four candidate interactors involved in cell wall stress response. Deletion of four of these candidate genes (*mapA, gel2, nsdD,* and *bgt1*) resulted in increased susceptibility to caspofungin and moderately reduced viability after drug exposure.Our data suggest that AspB specifically helps coordinate the fungal response to caspofungin, resulting in a fungistatic effect. These results also indicate the potential of a septin-targeted therapeutic to enhance the outcome of patients treated with caspofungin.

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Mitochondrial Drug-Binding Strategy Underlies Caspofungin Resistance in *Cryptococcus neoformans*

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Cryptococcus exhibits inherent resistance to echinocandins, which inhibit the synthesis of (1,3)-β-D-glucan, a key component of the polysaccharide cell wall. Although the essential FKS1 gene encodes the catalytic subunit of (1,3)-β-D-glucan synthase and caspofungin effectively inhibits this activity *in vitro* using whole-cell lysates, the drug remains ineffective against Cryptococcus, suggesting mechanisms beyond target insensitivity. Although altered regulation of cell-wall remodeling genes and drug efflux pathways has been suggested, the underlying mechanisms of caspofungin resistance remain unknown. Using boron dipyrromethene (BODIPY-) and fluorescein isothiocyanate (FITC-) labeled caspofungin, we demonstrate, for the first time, that the drug enters the cryptococcal cell wall but then primarily accumulates in the mitochondrial inner membrane rather than the plasma membrane. This unforeseen localization indicates that mitochondrial stress responses play a role in echinocandin resistance and that mitochondrial sequestration diminishes the effective drug concentrations at the intended target. We further establish that this mitochondrial accumulation is driven by caspofungin's strong affinity for cardiolipin. Notably, the interaction between caspofungin and cardiolipin also takes place in human cells, establishing a mechanistic connection to caspofungin-related cardiotoxicity. Our findings reveal a previously unrecognized mechanism of echinocandin resistance in Cryptococcus and emphasize cardiolipin as an important factor in caspofungin distribution and toxicity.

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Architecture of Fungal Cell Wall and Remodeling by Antifungals: New Insight from Solid-State NMR Spectroscopy

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Invasive fungal infections have emerged as a major threat to human health, with high mortality rate persisting even after treatment. To combat rising drug resistance, substantial efforts have been directed toward developing novel antifungal agents that specifically target the unique polysaccharides present in fungal cell walls. Despite five decades of molecular and biochemical research, our understanding of many structural aspects of the fungal cell wall remains limited due to the challenges of analyzing the structure and interactions of polysaccharides, such as chitin, glucans, and mannan, within a cellular context. Here, we discuss the recent advances achieved through cellular solid-state Nuclear Magnetic Resonance (NMR) that sheds light on the structural dynamics of cell wall polysaccharides in pathogenic fungal species. This approach allows for the use of living fungal cells while obtaining atomic-level information on polysaccharides within the cell wall. First, the integration of NMR-informed dynamic characteristics of carbohydrate polymers with data on linkage, alkali solubility, and localization from conventional chemical and imaging assays, assisted by functional genomics, have provided an updated perspective on the molecular organization of carbohydrate polymers in the human pathogen *Aspergillus fumigatus* [1]. Second, we identified the structural mechanisms utilized by fungi, including *A. fumigatus* [2], *Mucorales* species [3], as well as *Candida albicans* and *Candida auris* [4] to use polysaccharides for remodeling their cell walls in response to treatment with glucan and chitin inhibitors such as echinocandins and nikkomycin. Third, we have identified five distinct types of α-1,3-glucan structures, each playing diverse roles in *Cryptococcus neoformans* [5]. These structures contribute to both the rigid and mobile domains of the cell wall, facilitate capsule attachment to the cell surface, and support melanin deposition within the inner wall domain. Together, these studies provide an in-depth understanding of fungal cell-wall architecture and the functions of its diverse polysaccharides, as well as the adaptive strategies that fungi employ to cope with stress and endure harsh environmental conditions and antifungal treatments.

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A Coordinated Breakdown of Cell Wall Homeostasis Underlies the Antifungal Action of Chitosan

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The fungal cell wall is a complex and dynamic structure essential for cellular integrity, morphogenesis and pathogenicity. Here we present advances of how the natural polymer chitosan affects cell wall integrity and the infection capability of the rice-blast fungus *Magnaporthe oryzae*. Earlier studies demonstrated that chitosan impairs appressorium-mediated plant infection by inhibiting septin-dependent F-actin reorganization at the appressorium pore, promoting plasma membrane permeabilization and disrupting reactive oxygen species (ROS) homeostasis. These effects involve key components of the cell wall integrity (CWI) pathway, including the protein kinase C pathway (*pkc1*), the MAP kinase *mps1* and also NADPH genes.

Building on this foundation, our new transcriptomic data reveal a rapid and global reprogramming of genes related to cell wall biosynthesis and remodeling following chitosan exposure. We observe reduced expression of genes involved in chitin and glucan synthesis, septin organization, actin cytoskeleton regulation and cell wall construction, alongside the induction of stress-response genes and pathways associated with cell wall repair and the CWI salvage response. This transcriptional pattern suggests an orchestrated attempt to counteract cell wall damage that remains insufficient under chitosan stress, leading to defective appressorium maturation and failure to initiate host penetration.

Our combined molecular, genetic and cellular analyses support a model in which chitosan acts as a multi-target disruptor of fungal cell wall integrity through coordinated effects on membrane permeability, cytoskeletal dynamics, ROS signaling and transcriptional regulation. These findings highlight the fungal cell wall as a genetically vulnerable structure and underscore the potential of chitosan-based strategies for sustainable fungal disease control. Understanding pathogen responses to natural antifungal compounds will inform the rational design of new cell wall–targeting interventions.

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Pmk1-dependent regulation of a Mst12–Bip1 transcriptional network governing pathogenesis by the rice blast fungus, *Magnaporthe oryzae*

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Rice blast disease, caused by the filamentous fungus *Magnaporthe oryzae*, poses a major threat to global rice production. To invade its host, *M. oryzae* undergoes tightly regulated morphogenetic transitions that enable penetration and secretes effector proteins to suppress host immunity and promote colonisation. Effective infection, therefore, depends on mechanisms that translate external cues into transcriptional programmes controlling both morphogenesis and effector deployment. The pathogenicity of the blast fungus has been linked to several interconnected signalling pathways, including the cyclic AMP–dependent protein kinase A pathway, the Pathogenicity Mitogen-Activated Protein Kinase (Pmk1) cascade, the cell wall integrity pathway, and epigenetic regulators, but the transcriptional networks that act downstream of these pathways remain poorly defined.

Our results show that Pmk1-dependent phosphorylation of the transcription factor Bip1 (B-ZIP Involved in Pathogenesis-1) is required for rice blast infection, placing Bip1 as a key effector of this conserved MAPK pathway during pathogenesis. We also identify a *BIP1* promoter element required for infection and potentially regulated by the transcription factor Mst12. Global transcriptomic analyses reveal that Bip1 and Mst12 co-regulate a common subset of genes, including multiple effector genes, and other Pmk1-dependent transcription factors during plant infection. Together, these findings support a model in which Mst12 functions as a central regulator of pathogenesis-associated gene expression, operating within a hierarchy of transcription factors that couple upstream signalling to the transcriptional reprogramming required for successful host colonisation by *M. oryzae*.

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Deciphering the Role of Carbohydrate-Active Enzymes in the Pathogenicity and cell degradation of *Fusarium oxysporum* Infecting Banana Plant

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Banana wilt caused by *Fusarium oxysporum* is a major threat to global banana production, yet the enzymatic tools that this pathogen uses to colonize and degrade host tissues remain poorly resolved. Carbohydrate-active enzymes (CAZymes) are central to both host specificity and plant cell wall degradation, and comparative CAZyme profiling can provide insight into the evolution and virulence strategies of banana-infecting lineages. In this study, we analyze the CAZyme repertoires of 73 *F. oxysporum* strains to characterize their overall CAZyme composition and identify CAZy genes that may contribute to host specificity and banana cell wall degradation. Predicted proteomes were annotated using the dbCAN pipeline and InterProScan, and CAZymes were classified into the following families: GH, GT, PL, CE, AA, and CBM. We then compared CAZyme copy numbers across strains and focused on families known to target cellulose, hemicellulose, and pectin in banana cell walls. Preliminary analyses reveal that banana-infecting strains exhibit highly similar total CAZyme repertoires and PCWDE family abundances, whereas strains isolated from non-banana hosts display markedly greater variation. Ongoing work will link these candidate CAZy genes to genomic context and pathogenicity-related regions. Overall, our comparative CAZyme framework provides testable hypotheses about how *F. oxysporum* evolves host specificity and damages banana cell walls, with implications for resistance breeding and disease management.

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Elucidation of basidiomycete-specific chitin synthase roles in the white-rot fungus *Pleurotus ostreatus*

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Chitin is a key structural component of fungal cell walls, produced by chitin synthases (CHSs). CHSs have a broad range of reported roles in ascomycetes; however, remain poorly understood in basidiomycetes, despite large differences in their cell wall structures. White-rot species like *Pleurotus ostreatus* also have sustainable biomaterial applications as they can grow on agricultural waste. We previously identified nine *P. ostreatus chs* genes including two myosin motor domain (MMD) *chs*s in conserved clades V and VII, one in conserved clade IV, two *chs*s in the filamentous clade III, and four novel *chsb* genes in clades BI-III. This research aims to investigate the roles of basidiomycete-specific CHS (CHSB) functions.

Through homologous recombination-based gene disruption, we isolated disruption strains for all *chsb* genes in *P. ostreatus* (*chsb1-4*) and simultaneously disrupted *chsb*s with a shared clade. Morphology and cell wall analysis revealed that clade BI (*chsb1*) disruption caused severe mycelial growth defects, reduced cell wall thickness and significantly altered chitin/β-glucan content; clade BII (*chsb2* and *chsb3*) single disruption had mild effects, whilst double disruption caused severe growth defects, cell wall remodelling, almost no aerial hyphae production and abnormal septation; and clade BIII (*chsb4*) disruption had minimal effects aside from slightly thinner cell walls and slight aerial hyphae defects, suggesting a more specific role in aerial hyphae development. These results highlight the importance of *chsb*s in growth and suggest clade-specific functional differentiation between *chsb*s. Preliminary assessments of mycelium mats derived from *chsb* mutants revealed differences in flexibility and texture, suggesting that *chsb*s have the potential to alter the physical properties of mushroom materials. Additional work to understand the impacts of *chsb* disruption on fruiting body formation as well as *chsb* overexpression are ongoing.

Preliminary analysis of the remaining *chs* genes (*chs5-9*) demonstrates reduced growth and cell wall integrity, though less severely than *chsb1* and *chsb2chsb3* disruption. Disruption strains of *chs5*, the second MMD *chs* have not yet been obtained despite numerous attempts, suggesting some essential function. These findings highlight the importance of understanding basidiomycete *chs*s, clear functional differentiation among *chsb* clades, and the potential for altering mycelium biomaterials through *chs* gene targeting

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Invading in full force: substantial pressure from Botrytis cinerea infection cushions breaches plant surfaces

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Botrytis cinerea is a broad host-range plant pathogenic fungus that can develop two distinct structures for penetrating the plant surface. The unicellular appressorium (UA) facilitates the penetration of a single host cell, while the highly branched infection cushion (IC) penetrates at multiple sites leading to enzymatic destruction of the host surface. We hypothesized that the substantial enzymatic degradation of the host surface would make turgor pressure less important for IC-mediated penetration compared to UA-mediated penetration, where high pressures were previously reported. To investigate the contribution of directed turgor pressure in IC-mediated host penetration, we used a poly-dimethylsiloxane surface, with similar properties to the plant cuticle. By measuring the depth and contact perimeter of indentations made on the surface by *B. cinerea* ICs, we investigated the spatiotemporal dynamics of host penetration. We observed a sequential asynchronous penetration of several hyphal branches of the IC, that also increase in size as they penetrate more deeply, which might increase the contact surface with the plant host allowing for greater secretion of effectors and cell wall degrading enzymes.

Using a Hertzian contact model, we showed that substantial pressure of 6.4 bar is exerted on the surface, greater than the 5 bar of pressure exerted by the UA. This suggests that the more complex ICs may be more effective at penetrating host surfaces that could not be penetrated by UA.

To investigate this further, we used CRISPR-Cas9 mediated mutagenesis to analyze the relationship between cell wall structure of *B. cinerea* and pressure generation. In contrast to melanin-deficient mutants in other plant pathogenic fungi, the melanin-deficient pks12/pks13 mutant is still able to develop ICs, but applies significantly less force on the surface. Additionally, a reduced penetration force was observed in cda1 and cda3 mutants, characterized by reduced chitin deacetylation in the infection cushion. These results highlight how the modulation of the cell wall structure is important for plant pathogenic fungi to enable the proper direction of internal turgor pressure onto the penetration site, and how the more complex infection cushion not only allows for greater enzymatic degradation of the host surface, but also greater penetrative force.

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The role of mutation rate and drug stress in the evolution of antifungal resistance within *Aspergillus fumigatus*

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Serious fungal infections pose a unique challenge to human health, causing severe and life-threatening disease. Antifungal medications are essential for the treatment of fungal disease, but resistance is rapidly emerging across the globe. This is particularly concerning for Aspergillus fumigatus which is responsible for more deaths than any other fungal pathogen. Understanding the factors driving the remarkable adaptability of *A. fumigatus* is essential for developing effective strategies to mitigate the risk of resistance emergence. We have shown that genetic variants within the DNA mismatch repair system of multi-drug-resistant *A. fumigatus* strains increase mutation rates by 4 to 9-fold, meaning that it is highly likely that spontaneous resistant mutants will arise as *A. fumigatus* produces vast numbers of mononucleated spores. However, how resistance evolves during the growth of multinucleated hyphae exposed to stress is unknown. Through tracking the spatial and temporal evolution of resistance across drug stress environments ranging from sub-inhibitory to lethal concentrations of antifungals, we show how phenotypic and genetic diversification leads to competition between lineages and spatial exclusion, ultimately leading to the evolution of high fitness, highly resistant lineages. In contrast to high level lethal exposure to antifungals and resistance evolution during sporulation, extended exposure to sub-inhibitory concentrations lead to multiple independent evolutionary trajectories to antifungal resistance within hyphae.

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A genomic view of hypermutation potential in *Candida auris* drug resistance

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Background: *Candida auris* is a globally emerging pathogen causing invasive, life-threatening infections, with up to 70% mortality. One major concern is its association to outbreaks with unusually high levels of multidrug resistance. A potential driver of drug resistance evolution is hypermutation, which is the accumulation of mutations at an elevated rate due to errors in DNA repair mechanisms, such as the mismatch repair (MMR) system. This elevated rate leads to the accumulation of beneficial mutations including conferring drug resistance and virulence. A global characterization of *C. auris* hypermutation is therefore essential to understanding the rapid emergence of multidrug resistance.

Methods: Phylogenies were resolved for over 900 clinical isolates with published drug resistance data, spanning all six clades of *C. auris*. Candidate hypermutants were identified by monitoring for signatures of elevated mutation rates, including elongated terminal branches, larger tip-to-root distances, and overrepresentation of private mutations along each branch. Variant analysis with functional impact annotation was performed to reveal DNA repair gene mutations which may induce hypermutation. Finally, drug resistance profiles were mapped to candidate hypermutators to assess the contribution of hypermutation to resistance.

Results: Candidate hypermutators were identified across all six clades. The largest cluster of hypermutators was in clade I, with all isolates harbouring frameshift mutations in the MMR gene *rad53*. Notably, these *rad53* mutants were associated with increased multidrug resistance. Conversely, candidate

clade VI hypermutators exhibited *mlh3* and *msh3* frameshift mutations, but lacked associations with multidrug resistance. All other candidates had moderate to high-impact missense mutations in MMR genes but showed no association with elevated resistance.

Conclusion: This study offers insight into the prevalence of genes that can potentiate hypermutation and their associations with drug resistance across all six clades of *C. auris*. This work will be expanded to a global dataset of over 20,000 isolates, enabling a more comprehensive analysis of the prevalence of hypermutation, its genetic causes, and its impact on drug resistance. These findings will then be validated in vitro using CRISPR-Cas9 gene editing and experimental evolution. Overall, this study lays the groundwork for understanding the relationship between hypermutation and drug resistance in *C. auris*.

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A new reference graph-pangenome for *Aspergillus fumigatus* recovers gene expression of accessory genes absent from the reference strain

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Fungi harbour an incredible amount of genomics and phenotypic diversity, even within the same species. An example of this is the ubiquitous saprotrophic fungus *Aspergillus fumigatus*, which is responsible for the pulmonary infection aspergillosis, causing about 1.5 million deaths per year. Approximately 31% of the whole gene content of this species is considered accessory, exhibiting a presence-absence variation among isolates. This diversity also extends to phenotypes, with isolates displaying marked variation in their ability to withstand stresses such as iron starvation, hypoxia, and oxidative stress, and potentially making them more prone to infecting humans and/or more resistant to antifungals. Currently, the function of these accessory genes and their potential role in these phenotypic differences are not well understood. Part of this is due to the extensive use of a linear reference during bioinformatics analysis: a single strain of the species -here, Af293- is used as a guide for the mapping of genomics and transcriptomics reads, preventing the analysis of genes absent from this reference.

We propose here a new reference for *A. fumigatus*, called a reference graph-pangenome. Built using 26 genetically diverse long-read assemblies from a global distribution and environmental and clinical origin, the new reference graph-pangenome is almost the double of the current linear reference (55.6 Mb). It contains a total of 14,016 protein-coding genes, including 4,176 absent from the existing Af293 reference. To better characterize these accessory genes, we performed an RNA sequencing of seven different isolates across six different stresses. Using the graph-pangenome as a reference, we were able to recover up to 10% more reads aligned, leading to 984 additional genes expressed absent from the strain Af293. Furthermore, we identified up to 8% more differentially expressed genes in response to these stresses. In future work, we will perform a co-expression network analysis to assign putative functions to these non-functionally characterized genes. Altogether, this new tool provides not only a novel reference for the *A. fumigatus* community, but also establishes a reference pangenome framework for other fungal species, while offering novel insights into the function of accessory genes in virulence or antifungal resistance.

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Candida albicans genetic parallelism in recurrent vulvovaginal candidiasis

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Vulvovaginal candidiasis ("VVC", colloquially, "vaginal yeast infection") is a very common fungal disease that will affect ~70% of people with vaginas at some point in their life. The majority of infections are successfully treated and cleared, yet approximately 8-9% of individuals will experience recurrent symptoms 3 or more times a year ("RVVC"). Troublingly, 50% of RVVC cases are idiopathic. *Candida albicans* is the dominant causative species. Very few *in vitro* phenotypes have been identified that differentiate the yeast implicated in RVVC from pathogenic or commensal yeast isolated from other body sites. It thus remains unknown whether there is scope for the yeast populations to adapt to the vaginal environment (i.e., to become better vaginal pathogens). As an alternative to searching for differentiating phenotypes, we sought to examine whether there are genetic variants that are uniquely common among RVVC vaginal yeast isolates compared to: i) closely related non-vaginal isolates from within the same RVVC individual (non-vaginal RVVC, "nV-RVVC"); and ii) closely related non-vaginal isolates from a global isolate dataset ("global"). We obtained vaginal, rectal, and oral swabs from 30 women with a history of RVVC. We collected 150 vaginal and 73 non-vaginal isolates from 13 participants: isolates from all three sites from one participant, vaginal and rectal colonies from five, vaginal and oral colonies from two, and vaginal isolates from five. All isolates were whole-genome short-read sequenced and integrated into a global phylogeny containing ~1,100 strains. All RVVC isolates from the same participant clustered very closely together. There were ~400 vaginal-exclusive variants in each of the 13 global comparisons, and ~250 in the six nV-RVVC comparisons. Considerable genetic parallelism was identified in both datasets. In the global comparison, 61 genes contained variants in at least 75% of vaginal isolates sequenced from four or more populations. In the within-RVVC comparison, 96 genes contained vaginal-exclusive variants in two or more populations, with 38 genes shared across three or more populations. Eight genes were identified from both comparisons. Four were related to the FGR6 gene family, which has previously been identified as a key element in morphological switching, while the other four have previously had minimal characterization. This work provides a list of putative gene candidates that potentially provide an adaptive benefit in the vaginal environment, which warrants further study.

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Global analysis of genotypic and phenotypic variation in an NIH collection of 160 *Candida albicans* patient-derived isolates with paired clinical metadata

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Candida albicans is a common member of the human mucosal microbiota and an opportunistic pathogen, capable of causing mucosal and systemic disease. To improve our understanding, diagnosis, and treatment of *C. albicans* disease, a key question remains: how the host and the fungus directly contribute to this opportunistic pathogenesis. At the NIH Clinical Center, a library of 160 *C. albicans* isolates has been curated in a collection that includes detailed clinical metadata. These strains were isolated from 34 patients across 16 anatomical sites, including multiple isolates collected temporally within the same patient, from both commensal and disease states. A subset of these isolates (n=69) underwent whole-genome sequencing, and all isolates were analyzed for phenotypic heterogeneity. Strains were evaluated for their growth, morphogenesis, antifungal susceptibility, expression of key virulence-related genes, recognition and killing by human neutrophils and macrophages, and induction of pro-inflammatory mediators by human epithelial cells. Interestingly, a wide range of phenotypes were observed, with no clear clustering based on anatomical site of isolation or disease state. Even within a given host, strains were not necessarily concordant for phenotypes or clonality. For example, one patient with APECED syndrome, an inborn error of immunity caused by AIRE deficiency that manifests with mucosal candidiasis and multiorgan autoimmunity, had two *C. albicans* isolates collected one day apart from the esophagus and the mouth. Despite the oral isolate being constitutively hyphal with reduced neutrophil killing and broad-spectrum antifungal resistance, the esophageal isolate had no altered morphogenesis or decreased susceptibility to antifungals. Ongoing work is continuing to analyze phenotypic and genotypic data to identify any potential genetic basis for these results. Collectively, this work aims to define the multifaceted elements that contribute to phenotypic variation and pathogenesis in the leading human fungal pathogen, *C. albicans.*

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Evolution of invasive *Phytophthora ramorum* populations in Oregon's coastal forests

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The Sudden Oak Death (SOD) epidemic within the coastal forests of Oregon and California, USA, is an oomycete invasion characterized by multiple introductions, clonality, and aberrant mutational patterns. It has altered coastal forest ecology through widespread host tree mortality. Continued pathogen spread has necessitated costly management programs and the quarantine of productive timber resources. Many micro-organism invasions are likely inconspicuous, but plant pathogens causing this level of damage are highly visible. These present opportunities to understand the evolutionary and ecological dynamics of fungal and oomycete invasions. Phytophthora ramorum, the causal pathogen of SOD, has escaped from the live plant trade into Oregon's wild forests as 3 genetically-distinct lineages: NA1, NA2, and EU1, which persist as 3 clonal populations. NA2 and EU1 populations are each dominated by a single clone. But many NA1 isolates diverge from its dominant clone in a pattern of cryptic diversity. This diversity is largely explained by NA1's high frequency of genomic runs of homozygosity (ROH), which accumulate in unique patterns in individual isolates. Recently-accumulated ROH were inferred using non-conserved SNPs called from whole genome Illumina sequences of NA1, NA2, and EU1 isolates. Genome analysis of forest and nursery populations from multiple US states and Canada revealed that 29% of NA1 genomes had at least 5 recent ROH tracts, compared to 7% of NA2 and 9% of EU1 genomes. A post-hoc Tukey test confirmed higher proportions of NA1 genomes were affected by recent ROH ($p<0.001$) when compared to NA2 and EU1. NA1 and EU1 were compared using RNA-Seq to identify expression differences in vitro and in planta related to ROH. Differential expression analysis of mRNA identified XRCC4, a DNA double-strand break repair protein, upregulated in all NA1 isolates. Potential triggers of ROH genesis were investigated by exposing isolates to biological stress. Isolates were sequenced and phenotyped before and after exposure to identify new mutations and their effects. ROH frequency is correlated with phenotype changes and ROH can persist in populations, yet the evolutionary consequences are not yet understood. These unique pathogen populations are used as a model to understand how somatic mutations accumulate in invasive micro-organism populations and, therefore, how these populations adapt to invaded environments despite low initial genetic diversity.

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Stage-specific transposase activation as mechanism of genetic variation in the clonally propagating pathogen *Fusarium oxysporum*

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Transposable elements (TEs) are key drivers of genome evolution and adaptation in eukaryotes. The clonally propagating fungus *Fusarium oxysporum* (*Fo*) is a major plant pathogen and an opportunistic pathogen in humans, offering a powerful model to investigate adaptive processes in the absence of sexual recombination. Previous studies revealed that rapid adaptation of the tomato pathogenic isolate *Fol*4287 during short-term experimental evolution is largely driven by DNA TEs. More than 80% of the TE insertions detected in experimentally evolved lines were caused by *Hormin*, a non-autonomous miniature element derived from the *hAT* superfamily TE *Hornet*. Here we investigated the expression dynamics of the *Hornet* transposase, likely responsible for *Hormin* mobilization, during different stages of fungal development. RT-qPCR and RNA-seq performed during synchronized growth in liquid medium detected a marked upregulation of *Hornet* transposase transcript levels during early stages of sporulation, with a peak in freshly produced microconidia. Consistent with stage-specific TE activation, experimental evolution on complete medium plates of a *Fol*4287 clonal line passaged as microconidia resulted in significantly higher TE mobility and phenotypic diversity compared to parallel lines passaged as mycelium. These findings suggest that TE activation during early stages of conidiation promotes genetic variation and environmental adaptation in this clonally evolving fungal pathogen.

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Tracking plant pathogen emergence using thousand-genome panels

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Adaptation of plant pathogens proceeds at speeds that easily overwhelm the rate of resistant cultivar deployment and fungicide development. Low cultivar diversity and the application of single fungicides can exacerbate these dynamics. Understanding the molecular basis of pathogen adaptation is critical to define more sustainable containment strategies. Emerging traits such as virulence on resistant cultivars or fungicide resistance often appear in a geographically structured manner. Such geographic mosaics can be determined by regional variation in selection pressures or as a consequence of the pathogen's population history. Here, I will show how very large panels of pathogen genomes from the same species can recapitulate historic colonization patterns and reveal constraints on pathogen genetic diversity. In combination with phenotyping assays, large genome panels enable the tracking of fungicide resistance mutations across continents and reveal how effector gene loci are undergoing adaptive sequence rearrangements. Recent research has pointed to selfish elements (i.e. transposable elements) as key factors in pathogen evolution, facilitating adaptation to biotic and abiotic factors in their environment. Selfish elements, by nature, can also impact the integrity of genomes and lead to deleterious dynamics for the pathogen. In conclusion, large-scale genomic investigations of individual pathogen species unravel essential mechanisms of pathogen adaptation.

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Illuminating *in vivo* macrophage-*A. fumigatus* interactions across multiple days of infection

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When a healthy host is infected with *A. fumigatus*, innate immune cells are largely sufficient to thwart disease. A major cell type that prevents infection is tissue-resident macrophages, however, the interactions of these cells with *A. fumigatus* inside of an infected host have remained elusive. To investigate macrophage-*A. fumigatus* interactions *in vivo*, we use a larval zebrafish host model which allows for multi-day live imaging of fungal-host interactions. Zebrafish have a largely conserved genome with humans, and infection of larvae recapitulates pathogenesis in humans. CRISPR/Cas9 approaches in larval zebrafish also allow for high-throughput genetic manipulation. Using this model, we demonstrated that macrophages readily phagocytose injected *A. fumigatus* spores but do not efficiently kill spores. Macrophages inhibit spore germination and non-germinated spores can persist inside of macrophages for over 7 days. We are currently investigating phagosomal trafficking and phagolysosomal killing mechanisms that macrophages activate against spores. The goals of this research are to understand why spores are allowed to persist inside of macrophages and to identify pathways through which spore killing could be increased. Additionally, while neutrophils were thought to be the primary cell type that controls *A. fumigatus* post-germination, we identified a role for macrophages in controlling hyphal growth in larval zebrafish and we are also currently investigating extracellular killing mechanisms of macrophages against hyphae. Altogether, the larval zebrafish model allows us to genetically probe and directly image the function of macrophages against *A. fumigatus* inside of a living host, illuminating the importance of this cell type in the innate immune response to *A. fumigatus* infection.

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Machine learning identifies novel hallmarks of symbiotic evolution in *Burkholderia*-related bacterial endosymbionts of Mucoromycota fungi

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Interactions between Mucoromycota fungi and their bacterial endosymbionts offer a powerful model to study symbiotic evolution. Mucoromycota fungi often host *Burkholderia*-related endosymbionts (BREs) in the genera *Mycetohabitans*, *Mycoavidus*, and *Candidatus* Glomeribacter gigasporarum. In these systems, fungal-derived metabolites and the intrahyphal environment impose strong selective pressures that drive convergent bacterial genome reduction. To study the functional basis of fungal-endosymbiont interactions, our group has recently developed draft genomes from approximately 500 isolates of Mucoromycota fungi and their BRE. We leveraged phylogenomic and comparative genomic analyses of these new genomes and existing data to elucidate the evolutionary history, transmission modes, partner fidelity, and extent of metabolic interdependence in these systems. Within the Mortierellomycotina, our genomic analyses support several distinct *Mycoavidus* lineages each with unique gene loss patterns, transmission modes, and host associations. We also noted lineage specific patterns in reduction of endosymbiont amino acid, carbohydrate, nucleotide, and stress-response pathways across all BREs. Similar genomic contraction patterns in pathways relevant to host biology are also observed in bacterial endosymbionts of other eukaryotes, and are considered hallmarks of symbiotic co-evolution. We reasoned that we could identify novel metabolic determinants of fungal-bacterial interactions using machine-learning (ML) based classification of functional pathways in free-living, facultative, and obligate BRE. We applied an ensemble ML classification of KEGG pathway profiles in all available BRE genomes, including our new data and publicly available sequences. The models identified reduced bacterial central metabolic and stress-resilience modules as the strong predictors of obligate symbiosis, reinforcing the conclusion that fungal-driven metabolic provisioning consistently guide genome streamlining across BRE lineages. These results demonstrate that the intracellular fungal niche strongly shapes endosymbiont genomic evolution and that ML-based classification provides insight into the underlying mechanisms of bacterial adaptation to the fungal intracellular niche.

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Signal Reliability and Nutritional Rewards Drive Divergent Evolutionary Trajectories in Nematode-Trapping Fungus *Arthrobotrys oligospora*

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Nematode-trapping fungi (NTFs) and nematodes cohabit diverse soil environments, engaging in a predator-prey relationship driven by the fungal requirement for nitrogen. The NTF *Arthrobotrys oligospora* functions as a facultative predator: under nutrient-replete conditions, it grows as a saprophyte;

however, upon sensing low nitrogen and nematode signals (e.g., ascarosides), it initiates trap morphogenesis to develop specialized adhesive networks. This inducible strategy is considered an adaptation to conserve metabolic resources, ensuring energy-expensive traps form only when prey is present. Yet, the evolutionary forces and ecological trade-offs — specifically the balance between metabolic conservation and the sensory threshold required to initiate costly trap formation — remain experimentally underexplored. To investigate how nutritional reward and the predictive value of prey cues influence the evolutionary trajectory of these fungi, we employed a long-term experimental evolution approach. Here, we report findings from a 33-week snapshot where 12 replicate populations were evolved under three conditions: (1) Starvation Control; (2) Predation-Reward (continuous access to live prey); and (3) Interrupted-Induction (transient exposure to prey inducing traps sufficient to induce trap formation, followed by prey removal prior to capture). We observed high phenotypic divergence across the 36 evolved lineages. While the ancestor and Starvation Control lines largely retained inducible trap morphogenesis, spontaneous trap formation emerged as a distinct strategy in multiple lineages within both Predation-Reward and Interrupted-Induction groups. The emergence of this constitutive predatory lifestyle suggests that when prey is frequent, the need for rapid capture can override metabolic savings repressing trap production, driving a shift from a "reactive" to an "active" hunting strategy. The signal-exposed groups diverged in sensing strategies: unlike most Predation-Reward lines, the majority of Interrupted-Induction lines exhibited a secondary loss of pheromone sensitivity; maintaining expensive traps in response to transient, misleading signals would be maladaptive. In contrast, we identified a striking life-history trade-off between conidiation and predation in the Predation-Reward group. Analysis of conidiophore architecture indicated that investment in predatory traits limits reproductive potential. This trade-off was most pronounced in a divergent Predation-Reward lineage, which evolved a hyper-predatory phenotype characterized by robust spontaneous traps and heightened sensitivity, but severely reduced sporulation (forming single rather than multiple conidial clusters). Our results demonstrate that *A. oligospora* responsiveness to prey signals and its decision to produce traps rapidly evolve in response to selective pressures.

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Mycangial ultrastructure, temporal transcriptomics of fungal colonization, and engineering of the fungal symbiont community in *Xyleborus affinis* ambrosia beetles

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Ambrosia symbioses are reciprocally obligate mutualisms between wood-boring beetles and co-evolved groups of fungal symbionts. Beetles farm these fungi as their sole food source and the fungi rely on their hosts for maintenance and dispersal. These fungi are so important for the survival of their hosts that the beetles have evolved specialized organs, termed mycangia, to house their fungal partners. Mycangia serve as important bottlenecks of the symbiosis, regulating the composition and fidelity of symbiont communities. The ambrosia beetle *Xyleborus affinis* is native to the United States where it harbors fungal species mainly from the genus *Raffaelea*, forming a community with secondary filamentous and yeast symbionts. Recently, this beetle has been demonstrated to farm and vector the invasive fungus, *Harringtonia lauricola*, which is the causal agent of laurel wilt disease, which has been devastating Lauraceae forest ecosystems and the avocado industry. Little is known regarding how ambrosia beetles selectively maintain fungal partners within mycangia, including the role of mycangial ultrastructure in colonization, with limited knowledge concerning the genetic/molecular interactions between the fungus and host within these organs. We provide a systematic characterization of the *X. affinis* mycangia including novel ultrastructural elements, and provide a temporal gene expression profile of *H. lauricola* adaptation to the mycangial environment during colonization of the organ. Genetic tools for examining the broader *X. affinis* fungal symbiont community have also been developed. Characterization of *X. affinis* mycangia revealed features that play important roles in fungal colonization and maintenance. Temporal transcriptomic profiling identified unique sets of transcription factors, metabolic pathways, and effectors affecting hyphal vs yeast-like growth amongst other processes, differentially regulated during mycangial colonization. Transformation of native fungal community members with reporter genes driven by *H. lauricola* promoter sequences demonstrated the utility of genetic parts from this fungus in enabling molecular genetic studies in a broad range of ambrosia fungi, opening possibilities for such work in currently intractable ambrosia symbiotic systems.

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Environmental impacts on gene expression in the amphibian gut fungus *Basidiobolus*

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Basidiobolus is a genus of fungi with a complex life cycle of contrasting environmental niches and unique hyphal and spore morphotypes. It spends part of its life cycle as a free-living filamentous saprobe on plant detritus and exists in the anaerobic digestive systems of herptiles in a single-cell growth stage. *Basidiobolus* genomes exhibit a strong signal for biologically active specialized metabolites, which are predicted to provide an adaptive advantage. To elucidate functional variation and gain insight into the ecological relevance of this genus, we subjected three phylogenetically distinct strains of *Basidiobolus* to different pH levels and both aerobic and anaerobic conditions—abiotic factors that reflect the variation encountered in free-living environments and the herptile gut. In-vitro manipulation of oxygen and pH induced morphologies associated with these life stages, and fungal biomass was further assessed via RNA-seq to evaluate gene expression. Preliminary data indicate that aerobic conditions resulted in greater variation of specialized metabolism within and between species compared to anaerobic conditions, suggesting anaerobic conditions may promote a more conserved metabolism within the gut environment. Additionally, the anaerobic low-pH condition showed an overrepresentation of upregulated chitin CAZymes and serine- and metalloproteases, supporting the hypothesis that *Basidiobolus* is metabolically active in the herptile gut microbiome. We will present data and analyses exploring differential gene expression in response to environmental variables relevant to the life history of *Basidiobolus*, providing insights into the functional diversity of this complex fungal genus.

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Understanding *Basidiobolus*-bacterial interactions using multi-replicated enrichment communities

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The life cycle of the fungus *Basidiobolus* includes a stage where the organism lives as a gastrointestinal symbiont of herpetofauna. In this environment, *Basidiobolus* is abundant, prevalent, and co-structured with the bacterial community. The *Basidiobolus* genome is enriched in genes annotated to encode for non-ribosomal peptide synthetases and other specialized metabolites. While many of these compounds are predicted to be involved in interactions with bacteria, their impact on the structure, function, and general ecology of the gut environment is not yet understood. We hypothesize that specialized metabolites produced by *Basidiobolus* impact compositional and functional diversity of the bacterial community in a non-random manner. To test this, a fecal sample from an individual wood frog (*Lithobates sylvaticus*), raised in the absence of *Basidiobolus*, was cultured in multiple media conditions and serially transferred into fresh media every 48 hours. After the seventh passage of cells, composition of the cultures was assumed to be stable, and two additional transfers were conducted and treated with *Basidiobolus* crude extracts from aerobic and anaerobic growth conditions. Sequencing results show that stable, multi-species consortia emerge by the third passage and that *Basidiobolus* crude extract selectively inhibits growth of Gram-positive bacteria. We will also present metagenome-assembled genomes (MAGs) data to examine functional shifts in the enrichment communities and LC-MS/MS results from the crude extract to characterize metabolites that may function in bacterial-fungal interactions.

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Understanding Diversity of Endohyphal Bacteria in Clinical Mucorales Isolates

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Fungal–bacterial interactions are increasingly recognised as important but understudied contributors to fungal pathobiology, virulence, and clinical outcomes. Members of the Mucorales are known to harbour endohyphal bacterial partners, particularly members of the Mollicute-Related or *Burkholderiaceae*-Related Endobacteria, commonly identified in environmental Mucorales isolates. Yet, for patient-derived isolates, the breadth, prevalence, and clinical relevance of these associations remain largely unexplored. To address this gap, we are taking combined computational and experimental approaches to investigate endohyphal bacteria (EHB) across both clinical and environmental Mucorales isolates. By mining publicly available sequencing datasets and analysing newly collected patient isolates from the UK, Mexico, and France, we aim to broaden the spectrum of bacterial associates present in medically relevant Mucorales species. Our holistic EHB detection strategy integrates metagenomic screening, PCR-based detection, fluorescence in situ hybridisation, and whole-genome sequencing to sensitively and specifically identify candidate endosymbionts across genera, including but not limited to *Mycetohabitans*. Initial findings reveal previously recognised symbioses as well as novel endohyphal species in clinical and environmental samples. These results suggest that endohyphal associations in clinical isolates are more widespread and diverse than currently appreciated, with potential implications for fungal physiology, virulence, and treatment response. Together, this work establishes a framework for systematic discovery of fungal–bacterial symbioses in clinical settings and lays essential groundwork for future mechanistic studies. Ongoing efforts include functional characterisation of select Mucorales-bacteria pairs, with the long-term goal of understanding how these interactions can shape pathogenic traits and may ultimately be manipulated for experimental and therapeutic purposes.

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Analyzing differences in codon usage patterns in *Rhizopus micosporus*, its bacterial endosymbiont *Mycetohabitans* sp., and the novel prophages residing in the bacterial genome

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Mycetohabitans spp. are gram negative bacterial endosymbionts of *Rhizopus microsporus,* a known plant pathogen and opportunistic human pathogen. Though related to free-living *Burkholderia spp.*, *Mycetohabitans* strains have smaller genomes and are considered a separate genus undergoing genome contraction. Despite their reduced genome, many of the individual *Mycetohabitans* strains appear to have intact bacteriophage within their genomes. We identified 36 novel prophages with the computational tool GeNomad across 28 strains of *Mycetohabitans*. Preliminary results indicate that UV light can induce the prophage to produce viral-like particles. Computational analysis revealed two separate phage families based on average nucleotide identity, with one prophage falling in neither category. In order to better understand the pathogenicity and the symbiotic relationship of the fungus, bacterium, and bacteriophage, we investigated the differences in codon usage patterns between the various organisms compared to the tRNA pools. Uncovering these differences and how it relates to available tRNAs could pave the way for future fungal control mechanisms targeting codons exclusive to the bacterium and prophage.

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Linker histone H1.52 governs facultative heterochromatin and metabolic adaptation in Cryptococcus neoformans

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The opportunistic pathogen *Cryptococcus neoformans* causes over 100,000 deaths annually, yet the epigenetic mechanisms enabling metabolic adaptation within host environments remain unclear. Phylogenetic analysis across 50 basidiomycete species revealed that an ancient linker histone duplication is uniquely preserved in *Cryptococcus*. Molecular evolution analyses showed asymmetric subfunctionalization. H1.51 experienced reduced functional constraint with episodic positive selection in clinical isolates, whereas H1.52 evolved under sustained purifying selection. We characterized functional divergence using integrated RNA-seq and ChIP-seq. H1.51 deletion produced minimal transcriptional effects, whereas H1.52 loss dysregulated 1,561 genes and triggered genome-wide chromatin reorganization. Under host-mimicking conditions, wild-type cells established facultative H3K9me2

heterochromatin islands over biosynthetic genes while upregulating heterochromatin machinery. H1.52 deletion prevented this adaptation. The mutant failed to upregulate heterochromatin machinery, instead elevating anti-silencing factors. ChIP-seq revealed loss of facultative H3K9me2 alongside twofold expansion of H3K4me2, leaving growth genes inappropriately accessible. Despite lower expression of biosynthetic genes, mutant cells aberrantly activated catabolic pathways including amino acid degradation, lipid oxidation, and autophagy. H1.52 deletion cells failed to establish quiescence, maintaining elevated metabolic activity and showing accelerated growth recovery upon nutrient readdition. Genetic complementation reversed these phenotypes. These findings establish H1.52 as a critical regulator of facultative heterochromatin formation and metabolic adaptation during host stress.

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Beyond a reference genome: how cereal rusts pangenomes are changing our research approaches

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The adoption of new sequencing and computational technologies such as long read DNA sequencing coupled with Hi-C data have accelerated our understanding of the molecular mechanisms behind the evolution of complex dikaryotic organisms like cereal rusts. Cereal rusts represent pathogens of economic importance, whose management is essential for food security. Through the phasing of nuclear haplotypes, we uncovered the role of somatic hybridisation which allows for the transfer of intact nuclei between strains to generate novel haplotype combinations. We now recognise that this phenomenon is widely prevalent within the rusts and is linked to the rise of epidemiological significant strains. Most recently the construction of haplotype-phased pangenomes in various species is allowing us to formulate hypotheses to investigate the biology and movement of these organisms and document diversity in virulence loci. Together, these resources and our analyses represent a paradigm shift in the way we monitor cereal rust fungi and record changes in virulence within their populations.

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Distribution of haploid chromosomes into separate nuclei in two pathogenic fungi

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Nuclei define eukaryotes, enabling macromolecular compartmentalization and cellular regulation. Each nucleus is believed to contain one or more haploid sets of chromosomes (1N). However, we discovered that haploid cells of the pathogenic fungi *Sclerotinia sclerotiorum* and *Botrytis cinerea* distribute their chromosomes such that each of their nuclei contains only a subset of the haploid chromosomes (≤½N). The unusual chromosomal distribution was confirmed by cellular and molecular methods including chromosome counting, fluorescence in situ hybridization, flow cytometry–based DNA measurements, and single-nucleus polymerase chain reaction experiments. This phenomenon challenges fundamental assumptions about nuclear organization and opens fresh avenues in chromosome biology.

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Computational tools for chromosome conformation capture

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Chromosome conformation capture (3C) technologies, including Hi-C, generate maps that reveal the incredible diversity of genome organization across organisms and cellular states. Methodological advances now generate of maps with increasing size, depth, and resolution. Ever larger datasets add challenges at each step of computational analysis, from storage and memory constraints to researchers' time. In this talk, I will present open-source computational tools for 3D genomic analysis developed in collaboration with Open2C (Open Chromosome Collective). These include: bioframe (https://github.com/open2c/bioframe), pairtools (https://github.com/open2c/pairtools), and cooltools (https://github.com/open2c/cooltools). Together these Python packages enable flexible, scalable, and reproducible analysis of high-resolution chromosome conformation datasets. To illustrate the broad utility of these tools, I will draw upon examples of how they can be used to gain insight about the distinct genome conformations displayed across organisms and cellular states.

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A CRISPR activation platform to identify genes involved in trisomy-associated antifungal drug resistance in *Candida albicans*

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Candida species are among the leading causes of invasive infections and the fourth most common cause of hospital-acquired bloodstream infections. There are limited therapeutic options for treating *Candida* infections and other fungal diseases, and treatment is further hindered by the evolution of resistance to antifungal therapies. One way in which *Candida* rapidly adapts to antifungal drugs is via large genome rearrangements, including aneuploidies such as trisomies. These chromosomal trisomies are frequently identified in drug-resistant *Candida* strains, and are responsible for conferring resistance and tolerance, although the precise mechanism by which these trisomies lead to resistance is not always understood. We are using a novel approach to dissect the genetic mechanisms underlying aneuploidy-linked antifungal drug resistance in *Candida albicans*. Using CRISPR activation (CRISPRa)-based overexpression techniques, we have generated chromosome-wide libraries of overexpression strains to identify key genes that, when overexpressed, confer increased resistance to diverse antifungal agents. We have further developped multiplexing Cas12-CRISPR tools to validate genes singly and in combinations, that are involved in trisomy-mediated drug resistance and tolerance. Together, this work is exploiting novel functional genomic approaches to characterize the fundamental genetic mechanisms by which important fungal pathogens adapt and evolve resistance to antifungal drugs.

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FLASH, a new statistical machine learning algorithm, can accurately predict and link novel genes to antifungal resistance in diverse clinical and environmental fungi

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The evolution of resistance to antifungal drugs in fungi, specifically to azoles such as fluconazole and polyenes such as amphotericin B, has been partially characterized in several clinical and environmental studies, but variation in the main target of these drugs, the ergosterol synthesis pathway, does not explain all resistance. We show that resistance phenotypes across diverse fungal lineages including *Candidozyma auris, Candida tropicalis, Aspergillus fumigatus* and *Nakaseomyces glabratus*, as well as the y1000 dataset of over a thousand species of yeast from both clinical and environmental isolates, can be accurately predicted directly from raw DNA sequencing data using a machine learning statistical algorithm called FLASH that bypasses the need for alignment. This analysis points to novel genes that are predictive of resistance in addition to ones previously associated with resistance, lending support to the hypothesis that mechanisms of resistance may be diverse and vary in different environments and lineages. This method can also be used to survey the landscape of predicted antifungal resistance from leaf and soil metagenomes.

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Development of a synthetic biology toolkit for heterologous gene expression in the oyster mushroom, *Pleurotus ostreatus*

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Basidiomycota fungi are prolific producers of high-value metabolites including terpenoids, alkaloids and sugar derivatives. Although many fungal-derived natural products are known today, the production of these compounds in the native hosts is limited primarily due to the low/cryptic expression level of biosynthetic genes under the laboratory conditions. In recent years, new genetic and cultivation-based strategies for engineering of Basidiomycota fungi have become available, yet these are often species-specific, and genetic engineering techniques require a sophisticated knowledge of the biology of the producing organisms. Heterologous expression of Basidiomycota biosynthetic pathways in Ascomycota fungi such as *Aspergillus* spp. is feasible, however, more often they are unable to process numerous introns found within Basidiomycota genes.

Here in, we aim to develop a robust synthetic biology toolkit for the heterologous expression of Basidiomycota genes in a tractable basidiomycete, *Pleurotus ostreatus* (oyster mushroom), for which several protocols for introducing exogenous DNA as well as genetic manipulation have been established.[1,2] To date, we have tested *P. ostreatus* strains N001 (dikaryon), PC9 and PC15 (monokaryons) for growth on selected culturing media and performed RNA-seq analyses to identify genes which are highly and constitutively expressed or expressed in selected growth conditions. We are currently testing their respective promoters and terminators to express reporter genes in *P. ostreatus*.

We hope that the toolkit would benefit both the synthetic biology community as well as fungal natural products researchers in order to exploit Basidiomycota genomes as the source for the production of high-value compounds and enzymes.

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Facultative heterochromatin mediated by core and accessory chromosome-encoded H3K27-specific methyltransferases controls virulence in a fungal phytopathogen

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In many fungal phytopathogens, infection is regulated by accessory genomic regions enriched in facultative heterochromatin, but the precise role of this chromatin state in virulence is poorly understood. Here we examined the two facultative heterochromatic marks H3K27me and H3K36me in the tomato-pathogenic *Fusarium oxysporum* isolate *Fol* 4287, by genetically analyzing the corresponding histone methyltransferases Kmt6 and Ash1. *Fol* has three *KMT6* homologs, two of which, *KMTB/C*, are encoded on an accessory chromosome. Loss of the core gene *KMT6A* resulted in strongly reduced but not abolished H3K27me, revealing that methylation of subtelomeres can be taken over by the accessory copy Kmt6b. To the best of our knowledge, this demonstrates for the first time that accessory fungal histone modifiers can contribute to the overall epigenetic profile. Deletion of *KMT6A* or *ASH1* resulted in derepression of *in planta*-induced effector genes under axenic conditions. In the case of Ash1, H3K36-mediated H4 deacetylation is likely involved in gene silencing under non-inducing conditions. *KMT6A* deletion mutants were blocked at early stages of root infection and non-pathogenic on tomato plants, but were still able to kill the animal host *Galleria mellonella*. Constitutive overexpression of the pathogenicity-related transcription factor *FTF1* in the Δ*kmt6a* background restored effector gene activation *in planta* but not pathogenicity, suggesting that additional facultative heterochromatin-regulated virulence factors are essential for plant infection.

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Towards a mechanistic understanding of novel defense proteins from maize against U. maydis

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Understanding the molecular interplay between fungal pathogens and their host plants is crucial for preventing plant disease. Using the *Ustilago maydis*–*Zea mays* pathosystem, we aim to identify novel plant defense proteins and dissect their molecular functions. Previously, we discovered that maize Kiwellin proteins act as unexpected defense factors by inhibiting a secreted chorismate mutase from *U. maydis*, thereby reducing fungal virulence.

Here, we present evidence that this defense mechanism is conserved in other plants infected by related fungi. In addition, we introduce a screening approach that enables the systematic discovery of new maize defense proteins. Using this tool, we identified a family of related proteases with distinct antifungal activities. Their defensive role also appears to be conserved across multiple plant species.

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Cross-kingdom Interaction with *Candida albicans* Promotes Gut Colonization and Pathogenesis of *Salmonella* Typhimurium

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Commensal fungi are an important part of the gut microbiome, but researchers currently only have limited information about their role during enteric infections. The bacterium *Salmonella enterica* serovar Typhimurium (STm) causes acute intestinal inflammation in over 1.35 million people in the United States each year. STm pathogenesis is influenced by numerous factors, including host immune status and gut microbiome composition. *Candida albicans* is a commensal yeast that colonizes the gut of more than 60% of humans and is associated with diseases such as Inflammatory Bowel Disease. STm and *C. albicans therefore* both thrive in an inflamed intestine and are likely to co-occur frequently. Indeed, a clinical study recently showed that colonization with *Candida* species increases the recurrence of *S*. Typhi and *S*. Paratyphi infection*,* further suggesting the potential involvement of *C. albicans* during STm infection. In this study, we found that the presence of *C. albicans* significantly increased STm colonization in the cecum, spleen, and liver of mice and resulted in higher weight loss than STm infection alone. We determined the underlying mechanism and uncovered the amino acid arginine as a central molecule in the interaction between *S*Tm, *C. albicans, and the host*. We showed *in vitro* that the STm Type 3 Secretion System and the effector SopB were required for STm interaction with *C. albicans*. Presence of SopB caused increased arginine biosynthesis in *C. albicans* and resulted in the release of millimolar amounts of arginine into the extracellular environment. The released arginine was sufficient to induce activation of the *Salmonella* Pathogenicity Island 1 (SPI-1) and increase epithelial cell invasion of STm. Exogenously supplied arginine increased STm virulence in the mouse model, while *C. albicans* deficient in arginine production did not. Arginine, either supplied exogenously or produced by *C. albicans,* also significantly dampened the immune response to STm infection. The lower inflammatory response might further benefit STm by inefficient clearance, causing increased dissemination. This study thus highlights *C. albicans* colonization as a potential comorbidity for STm infection and identifies arginine as a key player in important cross-kingdom interactions between gut commensal fungi, STm, and host.

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Uncovering cell-specific responses of barley during *Magnaporthe oryzae* infection

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Plants rely on the defense responses of individual cells to counter pathogen attack. To overcome host resistance, pathogens secrete specialized molecules, known as effectors, that enable them to evade, suppress, or manipulate the immune responses of penetrated cells. However, from microscopic analyses it is known that even when a pathogen successfully suppresses immunity in a single cell, adjacent cells may still resist invasion. Thus, to obtain a comprehensive understanding of the transcriptomic and metabolic differences between susceptible and resistant plant cells, and of the mechanisms by which pathogens manipulate host immunity, analyses at the whole-tissue level are insufficient, instead investigations at the single-cell level are essential.

In this study, we aimed to establish a strategy to selectively identify barley cells colonized by the fungal pathogen *Magnaporthe oryzae* and to characterize their transcriptomic profiles in comparison to uninfected cells within the same tissue. Therefore, protoplasts were generated from infected barley leaves, followed by barcoding of RNA derived from individual cells and subsequent RNA sequencing. Cells exhibiting similar gene expression profiles were grouped into distinct clusters. These clusters were then analyzed for infection-associated marker genes and for the presence of *M. oryzae*- specific transcripts. Through this approach, we will identify plant and fungal genes that play crucial roles in immune response and virulence, respectively.

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Epigenetic rewiring of the low-oxygen response by an Inhibitor of Growth (ING) family Protein drives *Aspergillus fumigatus* disease progression

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Adaptation to dynamic oxygen microenvironments in the lungs is critical for *Aspergillus fumigatus* disease progression. Higher oxygen levels are available in the large airways, but decline progressively in the alveoli, eventually leading to severe hypoxia in deep tissues. While epigenetic mechanisms are known to play an essential role in low-oxygen adaptation in mammals, their function in fungi is ill-defined. To address this gap in knowledge, we screened mutants

in chromatin regulatory genes from the *Neurospora crassa* deletion library and identified an Inhibitor of Growth domain-containing Protein (IngA) required for low oxygen growth. The IngA ortholog in *A. nidulans* is part of the RpdA-L histone deacetylase complex. Loss of *ingA* in *A. fumigatus* resulted in stunted biofilms at both 21% and 10% atmospheric oxygen levels. A significant reduction in the Δ*ingA* submerged biofilm biomass at 10% oxygen was observed compared to wild-type and complemented strains.

Under hypoxic conditions (0.2% oxygen), both shaking batch cultures and submerged biofilms of Δ*ingA* exhibited a severe growth defect. Our results suggest that Δ*ingA* is metabolically limited, as it cannot grow on plates containing ethanol and 2-deoxy-D-glucose under normoxic conditions. Moreover, transcripts of genes encoding proteins involved in ribosome biogenesis and protein translation are significantly increased in Δ*ingA* in hypoxic conditions. We are currently testing the hypothesis that the growth failure of Δ*ingA* in hypoxic conditions arises from its inability to sense oxygen changes and adjust protein translation.

We also observe that IngA is a positive regulator of ergosterol biosynthesis, and Δ*ingA* is less susceptible to voriconazole. Critically, the loss of *ingA* results in a striking reduction in fungal burden and attenuated virulence in the triamcinolone murine model of invasive aspergillosis. Histological analysis reveals that the mutant can grow in the larger airways but cannot grow in deep tissues, where oxygen levels are much lower. Therefore, while IngA is not required for disease initiation, it is essential for disease progression in low-oxygen environments. As antifungals are typically administered after disease establishment in patients, identification of novel disease progression factors is critical for developing new antifungals to treat this deadly disease.

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How fungi exploit host defense activity for pathogenic development: Insights from a new peptide-GPCR system

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Fungal pathogens rely on precise mechanisms to sense when they have entered the host environment to coordinate the developmental transitions required for disease progression. G-protein coupled receptors are key regulators of fungal development and GPCR-mediated signaling is widespread in fungi, but the origins of their ligands and the logic of their activation remain surprisingly underexplored. To address this gap in smut fungi, we performed a structure-guided screen for putative GPCRs in the maize pathogen *Ustilago maydis*. This led to the identification of more than 30 potential GPCRs, many of them belonging to unknown GPCR classes. Among those we investigated Gpe1, a previously uncharacterized GPCR essential for establishing a successful plant infection. Strikingly, Gpe1 is activated by a peptide encoded by the adjacent gene *pit2*. Upon host entry, the secreted Pit2 protein is processed by plant-derived papain-like cysteine proteases, releasing a mature peptide ligand that activates Gpe1. GPCR activation strongly promotes fungal proliferation suggesting that the Gpe1/Pit2 system coordinates release of the G2 cell-cycle arrest thereby enabling fungal expansion *in planta*. We show that the Gpe1/Pit2 pair is conserved across related fungi but exhibits co-evolutionary signatures preserving receptor-ligand specificity. While this GPCR/peptide system appears to be exclusive to smut fungi, our findings uncover a novel concept in GPCR biology - a host-triggered, peptide-based activation mechanism - with potential parallels in other fungal systems. This work provides a new framework for understanding host-responsive fungal adaptation and expands the landscape of GPCR-based sensing mechanisms.

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Trehalose biosynthesis pathway-mediated thermotolerance via tight regulation of glycolytic flux

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Trehalose has been shown to play a key role in the fungal response to high temperature stress, as is found in the transition from ambient temperatures to human physiological conditions. Trehalose is synthesized from glucose-6-phosphate (G6P) and uridine-di-phosphate-glucose (UDPG) in two steps catalyzed by the enzymes trehalose-6-phosphate synthase (Tps1) and trehalose-6-phosphate phosphatase (Tps2) in most fungi. Given the importance of trehalose in the response to high temperature stress, loss of Tps1 or Tps2 leads to loss of thermotolerance. I will present data on the genetic suppression of the *tps1* mutant phenotype under high temperatures in *Cryptococcus deneoformans*, a species in the *Cryptococcus* pathogenic species complex. We found that, as has been found in other fungi, Tps1 is essential for growth at 37°C in *C. deneoformans* and identified spontaneous suppressor mutations that restore the growth of *tps1*Δ mutants under high temperatures. Whole-genome sequencing followed by variant calling analysis led to the identification of several causative loci. I will present the identity and characterization of one of these genes encoding hexokinase 1 (Hxk1), one of two hexokinases in *C. deneoformans*. Several independent mutations were identified in the gene including amino acid changes, protein truncation, and deletions. Biochemical analysis shows that these amino acid changes result in loss of Hxk1 activity. By combining genetics and measurements of metabolic flux in the glycolysis pathway, we will show that overall hexokinase activity increased in the *tps1*Δ mutants and was significantly restored to wild-type levels in the suppressor mutants. By generating gene deletion mutants, we will further present data that deletion of either *HXK1* or *HXK2* can bypass *tps1*Δ in a carbon source-dependent manner. Deletion of *HXK1* or *HXK2* partially restored G6P, ATP, and glycogen levels in the *tps1*Δ mutant to wild-type levels, affecting the glycolytic flux in these mutants. Overall, our study reveals a role for Tps1 in high temperature growth, genetic mutations that bypass this function, and a metabolic basis for genetic suppression in *C. deneoformans*. This presentation will show that the role of trehalose in thermotolerance extends beyond simply stabilizing proteins and membranes.

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Bacterial metabolites induce cell wall remodeling, antifungal resistance, and immune recognition of commensal fungi

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The fungus *Candida albicans* commensally colonizes mucosal surfaces in healthy individuals but can cause both superficial mucosal and life-threatening disseminated infections. The balance between commensalism and pathogenicity is complex and depends on factors including host and fungal genetic background, the host environment, and fungal interactions with local microbes. The major interaction interface of *C. albicans* with the host is its multilayered cell wall, which is dynamic and highly responsive to the surrounding environment. Therefore, factors that influence the fungal cell wall will directly impact *C. albicans*-host interactions. Our work demonstrates that multiple physiologically-relevant gastrointestinal bacteria influence fungal cell wall composition during co-culture with *C. albicans,* including as complex communities derived from the gut. Using *Escherichia coli* as a model, we show that bacterial-induced fungal cell wall remodeling occurs rapidly and is mediated by secreted bacterial metabolite(s). Fungal mutant analysis revealed that the high osmolarity glycerol (HOG) pathway, which is critical for responding to environmental stresses, has an important role in regulating this cell wall remodeling phenotype through the Sln1 histidine kinase. Importantly, bacterial-mediated fungal cell wall remodeling increases *C. albicans* resistance to the echinocandins, increases macrophage phagocytic rates, and decreases recognition by human IgA. Overall, this work comprehensively characterizes an interaction between *C. albicans* and common gastrointestinal bacteria that has important implications for fungal biology and host interactions.

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Dissecting turgor-sensing mechanisms required for appressorium-mediated plant infection by the rice blast fungus *Magnaporthe oryzae*

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Appressorium-mediated infection by *Magnaporthe oryzae* relies on the ability of the fungus to generate, sense, and respond to extreme turgor pressure in its infection cell. The histidine kinase Sln1 functions as a central turgor sensor that triggers appressorium repolarisation and host penetration, yet the molecular mechanisms underlying its function remain poorly understood. To define the signalling networks by which Sln1 coordinates turgor-dependent developmental transitions, we mapped the Sln1 interactome using an ultra-high-throughput yeast two-hybrid (Y2H) screen of an appressorium-stage cDNA library. Screens using full-length Sln1 (minus the transmembrane domain) and its isolated response-regulator domain identified 11 and 66 candidate interactors, respectively. These include β-oxidation enzymes, glycerol-generating enzymes, cytoskeletal motors, cell wall–modifying proteins, and COP9 signalosome components, highlighting a broad regulatory network linking metabolism, cytoskeletal dynamics, and cell wall remodelling to turgor sensing.

Additionally, complementary immunoprecipitation–mass spectrometry (IP/MS) with in-locus Sln1-3xFLAG and Ypd1-3xFLAG *M. oryzae* strains in both wild-type and turgor-deficient (*rsy1*–) backgrounds revealed 47 significant interactors. Sln1 associates with distinct protein cohorts depending on turgor status. In Guy11, interactors are enriched for wall-modifying enzymes and signalling factors, whereas in Δ*rsy1 mutants*, Sln1 engages proteins involved in wall biosynthesis and vesicle trafficking, including kinesin II and chitin synthase 1. Sln1 also associates with core melanin biosynthesis enzymes, and Δ*sln1* mutants fail to repress melanin deposition, linking phosphorelay signalling to the regulation of melanin biosynthesis and turgor maturation. These findings support a model in which Sln1 dynamically rewires its interactome in response to mechanical cues.

Finally, in yeast, Sln1 controls Hog1 MAPK activity through a conserved phosphorelay, and *SLN1* deletion is lethal due to constitutive Hog1 phosphorylation. Leveraging this conserved mechanism, we are testing whether Osm1 phosphorylation can serve as a quantitative proxy for Sln1 dephosphorylation and activation during turgor generation. Establishing this relationship will enable time-course assays to pinpoint when Sln1 becomes active as appressoria mature and mechanical pressure accumulates, as pre-requisites to plant infection.

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Light-dependent regulation of development by the velvet complex in *Neurospora crassa*

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Regulation of development is an essential biological process during fungal reproduction. The velvet complex is a fungal-specific protein complex that participates in the regulation of gene expression during development, pathogenesis, and secondary metabolism in response to environmental signals such as light. In *Neurospora crassa* three developmental pathways coexist: sexual development, macroconidiation (the main asexual pathway, alse called conidiation), and microconidiation. In *N. crassa*, there are three velvet proteins (VE-1, VE-2 and VOS-1) and a fourth truncated version (VEL-3), belonging to a paraphyletic subfamily of velvet proteins. VE-1 and VE-2, together with LAE-1 interact to form a velvet complex. Deletion of *ve-1* and *ve-2* in *N. crassa* cause similar but not identical alterations in both sexual development and conidiation, while deletion of *lae-1* results in milder alterations. In the presence of light, strains lacking VE-1 and/ or VE-2 display increased macroconidiation as well as a notably delayed and reduced sexual development with fewer protoperithecia when compared to the wild-type strain. All the components of the velvet complex are detected in aerial hyphae, the early stages of macroconidiation, and in protoperithecia, the initial stages of sexual development. However, the VE-1 protein, but not VE-2 or LAE-1, shows an increase instability in light compared to dark. In the wild-type strain, the transcriptional response to sexual and asexual development is larger than the response to light. Indeed, VE-1 regulates the expression of hundreds of genes during conidiation and thousands of genes during sexual development. In some cases, the regulation is direct by binding of the velvet complex to the promoters of the genes, for example, MAPKs during sexual development, and *vib-1* and *fl* during conidiation. Interestingly, the combined deletion of *ve-1* and *fl* triggers microconidiation, and suggests that these transcription factors act in coordination to promote macroconidiation as the main pathway for asexual development.

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A co-evolved peptide-GPCR system senses host entry to drive fungal infection

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Interactions between fungi and their hosts rely on precise sensing mechanisms for environmental cues, signaling molecules, and nutrients detection, often mediated by G-protein coupled receptors (GPCRs). In plant-associated fungi, GPCRs play a crucial role in sensing host-derived signals that trigger transitions lifestyles. Yet, the precise mechanisms by which fungal GPCRs recognize and respond remain largely unexplored. Based on recent developments in structure prediction, we investigated the presence of seven-transmembrane (7TM) proteins in the plant pathogen *Ustilago maydis*. Our analysis identified potential GPCRs candidates, one of them being previously reported as an important virulence factor. Interestingly, a secreted protein is neighboring this GPCR candidate on the genome. This secreted protein, called Pit2, is then processed by plant proteases during host infection and we demonstrated that one of its cleavage products, a 9 amino acid peptide-like pheromone contained within its core, activates the G-protein-coupled receptor promoting fungal in planta expansion (Gpe1). Our results show that this GPCR acts as a molecular gatekeeper, detecting host entry by recognizing a highly hydrophobic peptide motif. We solved the crystal structure of Pit2 at 2.3 Å resolution and show that Pit2 forms a tightly intertwined homodimer that buries the conserved WWFGFTGSL motif in its dimer interface, supporting a scaffold role that shields the ligand before host-dependent release. AlphaFold3 modelling of the Gpe1–peptide complex implicates residues in Transmembrane Helix 1 and Extracellular Loop 2 in ligand binding confirmed by structure-guided mutagenesis of these residues compromises both receptor activation and virulence in maize plant infections. Comparative analyses also reveal that the Gpe1/Pit2 module is conserved in related smut fungi, with species-specific peptide–receptor pairs that limit cross-activation. Furthermore, live-cell imaging in axenic cultures shows in peptide presence it triggers Gpe1-dependent changes in endomembrane dynamics and promote fungal proliferation by enhancing cell division. Together, our findings reveal a co-evolved mechanism between fungus and host that encodes environmental context into a protein scaffold, establishing a novel paradigm for host-dependent signaling with implications for inter-organismic communication.

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TORC1 integrates multiple environmental cues to regulate appressoria formation in the rice blast fungus

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The rice blast fungus, *Magnaporthe oryzae*, is the causative agent of one of the most widespread and serious disease of cultivated crops, severely impacting cultivars in the *Poaceae* family. To gain entry to plant hosts, airborne conidia attach to the **hydrophobic** and **nutrient-free** leaf surface where they develop a specialized infection structure, the appressorium. During differentiation the 3-celled conidium undergoes autophagy mediated cell death while the remaining cell becomes an appressorium – a highly pressurized structure designed to provide the mechanical force needed to breach the leaf cuticle. Better understanding of the molecular mechanisms underlying appressoria formation could identify vulnerable signalling nodes and thus generate novel strategies to tackle blast disease. In this vein, we investigate the role that Target Of Rapamycin (TOR) signalling plays in appressorium formation as components of this otherwise well conserved signalling network are present in fungi, but absent in plants, and could thus represent suitable targets for effective control of blast disease.

Using an optimized CrispR protocol, we can now manipulate the *M. oryzae* genome with relative ease. This facilitated affinity purification of *Mo*TOR Complex1 (*Mo*TORC1) and *Mo*TORC2. Subsequent proteomics analyses in combination with cryo-electron-microscopy (cryo-EM) allowed us to identify the composition and to obtain high-resolution structures of both native complexes. Deletion *MoTCO89*, encoding a non-essential *Mo*TORC1 subunit, yielded “blind” conidia, no longer able to transduce environmental cues, including nutrients and hydrophilic surfaces, which in *WT* conidia would prevent appressoria development. Moreover, inhibition of *Mo*TORC1 with caffeine, but not rapamycin, restores appressoria formation in these non-inductive conditions. These observations suggest upstream factors that inhibit *Mo*TORC1 signalling could serve as drug targets to block plant infection.

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From spore to attack mode: what molecular switches trigger appressorium development in *Magnaporthe oryzae*?

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The filamentous fungus *Magnaporthe oryzae* causes the blast disease in cultivated rice and destroys enough rice to feed 60 million people in the world annually. *M. oryzae* it is widely known for being highly variable, undergoing host jumps and causing new outbreaks, constituting a threat to global food security. In the field, a single lesion can produce more than 50,000 genetically identical *M. oryzae* spores in under 24 hours. The spores are teardrop-shaped with three cells arranged along their length: a pointed apical cell at the tip, followed by a middle cell and a rounded basal cell. Each cell contains a single nucleus. Upon contact with the leaf, spores germinate only, and always, from the apical cell. After the germ tube is produced, a single round of mitosis occurs, coupled with the formation of a specialized dome-shaped infection called appressorium which the fungus uses to penetrate the plant surface and cause infection. Appressorium development, is tightly regulated by cell cycle via cell cycle checkpoints, which are mediated by the DNA damage response pathway. The appressorium developmental pattern follows a defined sequence, as the germination only occurs from the apical cell, leading to a single round of mitosis and finalising with the degradation of the spore via an autophagy-mediated cell death process. It remains unclear which cellular mechanisms control this developmental program coupled with cell cycle progression and DDR, and which factors determine spore identity. By combining phosphoproteomics, cell biology, chemical genetics, transcriptomics, and classical genetics, we have uncovered molecular mechanisms that govern the developmental cell-cycle program underlying infection and define spore identity.

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Phosphoproteomic analysis of appressorium development identifies Vts1, a novel RNA-binding protein required for pathogenicity in the blast fungus *Magnaporthe oryzae*

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How fungal pathogens sense their host and initiate infection-related development is still poorly understood. We have employed a quantitative phosphoproteomic approach to investigate appressorium development by the blast fungus *M.oryzae*. Our study has mapped 8,005 phosphopeptides on 2,062 fungal proteins, providing a detailed landscape of the phosphorylation events that orchestrate appressorium-mediated plant infection. This reveals a profound re-wiring of phosphorylation-based signalling cascades during development, shedding light on the dynamic molecular responses elicited in the fungus during appressorium morphogenesis. The Pmk1 MAP kinase is a key orchestrator of appressorium formation which prompted us to carry out comparative phosphoproteomics and parallel reaction monitoring to identify substrates of Pmk1. In this way we identified 32 putative Pmk1 MAPK targets, revealing a complex network of regulatory interactions. One of these substrates is a novel regulator, Vts1, which undergoes Pmk1-dependent phosphorylation and is required for virulence. Vts1 is a Sterile Alpha Motif (SAM) domain protein that plays an important role in appressorium morphogenesis and pathogenesis. We show that a single Pmk1-dependent phosphorylation event is necessary for Vts1 to function as a virulence determinant and that truncation of the SAM domain of Vts1 also impairs its function. Structural modelling and structure-based phylogenetic analysis provide evidence that Vts1 is an RNA-binding protein, consistent with transcriptional profiling which identified many Vts1-dependent differentially expressed genes associated with RNA-binding and processing. Using RIP-seq analysis we defined a set of Vts1-associated mRNAs many of which have been previously implicated in fungal pathogenesis. Mutation of RNA-binding residues in Vts1 prevents complementation of a *Dvts1* mutant in virulence assays, consistent with RNA-binding by Vts1 being essential for its function in plant infection, potentially as a spatial regulator of translation of a sub-sety of mRNAs during the pre-peentration stage of development. Taken together our data provide an example of how quantitative phosphoproteomic analysis of fungal infection can identify novel virulence functions and help to define new signalling mechanisms required for appressorium-mediated plant infection.

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Capture of the SmSTRIPAK proxiome identifies the Greenbeard proteins SmDOC1/2 as regulators of sexual development in *Sordaria macrospora*

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Hyphal fusion and sexual development in filamentous fungi depend on the coordinated signalling of numerous conserved signaling complexes, such as the Striatin-Interacting Phosphatase and Kinase (STRIPAK) complex and the Pheromone Response (PR) MAPK cascade (MIK2, MEK2, MAK2 and HAM5). The homothallic ascomycete *Sordaria macrospora* (Sm) was used here to screen for putative protein interactors of the SmSTRIPAK complex. We enriched and identified canonical SmSTRIPAK components and a DOC protein using the STRIPAK complex interactor 1 (SCI1) subunit of the complex as bait. DOC proteins have been described in the closely related, heterothallic species *Neurospora crassa*, where they function in allorecognition of germlings and hyphal fusions. We generated single deletion strains ΔSmdoc1 and ΔSmdoc2 and a double deletion mutant ΔSmdoc1ΔSmdoc2 in *S. macrospora*. Deletion phenotypes were paradoxical. Single knockouts (ΔSmdoc1 or ΔSmdoc2) were nearly sterile. Yet the double mutant (ΔSmdoc1ΔSmdoc2) exhibited wild-type fertility and fruiting-body development. This demonstrates non-redundant and mutually antagonistic roles of DOC proteins. Similarly, we demonstrated an impairment of sexual development in the *N. crassa* Δdoc-2 mutant. Using gene tagging at the native locus, we performed TurboID-based proximity mapping with SmDOC1 and SmDOC2 as the bait proteins. This proximity mapping revealed a close association between SmDOC1/2 and components of the PR MAPK pathway, as well as mutual SmDOC1-SmDOC2 proximity. Yeast two-hybrid experiments confirmed a direct interaction between SmDOC1 and the MAP kinases MEK2 and MAK2. Fluorescence microscopy revealed that SmDOC1-TagRFP-T localized to ring-like structures around septal pores. Our results show that the DOC system is not only found in heterothallic *N. crassa* but also plays an important role in the development of fruiting bodies in fungi. These findings suggest that the DOC1/2 proteins form a novel system integrating the STRIPAK and PR pathways. This provides a possible mechanistic explanation for the non-additive deletion strain phenotypes observed.

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Bridging Continuum Mechanics and Fungal Biology to Enable Mycelium-Based Biosensing Technologies

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Filamentous fungi form spatially distributed networks capable of detecting and responding to diverse chemical cues, yet the physical mechanisms governing how these signals move through the mycelium remain incompletely characterized. We seek to develop predictive models of fungal chemical sensing—of both nutrients and hazards—using tools from continuum mechanics traditionally applied to synthetic polymeric materials.

Our early-stage work explores two complementary modeling approaches. The first is a hyphal-resolved description that treats individual hyphae as directed one-dimensional conduits for transport and reaction, allowing explicit representation of geometry-dependent bio-chemical propagation of chemical information. The second is an effective three-dimensional continuum model that homogenizes the network architecture into mesoscale parameters, enabling analysis of signal dynamics at the scale of the colony without requiring detailed hyphal reconstruction. Together, these models aim to characterize how local chemical inputs evolve across space and time within a growing, branching network and to identify the structural and physical features that dominate information flow.

In parallel with these modeling efforts, collaborators are generating experimental data to refine model assumptions and parameter ranges. By integrating mechanistic modeling with biological insight, we aim to establish a framework that can ultimately inform the design and control of fungal systems for biotechnology and living material applications.

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RNA isoforms and structure rapidly respond to temperature changes in *Histoplasma*

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Temperature is a key signal sensed by thermally dimorphic fungi, including *Histoplasma ohiense* (*Ho*), to elicit developmental programs that facilitate pathogenesis. Since RNA is a classic thermoresponsive molecule, we hypothesized that fungal transcriptomes harbor temperature-responsive RNA structures that could regulate fungal biology. Since *Ho* displays dramatic temperature regulated biology, growing as hyphae at ambient temperature and as yeast at host temperature, it is an ideal system to identify putative RNA thermometers.

To interrogate the role of RNA molecules in thermosensation, we generated the first isoform-level transcriptomic atlas of *Ho* yeast (37˚C) or hyphae (22˚C) using Nanopore direct RNA-seq and short-read RNA-seq, with validation of transcription start-site annotations by 5'-seq. We annotated 14,014 genes and 50,791 isoforms, including those that displayed differential splicing or alternative 5' leaders and 3' trailers that were differentially enriched in yeast or hyphae. Initial annotation of differentially expressed isoforms revealed that one of the splice events increased the length of the transcript region encoding a phase separation domain in a key regulatory transcription factor that is required for yeast-phase growth, suggesting that differential RNA isoforms could play important regulatory roles in the response of *Ho* to temperature. Differential leader and trailer isoforms were equally provocative. We identified 2,140 and 3,516 protein coding genes with longer leader and longer trailer isoforms, respectively. A time course following a shift from 37˚C to 22˚C revealed that some longer leaders and trailers arise or disappear as early as 2 hours post shift. Rapid regulation of leader and trailer isoform levels post temperature shift and at various developmental stages suggest isoform-driven regulation of temperature-dependent development. We are currently exploring the effect of these differential isoforms on translation.

To extend these studies to identify thermoresponsive RNA structures, we adopted a transcriptome-wide RNA structure probing approach, DMS-MaPseq. Preliminary analysis revealed 5' leader and 3' trailer regions that have different structures at either 37˚C or 22˚C, including temperature-responsive structural elements in the mRNA leader structures of ubiquitin and BiP. Molecular characterization and structure-function assays of these and other putative thermosensory structural elements are currently underway.

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The (mis)use of sex categories in fungi

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The exclusion of fungi from evolutionary thinking has led to an incomplete and inaccurate understanding of the evolution of sexual reproduction. To place fungi within theories developed for animals, mycologists focused on fungal sex adopted ideas of animal sex and used the sex categories female and male to label fungal individuals and parts. Definitions of the fungal female and male are based on anisogamy, sex roles, or body parts. But attempts to "sex" fungi based on structures or behaviors are biologically inaccurate, non-explanatory, and biased; in some cases, the definitions are mutually exclusive. We explain how sexed language can be replaced with more accurate, descriptive and neutral terminology. The incoherence of sex categories in fungi illustrates a larger problem: uncritically adopting terminology and definitions developed for one group of taxa across the tree of life obscures biological variability, distorting evolutionary theory and preventing useful comparisons across kingdoms.

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The complex evolution and genomic dynamics of mating-type loci in *Cryptococcus* and *Kwoniella*

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Sexual reproduction in basidiomycete fungi is governed by *MAT* loci (*P/R* and *HD*), which exhibit remarkable evolutionary plasticity, characterized by expansions, rearrangements, and gene losses often associated with mating system transitions. The sister genera *Cryptococcus* and *Kwoniella* provide a powerful framework for studying *MAT* loci evolution owing to their diverse reproductive strategies and architectures, spanning bipolar and tetrapolar systems with either linked or unlinked *MAT* loci. Building on recent comparative genomic analyses, we generated additional chromosome-level assemblies and uncovered distinct trajectories shaping *MAT* loci organization. In contrast to the small-scale expansions and gene acquisitions observed in *Kwoniella*, our analyses revealed independent expansions of the *P/R* locus in tetrapolar *Cryptococcus*, possibly driven by pheromone gene duplications. Notably, these expansions coincided with a pronounced GC-content reduction best explained by reduced GC-biased gene conversion following recombination suppression, rather than relaxed codon usage selection. We also identified diverse modes of *MAT* locus linkage, including three previously unrecognized transitions: one resulting in a pseudobipolar arrangement and two leading to bipolarity. All three transitions involved translocations. In the pseudobipolar configuration, the *P/R* and *HD* loci reside on the same chromosome but remain genetically unlinked, whereas the bipolar transitions additionally feature rearrangements that fused the two loci into a nonrecombining region. Mating assays and progeny analysis in *Kwoniella mangrovensis* revealed substantial ploidy variation and aneuploidy, likely stemming from haploid–diploid mating, yet evidence of recombination and loss of heterozygosity indicates that meiotic exchange occurs despite irregular chromosome segregation. These findings underscore the importance of continued diversity sampling and

reinforce the convergent evolution of fused *MAT* loci in basidiomycetes, offering new insights into the genetic and chromosomal changes driving reproductive transitions.

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Unidirectional mating-type switching as a programmed genome-editing system in filamentous fungi

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Sexual identity in filamentous ascomycetes is encoded at the *MAT1* locus, a region long assumed to be structurally stable and evolutionarily conserved. Unidirectional mating-type switching as an alternative mating strategy, defies both expectations. In these fungi, self-fertile individuals generate progeny that segregate into two morphotypes. One is stable and self-sterile, while the other is unstable and self-fertile. This sexual variability has remained unexplained for decades.

Genome sequencing and subsequent genetic studies have recently shown that an irreversible DNA deletion governs the transition from self-fertility to self-sterility. Species capable of mating-type switching share a distinctive *MAT1* architecture. In all cases the *MAT1-2* genes are positioned within *MAT1-1-1*, disrupting the expression of this gene. The *MAT1-2* genes are flanked by identical direct repeats, and a recurring recombination event irreversibly excises these genes. This results in a self-sterile nucleus containing only *MAT1-1* genes in which *MAT1-1-1* expression is restored. Although the locus structure and associated deletion accounts for the unidirectional nature of the switching event, the molecular mechanism as to how this occurs remains unresolved.

Accumulating evidence points to a mixed nuclear state that distinguishes switching species from typical homothallic fungi. The self-fertile morphotype appears to maintain a mixture of nuclei, some of which retain the full *MAT1-1*/*MAT1-2* complement, while others carry only *MAT1-1* genes. It is possible that interactions between these genetically distinct nuclei enable self-fertility, while the stable, permanently switched morphotype may simply reflect segregants that receive only the self-sterile nuclear type.

Unidirectional mating-type switching redefines the *MAT1* locus as a site of programmed genome instability, which contrasts the classic, static view of this region. Ongoing advances in long-read sequencing and single-nucleus genomics now offer us the tools required to resolve the nuclear dynamics and recombination processes that drive unidirectional mating-type switching. In pursuing these approaches, we anticipate finding an answer to how unidirectional switching shapes reproductive biology, population structure, and the evolutionary trajectories of fungi.

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Adding pieces to the puzzle of *Aspergillus fumigatus* sexuality

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The filamentous ascomycete *Aspergillus fumigatus* is the major cause of a variety of fungal infections commonly termed aspergillosis. Besides vegetative growth and asexual spore formation, this mold is capable of cleistothecia formation accompanied by ascosporogenesis under specific environmental conditions. Knowledge about the sexual phase of its lifestyle and regulatory determinants is, however, incomplete. *A. fumigatus* sexuality is governed by a bi-polar mating-type system comprising an idiomorphic region that contains each one of the master regulator-encoding genes *MAT1-1-1* and *MAT1-2-1*. Transcriptional profiling data from strains overexpressing the mating-type regulators turned out to be a rich source in shedding light on a variety of fungal traits, such as secondary metabolism, antimicrobial defense, pheromone signaling, or fruiting body formation.

Assembling the corresponding transcriptopmes *de novo* allowed identification of the elusive **a**-factor-like mating pheromone encoded by the *ppgB* gene. The deduced peptide is 24 amino acids long and features a canonical CaaX farnesylation motif. Inspection of *Aspergillus* genomes from members of the section *Fumigati* revealed high conservation of PpgB sequence as well as of the α-factor-like PpgA, indicating that incompatibility factors other than solely pheromone discrimination are responsible for speciation.

With respect to cleistothecia development, an annotated gene encoding a conserved hypothetical protein and assigned as *tomA* (target of MAT1-1-1) was investigated in more detail. Transcription of *tomA* strictly depends on the *MAT1*-encoded master regulators and canonical MAT1-1-1 binding sites are present within the corresponding upstream regulatory region. Inspection of strains expressing GFP-tagged TomA indicates its nucleolar localization, supporting functionality of a deduced nuclear localization sequence in the *tomA* coding region. To explore any cellular function of the *tomA* gene product, deletion strains in either mating-type background were generated to reveal that the absence of TomA in the heterokaryon results in sterility, indicating that *tomA* is crucial for *A. fumigatus* sexual reproduction.

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Signs of ongoing reproductive incompatibility in *Aspergillus fumigatus*

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Reproductive isolation is the inability of two populations to produce viable, fertile, sexual offspring due to biological barriers. In Aspergillus fumigatus, an important human fungal pathogen, studies have shown that antifungal resistant isolates cluster separately from sensitive isolates in phylogenetic analyses irrespective of geographic location, indicating a lack of current gene flow. Reproductive isolation could occur through prezygotic barriers, which prevent fertilization, or postzygotic barriers which act after a hybrid zygote has formed. Crosses within- and between-clusters are equally fertile, indicating a lack of prezygotic barriers.

To identify potential reproductive barriers, we used 102 isolates to perform 286 sexual crosses, 60 of which produced offspring. Of the 60 crosses, 13 showed unfit progeny with reduced mycelial growth, no sporulation, or both. We identified three main phenotypic segregation ratios: (a) 1:1 segregation of sporulating and non-sporulating offspring, (b)1:1:1:1 segregation involving differences in both sporulation and size, and (c) a small percentage (5–20%) of non-sporulators. These repeated phenotypic ratios do not appear linked to resistance background, equally found within and between cluster crosses.

Surprisingly, these phenotypes are not found in either parent (both wild type), pointing towards a non-Mendelian mode of inheritance. We hypothesize that differential adaptation in phylogenetic clusters may lead to low-fitness hybrid progeny, which are outcompeted in nature.

To identify the genetic elements causing this segregation, we used BSA on a cross showing the 1:1 ratio of sporulating and non-sporulating offspring. We identified a 70-kb locus on chromosome 1 linked to the phenotype, containing 19 genes. Notably, in the brlA gene, a known regulator of asexual development, all non-sporulating offspring carried an identical deletion of ~45 bp, not shared with the sporulating offspring. This deletion is not found in either parent but only occurs in one parental genome. BSA analysis of the 1:1:1:1 ratio of offspring indicates a mechanism outside of brlA. Crosses of closely related species also show the same phenotype, but BSA also indicates a non-brlA mechanism. Our data shows for the first time a post-zygotic reproductive mechanism, that is surprisingly widespread in a sexually reproducing species. The importance of this mechanism will depend on the contribution of sexual and asexual reproduction in this species.

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Malassezia **parasexual hybridization, yeast-to-hyphae transition, and pathogenesis**

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The lipophilic yeast *Malassezia* is the most abundant member of the skin mycobiome, representing over 90% of all commensal skin fungi. While primarily commensal, *Malassezia* has also been associated with skin disorders including seborrheic dermatitis (SD), atopic dermatitis (AD) and pityriasis versicolor (PV), with the hyphal form often observed in diseased lesions. However, the role of *Malassezia* hyphae in pathogenesis as well as the mechanisms underlying their formation remains poorly understood. Recent genomic studies have revealed natural *Malassezia furfur* hybrids arising from independent hybridization events, suggesting that genome plasticity and noncanonical reproductive modes contribute to its evolution. Here we defined the conditions that promote yeast-to-hyphae transition in natural diploid strains of *M. furfur*, and found that the presence of a complete mating type system is a requisite for hyphal differentiation. We used these conditions to interrogate the genetic requirements for hyphal formation in both intra- and interspecific crosses of *M. furfur* haploid strains. Interspecific mating resulted in the generation of diploid heterozygous hybrids, some of which exhibited tracts of loss of heterozygosity consistent with recombination, while intraspecific mating resulted in cell-fusion products that undergo recombination and a process of haploidization that seems consistent with a parasexual cycle. Functional analysis of the *M. furfur* mating-type (*MAT*) genes revealed a role for the *MAT A (*pheromone/receptor, *P/R*) locus in cell recognition and fusion, and a role for the *MAT B (*homeodomain*, HD)* locus in hyphal development, although *MAT B* appears to have lost its mating-type determination function, as a single *HD* allele is sufficient for hyphal formation. Lastly, both in vitro and in vivo skin colonization models demonstrated that *M. furfur* hyphae display a greater capacity to invade the epidermis when compared than yeast cells, triggering a stronger inflammatory host response, indicating their contribution in pathogenesis. In conclusion, this study unveils a novel role of the *MAT* loci and mating in *Malassezia* leading to parasexual reproduction and the formation of hyphae that mediate pathogenic interactions with the host.

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Genetic variation in health and aging in spores isolated from *Schizosaccharomyces pombe*

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To survive in hostile environments, many organisms have evolved the ability to greatly decrease their metabolism and enter states of dormancy (e.g. diapause, hibernation). Interestingly, some aspects of aging are slowed during dormancy, and in multiple species, hibernators tend to live longer than non-hibernators. Many human cell types, such as neuronal stem cells, enter dormant states that are protective during injury and extend lifespan. Despite this prevalence, much remains to be learned about the molecular mechanisms of cellular dormancy.

Fungal spores represent a model to investigate cellular dormancy, as they are long-lived, metabolically inactive, and experimentally tractable. Here, we characterize spores produced by *Schizosaccharomyces pombe* and find that longevity and health of *S. pombe* spores declines over time and in response to heat stress. We characterize several traits associated with stressed and aged spores and use multiple assays (saturation mutagenesis screen, proteomics, and RNA-sequencing) to identify genes that either limit or promote spore longevity. We reveal parallels to aging cells in animals and important insights into a pervasive, yet understudied, cellular survival mechanism.

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A Bitter Tug-of-War: Exploiting fungal sexual reproduction for better blue cheese

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The fungus Penicilium roqueforti is used in the production of blue cheeses such as Stilton, Gorgonzola and Roquefort. All such cheeses go through a “bitter phase” during ripening, during which they are unpalatable. However, the timing, duration and final results of taste changes during this phase are highly variable, especially when using different strains of the fungus. Previous research has identified that small, hydrophobic peptides generated during cheese ripening are likely responsible for the bitter taste, but the exact chemical identities of these molecules in blue cheese is currently unknown. Linked to this, the genetic basis of variation in bitterness between different strains of the fungus P. roqueforti is poorly understood, which makes strain improvement challenging. There is also evidence that umami and salty tasting molecules can interfere with bitterness.

My work is investigating why P. roqueforti strains show different bitterness profiles, aiming to identify genes whose expression contributes to varying levels of bitterness in cheese products. An electronic tongue has been applied to investigate changes in taste over time in a cheese simulation medium, using

two strains known to generate more or less bitter cheese. This demonstrated how bitterness and umami levels vary between the two. Following this, 18 potentially-relevant candidate genes that might contribute to formation of bitter peptides have been identified and investigations into their expression in a cheese-like environment have been conducted. These include an aspartic protease AspA, which has already been identified as particularly important enzyme in the cheese maturation process, so investigations into its activity have been prioritised. Additionally, a Stilton cheese's taste and peptidome have been tracked over time using e-tongue and LC-MS-MS approaches to observe how maturation affects these qualities. Significant trends in taste were observed, and 938 unique casein peptide sequences were recorded and rated for likely bitterness using machine learning models. My current hypothesis is that the balance in expression levels of proteases and peptidases is responsible for determining bitterness levels in the final product. This is being investigated further using CRISPR Cas-9, qPCR, proteomic and e-tongue experimental approaches on both cheese simulation medium and actual cheeses.

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Evolution of complex life cycles in Pucciniales

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Complex life cycles—those that require abrupt prescribed ontogenetic changes, often including a change of host species—have arisen multiple times in the eukaryotic tree of life. Rust fungi (Pucciniales, Basidiomycota) are nearly unique in Fungi for evolving this life history strategy. Pucciniales are also the only plant pathogens with complex life cycles, which consist of two different thalli (the gametothallus and the sporothallus) and five functionally different spore stages. Intuitively, development of complex life cycles could be considered prohibitive for speciation. Yet, at ca. 8000 described species, the rusts constitute not only the largest natural group of phytopathogens, they also comprise the second largest known order of Fungi. In this study we attempt to understand how complex life cycles may have evolved in the rusts, and how the evolution of this strategy may have contributed to diversification in this lineage. The earliest diverging extant rust species, *Rogerpetersonia torreyae*, is only known from the gametothallus, which infects *Torreya californica* (Taxaceae). We generated genomic data for *R. torreyae* to infer whether it is also equipped to produce a sporothallus. We then used character mapping across the rust tree of life to infer when the different life cycle stages that constitute modern rusts evolved.

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Hidden in plain air: mapping airborne fungal resistance through citizen science

Eveline Snelders Wageningen University

The ecology of fungi remains largely an unexplored territory, despite their essential roles in Earth's critical processes. Infection rates differ substantially among fungal species with some causing disease far more frequently than others while, despite extensive research, virulence genes alone cannot explain this. Antifungal resistance also varies both between species and geographically within the same species. Air is a major transmission route for many pathogenic fungi affecting humans and other animals, yet the relative contribution of individual species, and their resistance profiles, to overall airborne exposure is rarely assessed. Although environmental factors such as urbanisation, land use, and climate strongly influence airborne fungal abundance, spore exposure remains understudied due to limitations in sampling methods. To address the lack of systematic large-scale air sampling data, we validated and implemented a new, cost-effective air-sampling method for *Aspergillus fumigatus*, a ubiquitous airborne opportunistic pathogen. Using a citizen-science approach, we collected samples throughout the Netherlands to examine how land use shapes airborne antifungal resistance. I will show the spatial patterns in airborne resistance to a common drug class, triazoles, and how land use classes drive these patterns. Not all high fungicide crops in the Netherlands showed a higher level of airborne resistance, but the greenhouse horticulture and flower bulb production land uses were major drivers. Because a selective agar medium is critical for the effectiveness of this low-cost method, we are now validating media for other Aspergilli and Mucorales species. New data show that, in the Netherlands, spore concentrations of *Aspergillus niger, Aspergillus flavus*, and *Mucorales* species are orders of magnitude lower than those of *A. fumigatus*. This difference in exposure may explain variations in which species cause the disease burden across geography. We can now uncover hidden patterns in transmission pathways by integrating air sampling with land use data. This flipped perspective embeds epidemiological thinking into studies of fungal ecology, virulence, and genomics. By making large-scale aerobiological surveillance feasible, we can extend this integrated, ecological strategy to any airborne fungus with a defined selective medium, uncovering previously unobserved fungal disease dynamics.

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Disentangling the evolutionary history of yeasts near its geographical origin

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The broadleaf Fagaceae forests of East Asia, particularly those in Taiwan, represent a key biogeographic origin of the baker's yeast *Saccharomyces cerevisiae* and a reservoir of extraordinary genetic diversity. Previous studies have shown that Taiwan harbours multiple ancestral lineages of *S. cerevisiae* predating human domestication, making it a natural refuge for early-diverging yeasts. Building on this foundation, we expand comparative population genomic analyses across multiple yeast species to elucidate their evolutionary histories and adaptive diversification in East and Southeast Asia.

We analysed multiple *S. cerevisiae*, *S. kudriavzevii*, *Lachancea thermotolerans* and many non-conventional yeast strains, including more than 300 newly sequenced genomes from Japan, Taiwan, the Philippines, and Vietnam. Phylogenomic reconstructions based on genome-wide SNPs and concatenated single-copy orthologues reveal that basal lineages of all three species persist in Taiwan, reinforcing its role as a refugium. The most divergent *L. thermotolerans* lineage exhibited pronounced genetic differentiation (Fst= 0.81–0.89) from other subpopulations, mirroring the deep divergence patterns observed in *S. cerevisiae*. These results indicate that not only *S. cerevisiae* but also non-*Saccharomyces* yeasts likely originated and diversified in East Asia.

Preliminary phenotypic assays across isolates from different climatic regions revealed clear patterns of thermotolerance variation that parallel genomic differentiation. *S. cerevisiae* and *L. thermotolerans* strains from tropical lowlands (e.g., the Philippines and southern Taiwan) exhibited higher growth

efficiency at elevated temperatures (35–37 °C), whereas northern or montane populations showed better performance under slightly cooler conditions. Genome–environment association analyses suggest that genes related to membrane composition, stress response, and mitochondrial function may underlie these local adaptations. Together, these findings suggest that temperature gradients across East and Southeast Asia have been a major selective force driving parallel adaptation in multiple yeast lineages.

By integrating high-resolution genomic and phenotypic data across species, this study provides a comprehensive perspective on yeast evolution in natural ecosystems, further establishing East Asia as a global centre of yeast biodiversity and adaptation.

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Starship transposons and the makings of a new framework to study fungal evolution

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Starships are a recently discovered superfamily of giant transposons found across hundreds of fungal species. *Starships* are fundamentally unlike typical eukaryotic transposons because they span hundreds of kilobases and mobilize dozens of genes, including those encoding phenotypes ranging from stress resistance to host-microbe interactions. Although we have known about the existence of *Starships* for only five years, sustained efforts and collaborations are rapidly establishing systems to move from identifying genomic patterns to characterizing the genetic processes underpinning *Starship* biology, including their roles as vectors of fungal-to-fungal horizontal gene transfer. We argue that the little we know about *Starships* already raises important questions about the mechanisms of adaptation across medically, agriculturally and economically important groups of fungi. Drawing on examples of *Starship* activity spanning individual species to entire phyla, we build on existing models of fungal genome biology to propose that *Starships* are a distinct genomic compartment whose dynamics must be considered to fully understand and predict the mode and tempo of fungal evolution.

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The shattered genome of *Basidiobolus*: a fungus from the gut of reptiles and amphibians

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Basidiobolus is a zygomycete fungus best known for being associated with the gut microbiome of amphibians and reptiles. Previous reports of two *Basidiobolus* species suggest genomes of ~100 Mb with more secondary metabolism genes than other zygomycetes. A significant proportion of these genes exhibit evolutionary relationships with non-ribosomal peptide synthases (NRPS) from Bacteria, including genes annotated to produce cyclosporin, surfactins, and siderophores. These evolutionary relationships are consistent with many of these genes being acquired by horizontal gene transfer (HGT) from bacteria. In addition to the complexity of the HGT in *Basidiobolus*, the genome assemblies suggest there are hundreds of chromosomes in each of the genomes, resulting in a fragmented architecture. Here, we present an analysis of the genome architecture of different *Basidiobolus* reference genomes we have assembled and strains we isolated from various species of amphibians and reptiles. Across eight isolates, genome size varied from 58 Mb to 143 Mb, with hundreds of scaffolds containing telomeric sequences. Genome-wide k-mer analyses revealed ploidy levels greater than haploid, and chromosome-level patterns indicated ploidy variation ranging from diploid to triploid to tetraploid, consistent with complex patterns of duplication and aneuploidy. *Basidiobolus* genomes exhibit an average repetitive sequence content exceeding 50% of their total length. Analysis of the age of the classes of transposable elements by Kimura-2 distances support multiple waves of invasion and expansion of the genome at different times and, Long Interspersed Nuclear Elements (LINEs) as one of the most relatively recent. Furthermore, LINEs were detected to be nonrandomly distributed with a bias for insertion within proximity to telomeric ends. This pattern within subtelomeric regions suggests that LINEs contribute a mechanism of fragmentation in *Basidiobolus* genomes, promoting the shattering of the genome into numerous small chromosomes. Our work focuses on establishing the baseline knowledge of *Basidiobolus* genome evolution, highlighting genome content characterized by HGT and genome architecture marked by complex ploidy and influenced by transposable elements.

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Harnessing Filamentous Fungi for Biomanufacturing of Animal-Free Milk Proteins

Lucy Sullivan 21st.Bio

Conversations surrounding alternative proteins are on the rise as global communities are working to address growing issues such as food insecurity and climate crisis. We, at 21st.Bio, are addressing the rising demand for nutritional and functional proteins by producing recombinant milk proteins in *Aspergillus oryzae* and *Aspergillus niger* using a state-of-the-art expression platform. This technology, coupled with the powerful platform of precision fermentation, allows us to offer alternative products that could supplement the protein market. Multiple generations of strain engineering have been made possible with our advances in developing an automated strain construction workflow, which has enabled us to generate strains that are producing more protein while still contributing fewer carbon emissions than traditional means of purifying these same proteins from milk. These advances in our high-throughput strain construction are being coupled with the ongoing development of a high-throughput screening system set up to improve the prediction of strain performance in bioreactors.

This talk will focus on recombinant β-lactoglobulin (BLG) as an alternative source of protein and the learnings from our journey starting with strain engineering all the way to 50m^3 scale-ups.

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Production of hen egg ovalbumin by *Trichoderma reesei* using precision fermentation technology and testing of its techno-functional properties

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Egg white protein ovalbumin is a highly functional food ingredient, yet replacing it with plant proteins is challenging due to its unique structural and functional properties. To provide a non-animal source of ovalbumin, we utilized the fungal host *Trichoderma reesei* for the biotechnical production of recombinant hen ovalbumin (TrOVA). The protein was expressed using both the strong synthetic SES promoter and the native *cbh1* promoter, reaching up to 2 g/L in lab scale bioreactors. The SES promoter strain produced fewer background proteins, simplifying downstream purification and providing higher product purity.

Biochemical studies revealed that TrOVA degradation was caused by a specific endogenous protease, which was identified and removed from the *T. reesei* genome, enabling stable accumulation of intact protein. Following purification, the TrOVA amino acid sequence and molecular weight were verified using mass spectrometry. Glycan analysis showed that TrOVA carried N-glycans typical of *T. reesei* proteins, consisting of mannose chains $Man_{5\text{-}11}$ linked by GlcNAc residues. Although minor differences in glycosylation were observed compared to native ovalbumin, overall similarity remained high, with $(Man)_5(GlcNAc)_2$ identified as the predominant glycoform shared by both.

Purified TrOVA displayed native secondary structure, excellent foaming characteristics, and heat-induced gelation. These results demonstrate that *T. reesei* is a promising host for precision-fermentation-based production of functional egg proteins.

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The impact of developmental structures on pure mycelial materials made from *Aspergillus nidulans*

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The vast majority of the world's manufactured materials are nonrenewable and energy-intensive to produce. Viable alternatives must be developed, and "mycelial materials" made from filamentous fungi are an exciting option. Companies manufacturing various mycelial materials (e.g., textiles, food products, packing foams, building supplies) have attracted hundreds of millions in commercial investment, and the global market is predicted to soon exceed $5 billion. Yet, the current approach to modifying material properties is typically through trial-and-error, using different fungal species, altering nutrients used for growth, or changing the growth environment. Because it's unclear how these inputs lead to *specific* changes in material properties, advances have been limited. To address this limitation, we are exploring the use of genetic approaches to modify fungi to be used in materials. Our central hypothesis is that genetic means can be used to tune fungal morphological phenotypes, and that these phenotypes can be combined to enable the design of mycelial materials with specific sets of material properties. Initial studies have involved the model fungus, *Aspergillus nidulans*. We initially hypothesized that disabling the cell wall integrity (CWI) signaling pathway, which leads to weaker-walled hyphae, would result in weaker material. Surprisingly, material from an *mpkA* deletion strain (D*mpkA*) was significantly stronger than material generated from an isogenic control. Comprehensive phenotype testing implied increased material strength was due to lack of fungal developmental structures. Subsequent experiments, with several developmentally-disabled strains (D*brlA*, D*flubA*, D*fluG*) confirmed this.

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A fungal community driven development of the oCelloScope™ image analysis platform to quantify growth using deep learning in Medical-, Crop Sci-, Food research and Strain Development

Soren Busch BioSense Solutions

Through years of strategic collaboration with mycology professionals in both academia and industry, the oCelloScope live-cell imaging platform has emerged as a comprehensive tool for analysing fungi, yeasts, and oomycetes across diverse applications from plant pathology to industrial strain development. Through this community-driven development, the technology combines a patented non-invasive time-lapse imaging system with advanced deep learning modules that recognize, count, and track spores, conidia, and mycelial networks in standard microtiter plates and on agar surfaces.

In this presentation, recent assay developments will be highlighted, including a no-stain live/dead assay for yeast and deep-learning-based workflows that quantify susceptibility of oomycete plant pathogens such as Plasmopara viticola (downy mildew) and Phytophthora infestans (late blight) in 96-well formats. Additional assays capture key stages of fungal development, from spore germination kinetics and early hyphal outgrowth to branching patterns and mycelial expansion, enabling high-throughput screening of antifungals, biological control agents, and engineered production strains in both "clean" and "dirty" samples.

Compared to conventional microscopy and optical density measurements, the oCelloScope platform provides faster readouts, automated image analysis, and robust handling of non-uniform fungal morphologies, while preserving cells under native growth conditions. These capabilities have already been applied in multiple peer-reviewed fungal studies and BioAg development projects, supporting rapid assay transfer from research to applied settings. The

aim of this presentation is to engage the scientific community in the ongoing development of essential assays for quantification of fungal processes, leveraging our technological capabilities alongside the extensive expertise available at Asilomar.

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Building the Future of Roads: Biotechnological Optimization of Mycelium-Based Binder

Line Kloster Pedersen, Oleksii Rebrov Visibuilt

Fungal biotechnologies are advancing and finding applications across many sectors. One rapidly growing area is the development of mycelium-based materials. While these materials have been explored in controlled, non-load bearing applications, their use as load-bearing materials in outdoor conditions with continuous mechanical and environmental stress remains challenging.

The motivation for this work comes from the growing need in the road industry to find alternatives to petroleum-based binder, bitumen, and to reduce the high CO_2 emissions linked to concrete production. We developed a mycelium-based binder, visiBINDER, which leverages the natural ability of fungal mycelium to cohesively bind the material it grows through. This biogenic binder offers significant advantages, lower energy consumption in pavement production, independence from fossil-based raw materials, and improved safety for paving workers.

In this work, we present the development of the mycelium-based binder for pavement construction, including the process of screening and selection of suitable fungus–substrate combinations, advances in fermentation optimization, binder integration into mineral aggregates, and the first industrial implementation of visiBINDER in an asphalt production plant with the results from real use-case testing.

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Deciphering the Chemical Language of the Fungal-Microbiome Interactions using Computational Omics

Marnix H Medema Bioinformatics Group, Wageningen University

Fungi and bacteria produce a wealth of specialized metabolites, which play important roles in ecology and provide a rich resource for natural product drug discovery. Genome sequence data has revealed that only a tiny fraction of the chemical diversity of these natural products has been unearthed. Here, I will highlight recent work performed in my research group and with the wider community on developing and applying computational and artificial intelligence approaches to accelerate natural product discovery using omics data. Specifically, I will highlight new methods to chart biosynthetic diversity, to predict chemical (sub)structures of metabolites directly from sequence data, to retrobiosynthesise chemical substructures into their constituent building blocks, and to then connect biosynthetic gene clusters to molecules based on this information. Furthermore, I will highlight new methods to predict functions of natural products based on various types of omics data, to aid in the prioritization of the most high-potential areas within biosynthetic space. To show the tangible value of these computational approaches, I will highlight recent work from our group on fungal biosynthetic diversity across global populations, and elucidating molecular mechanisms of bacterial-fungal interactions in the plant rhizosphere.

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A high(er) throughput method for using CRISPR/Cas9 to delete genes from *Coccidioides posadasii*

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Coccidioides posadasii and *immitis* are human fungal pathogens endemic in the American southwest. *Coccidioides spp.* are thermally dimorphic, growing as a hyphae under environmental conditions and a large, endospore filled spherule structure under host conditions. *Coccidioides spp.* are the causative agents of coccidioidomycosis, or Valley fever. They are pathogens of growing concern as reported cases of coccidioidomycosis have increased 20-fold in the last two decades. Despite their importance as pathogens, *Coccidioides spp.* are understudied, especially compared to other human fungal pathogens with similar infectious burdens. The reasons *Coccidioides spp.* are understudied are multifactorial, including requirement of high biocontainment, technical difficulties in lab-based culture growth, and a historic lack of genetic tools. Previous methods of genetic manipulation have been technically challenging, time-consuming, and inefficient. Updated methods to delete genes in *Coccidioides* are required to help identify genes important to virulence, the dimorphic shift from environmental to pathogenic phase, and resistance to anti-fungal drugs. We have developed reproducible and affordable methods of making high-quality protoplasts, or competent cells, using enzymes used in the wine-making industry. We also developed a high-efficiency method to delete genes from *C. posadasii* using homology-directed repair and CRISPR/Cas9 guides. To test the effectiveness of these methods, we selected 12 genes with hypothesized roles in anti-fungal resistance, virulence, and the dimorphic transition from hyphea to spherule. We deleted the genes and replaced each gene with a hygromycin resistance markers. We found that the transformation methods were highly efficient with 90% of hygromycin resistant colonies showing presence of the hygromycin gene. 60% of colonies showed evidence of gene deletion and integration of the repair construct in the targeted location. The 12 deletion mutants had varying phenotypes including changes to growth rate, virulence, and ability to make spherules. Overall, the protocols presented here will increase the efficiency and speed of research occurring in the *Coccidioides* field and open opportunities for novel studies and discoveries.

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Empowering Fungal Research: Cultivarium tools to accelerate study and engineering of non-model fungi

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Cultivarium, a not-for-profit research organization, is aiming to accelerate discovery in diverse non-model fungi by developing high throughput open access tools for the research community. Our goal is to provide inexpensive and accessible protocols, software, and devices to overcome challenges in fungal

cultivation, genetic manipulation, and data accessibility. For cultivation, we are developing an affordable incubator-imager designed to capture time-lapse images of fungal morphologies under standard conditions. Such visual reference databases will make it possible to rapidly compare and validate samples across labs and growth conditions. For fungal genetics, Cultivarium is developing high throughput screens to identify protocols for DNA delivery. In our preliminary results, we demonstrate successful electroporation into several established model fungal species. This success paves the way for applying the approach to novel non-model organisms. These organisms either pose potential threats to public health or possess valuable intrinsic properties, such as unique metabolic pathways or bioactive compounds.We offer free access to our organism portal and data search software tools. Finally, we offer access to our AI-guided protocol video-capture system called Protocol Recording for Improved Scientific Methods (PRISM) that helps reproduce, distribute and train on fungal lab techniques. By making our tools accessible to the fungal community, we hope to lower the barrier for genetic tractability in new fungal species, and empower researchers with accelerated research to discover new fungal biology, prepare for emerging fungal pathogens, and enable new fungal biotechnology applications.

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Light regulates alternative splicing and cell wall remodeling in *Trichoderma guizhouense*

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Light, as one of the most pervasive environmental cues, is perceived by photoreceptors in fungi and further plugs into the stress-activated MAPK HOG pathway to regulate many physiological and morphological processes[1]. In *Trichoderma guizhouense*, blue light can promote conidiation, and blue light signaling depends on the blue light receptor BLR1 and MAPK HOG1[2]. Recently, we found that light signal can induce alternative splicing through the photoreceptors and HOG pathway in different fungi, and blue light induced-alternative splicing in *T. guizhouense* regulates ergothioneine biosynthesis and conidiation[3]. We demonstrate that a putative serine/threonine protein kinase SRK1 interacting with HOG1 regulates the alternative splicing induced by light. On the other hand, we found that blue light exposure can induce a marked thickening of the cell wall in *T. guizhouense* as part of its photoadaptive response.

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A Guardian of the Cell: How Cryptochrome Balances Stress Responses in *A. nidulans*

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Cryptochromes are photoreceptors with functions in the entrainment of circadian clocks or as proposed magnetoreceptors in birds or as light-independent regulators of stress responses in plants. Here, we show that the fungal cryptochrome-like photolyase CryA from *Aspergillus nidulans* is induced by light and by oxidative stress and establishes negative-feedback loops (NFL) for light- and stress-activated genes. The NFL depends on CryA interaction with phytochrome and the HOG (high osmolarity glycerol) pathway transcription factor AtfA in nuclei. CryA translocated in less than one minute from nuclei to mitochondria in the presence of hydrogen peroxide suggesting mitochondrial functions and possibly mitochondrial-nuclear communication. The shuttle to mitochondria depended on an N-terminal extension (NTE) and a cysteine therein, which probably induce conformational changes of CryA upon oxidation. We propose CryA as sensor for oxidative stress. Such an NTE is also present in other photolyases and some cryptochromes, suggesting evolutionary conservation of the mechanism.

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Evaluation of a candidate MAT locus for *Linnemannia elongata* (Mortierellomycotina) using quantitative PCR methods

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Mortierellomycotina is a subphylum of fungi with beneficial, non-mycorrhizal interactions with plant roots. These ubiquitous organisms are sexual and produce meiospores (zygospores) with compatible partners or in a homothallic manner, depending upon the species. For the related Mucoromycotina (Mucoromycota), sexual compatibility is regulated by two idiomorphic high-mobility group (HMG) transcription factors (TFs), and involves the exchange of metabolic derivatives of beta-carotene, resulting in a functional, small organic pheromones called trisporic acids (TSAs). In the Mucoromycotina, the mating-type (MAT) locus structure underlying sexual compatibility is well described, yet the genetic source of compatibility in Mortierellomycotina has remained elusive to BLAST-based search approaches. Previously, we leveraged genomic data from *Linnemannia elongata* isolates with known compatibility interactions to identify candidate MAT loci for Mortierellomycotina. This candidate MAT locus contains either a HMG TF or homeodomain (HD) TF, and TF content correlates well with zygospore production data. We hypothesize that MAT locus TFs will be highly transcribed in sexual mating interactions compared to the same isolates cultured alone. To test this hypothesis, we collected mRNA and quantified MAT locus TF transcript abundance from two *L. elongata* isolates (JKU1 and JKU4) in monoculture and in coculture, using novel primers. Here, we present the preliminary results from these qPCR assays and discuss future directions for elucidating Mortierellomycotina sexual reproduction.

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DNA damage tolerant colonies of a fungal plant pathogen acquire mutator phenotype

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Fungi contribute disproportionately to emerging diseases, partly due to their remarkable ability to adapt to new environments and fungicides. While it is known that DNA damage induces mutations that lead to adaptation to new environments, very little is known about this process in fungal pathogens. To address this gap, we investigated the response of the fungal plant pathogen *Fusarium oxysporum* to UV irradiation.

Upon UV exposure of spores, we observed a distinct subpopulation that failed to develop even after 48 hours. When separated by size, this arrested population displayed reduced plating efficiency and increased mutation rate. This subpopulation showed the most dramatic response to UV at the transcription and phosphoproteome levels ever reported in yeast or fungi. When sequencing the entire genome of colonies that survived UV we found that applying selection pressure results in higher UV-induced mutation frequency.

Unexpectedly, ~10% of the surviving colonies failed whole genome sequencing attempts. Strikingly, these colonies carried persistent DNA damage, with up to 60% of the lesions originally inflicted on the total spore population still detectable—even after regrowth. This raises a fundamental question: how can colonies with such extensive DNA damage sustain replication and propagation and what are the mutagenic and evolutionary implications of this phenomenon. Interestingly, daughters of UV survival colonies showed higher mutation density than their parents, indicating that DNA damage tolerant colonies are a mutators that can drive adaptation to stress.

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Circadian clock control of translation fidelity in Neurospora crassa

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tRNA synthetases (RS) charge cognate tRNAs with the correct amino acids are key regulators of translation accuracy (translation fidelity), and we discovered that the levels of several tRNA synthetases, including the methionyl-tRNA synthetase (MetRS), are regulated by the clock in *Neurospora crassa* and peak during the subjective night. In mammalian cells and *Saccharomyces cerevisiae*, MetRS is phosphorylated by the mitogen-activated protein kinases, ERK1 and ERK2, when cells are exposed to oxidative stress. MetRS phosphorylation reduces the specificity of MetRS for tRNAMet leading to the charging of methionines (Met) to non-cognate tRNAs (Met-misacylation) and subsequent Met-misincorporation into polypeptides. These non-coded Met residues in polypeptides sequester reactive oxygen species (ROS) to help cells overcome oxidative stress. Because ERK1/2, as well as ROS levels, are clock-controlled in *N. crassa*, we hypothesized that the clock regulates the fidelity of translation through Met-misacylation, in part, to help cells overcome daily rhythms in ROS levels. To test this idea, we used a GFP-mCherry dual reporter to monitor the rates of Met misincorporation in *N. crassa*. We discovered that Met-misincorporation is clock-controlled with a peak during the night which is the time of day when internal ROS levels are high in cells. We found that rhythmic Met misincorporation is mediated by rhythmic P-MetRS and MAK1. Additionally, we are interested in understanding if Met misincorporation rhythms alter proteome function. To investigate this, we mined a rhythmic proteomics dataset to identify proteins with rhythmic Met misincorporation and identified 237 proteins with rhythmic Met misincorporation and are following up on these candidates. Taken together, these data supported that clock-controlled Met-misincorporation in polypeptides helps to not only protect the organism from circadian oscillations in ROS, but also to potentially extend the functional coding capacity of the genome providing an exciting mechanism of clock-regulation of the proteome.

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Circadian clock is critical for regulating pathogenicity in *Fusarium oxysporum*

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Circadian clocks have evolved to enhance the fitness of organisms by improving their ability to adapt to daily environmental changes. Circadian clocks are known to modulate host immune responses to pathogen infections, yet their role in influencing pathogen pathogenesis remains unclear. To investigate the specific role of the circadian clock in pathogenic fungi, we examined the presence of a functional circadian clock in *Fusarium oxysporum,* which has multiple circadian clock genes *frqs* due to gene duplication events, with *Fofrq1* being the primary circadian clock gene. The pathogenesis of *F. oxysporum* in plants is controlled by its circadian clock, with infections causing severe disease symptoms at dawn. Notably, disruption of clock genes dramatically reduces fungal pathogenicity. Mechanically, circadian clocks regulate fungal-plant interactions by controlling the expression of pathogenic factors, including FoZafA, which enables the pathogen to adapt to zinc starvation within the plant, and FoCzf1, which governs the production of the toxin fusaric acid. In summary, we elucidated the important function of the circadian clock in modulating the pathogenicity of *F. oxysporum* by regulating zinc starvation response and secondary metabolite production.

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Evidence that Entomophthora muscae controls the timing of host death via its own circadian clock

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Timing in the natural world is a matter of life or death, consequently, nearly all life on Earth has evolved internal circadian clocks. Many behavior-manipulating parasites exhibit striking daily timing, but whether this is clock-driven has remained unclear. Here, we leveraged the laboratory-tractable zombie fruit fly model, *Drosophila melanogaster* infected by the behavior manipulating fungus *Entomophthora muscae*, to tackle this long-standing mystery. Using an automated behavioral paradigm, we found that the timing of death of wild-type flies continues to occur with ~21 hour periodicity in the absence of environmental cues. Surprisingly, we also discovered that light is required within the first 24 hours of exposure for *E. muscae* to infect and kill flies. Experiments with circadian and photoreception mutants revealed that death is independent of host genotype, suggesting the fungus—not the host—drives this rhythm. Transcriptomic analysis of *in vitro* grown fungus revealed that *E. muscae* maintains rhythmic gene expression independent of the fly host that peaks at sunset and has a free-running period of ~22 hours. Among cycling genes, we identified a transcript encoding a protein with high homology to *white collar-1*, the blue light sensor and core component of the molecular oscillator in the model ascomycete fungus *Neurospora crassa*. Altogether, our findings suggest that *E. muscae* has an endogenous circadian clock that it uses to control the timing of host death. This study provides evidence that a fungal clock can influence fly outcomes, pointing to a new mechanism by which parasites temporally coordinate host manipulation.

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Illuminating transcriptional dynamics underlying circadian clock regulation and photoentraiment.

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Circadian clocks rely on transcriptional-translational feedback loops coordinated by cis-regulatory elements. In Neurospora crassa, the frq promoter is key to circadian regulation, yet its complex structure obscures the functional contributions of individual elements. To address this, we engineered Minimal Synthetic Oscillators (MiSOs) composed of various configurations of the frq promoter's two core cis-elements: the c-box (clock-controlled) and the pLRE (light-responsive). Using luciferase reporters and frq-luc fusion constructs in an arrhythmic background lacking the endogenous promoter, we systematically evaluated how promoter architecture influences rhythmicity, phase, light responsiveness, and temperature compensation.

MiSOs containing only the c-box and pLRE, with or without a core promoter (CP), generated robust oscillations, though performance varied by element arrangement and dosage. Constructs with two c-boxes enhanced light responsiveness and phase tuning. Most MiSOs preserved key circadian properties, including temperature compensation and the ability to synchronize downstream output pathways. However, light-pulse experiments revealed that promoter design determines not only the direction (advance vs. delay) of phase shifts, but also the oscillator's sensitivity to light duration and timing.

Our results show that minimal sets of cis-elements are sufficient to reconstitute key features of circadian regulation, and that promoter architecture exerts fine control over phase, amplitude, and environmental responsiveness. MiSOs provide a powerful synthetic biology platform for dissecting transcriptional control within biological oscillators and may offer new strategies to reprogram clock function.

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Aspergillus fumigatus conidial surface-associated proteome reveals factors for fungal evasion and host immunity modulation

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Aspergillus fumigatus causes aspergillosis and relies on asexual spores (conidia) for initiating host infection. There is scarce information about *A. fumigatus* proteins involved in fungal evasion and host immunity modulation. Here we analysed the conidial surface proteome of *A. fumigatus*, two closely related non-pathogenic species, *Aspergillus fischeri* and *Aspergillus oerlinghausenensis*, as well as pathogenic *Aspergillus lentulus*, to identify such proteins. After identifying 62 proteins exclusively detected on the *A. fumigatus* conidial surface, we assessed null mutants for 42 genes encoding these proteins. Deletion of 33 of these genes altered susceptibility to macrophage, epithelial cells and cytokine production. Notably, a gene that encodes a putative glycosylasparaginase, modulating levels of the host proinflammatory cytokine IL-1β, is important for infection in an immunocompetent murine model of fungal disease. We also characterized CyrA (a cysteine-rich protein), as a secreted protein important for the induction of pro-inflammatory cytokines and virulence in an immunocompetent murine model of fungal disease. *A. fumigatus* wild-type exposed to murine macrophages induces the production of eicosanoids/prostaglandins EPA2, PGE2, PGD2, and 15-oxo-ETE. The Δ*cyrA* mutant has increased levels of PGE2 and PGD2 suggesting CyrA is involved in their modulation. High CyrA-specific IgG responses were found in antisera from individual patients with invasive pulmonary aspergillosis, suggesting a role for CyrA in *A. fumigatus* pathogenesis. These results suggest that *A. fumigatus* conidial surface proteins are important for evasion and modulation of the immune response at the onset of fungal infection.

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The immune system of the mushroom *Schizophyllum commune*: identification of transcriptional regulators and effectors

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To survive in challenging ecological niches, fungi must deploy robust defense mechanisms against their competitors. While the chemical arsenal of mushroom-forming fungi is increasingly understood, considerably less is known about the regulation of the defense response. Here, we dissect the immune system of the model mushroom *Schizophyllum commune*, linking upstream transcriptional regulation to downstream effector activity.

Using transcriptomic profiling of *S. commune* during direct interaction with bacterial and fungal antagonists, we identified a substantial conserved reprogramming of gene expression. This involved the upregulation of 632 genes, including cytochrome P450s, glutathione S-transferases and lectins with a Ricin B domain. We also identified a group of three closely related genes encoding ABC-transporters, suggesting that the xenobiotic efflux mechanisms well-characterized in ascomycetes are conserved and pivotal in the basidiomycete defense strategy.

Beyond these conserved mechanisms, we identified numerous small secreted proteins, including CAZymes and novel effectors. Among these identified effectors, we characterized Tip8, a novel secreted CAZyme of the AA7-family, which exhibited potent oxidative antifungal activity. Furthermore, four additional novel proteins (Tip1, Tip3, Tip7, and Tip13) demonstrated significant antimicrobial activity, confirming their roles as frontline defense effectors.

To decipher the regulatory mechanism driving the defense response, we targeted transcription factors (TFs) Gat1, Fst8 and Ftr3, which are upregulated during interaction. Functional characterization of the deletion strains (Δ*gat1*, Δ*fst8*, Δ*ftr3*) demonstrated severely impaired defense, evidenced by significantly enhanced colonization by microbial antagonists. Transcriptomic analysis of these mutants identified a core set of 77 defense-associated genes

that failed to be upregulated in the absence of these TFs. This encompasses genes encoding secreted proteins, including three predicted thaumatins, which in plants are characterized as antifungal compounds.

Collectively, these data uncover both the regulatory and the effector machinery of *S. commune*. This fundamental insight into the fungal immune system offers critical targets for enhancing stress resilience and reducing crop losses in mushroom cultivation.

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Genetic Basis for a Bacterium Acting as a Toxin Sponge to Protect its Fungal Partner from Phenazines

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Phenazine-1-carboxylic acid (PCA) is a bacterially produced redox-active antifungal that is abundant in many environments. It was recently discovered that PCA-susceptible fungi, such as *Aspergillus calidoustus*, can gain access to PCA-abundant environments by physically associating with native phenazine-tolerant bacteria, such as the novel species *Paraburkholderia edwinii*. *P. edwinii's* protection response is characterized by the sequestration and reduction of PCA, rendering PCA less toxic to the fungus. Although *P. edwinnii's* protection response has been characterized, little is known about the molecular mechanisms involved. The deletion of the transcriptional repressor, *hrcA*, in *P. edwinii* enhances the protection response and allows for increased fungal growth, suggesting HrcA's involvement in protection response pathways. An RNA-seq comparing the Δ*hrcA* and wild-type strains of *P. edwinii* revealed that putative Csu-type pilus subunits (*csuA* and *csuB*) are upregulated in the Δ*hrcA* background. *csuA* and *csuB* are the first and last genes in a putative chaperon-usher pathway operon, respectively. The Δ*csuA* deletion strain and the double deletion strain Δ*csuA* Δ*csuB* are defective in protecting the partner fungus from PCA, as demonstrated by a significant decrease in total co-colony area. Moreover, the role of CsuA and CsuB is specific to the protection of the fungal partner and not a result of a bacterial growth defect in our conditions. Preliminary data indicates that Csu-type pili are involved in both fungal association and PCA detoxification. The Csu-type pili are required for bacterial adhesion to *A. calidoutus* spores in the presence of PCA, indicating these pili play a role in fungal partner association. Additionally, the Δ*csuA* and Δ*csuA* Δ*csuB* strains failed to produce the same low redox potential that wildtype uses to detoxify PCA, suggesting a defect in reducing toxic molecules. Understanding how *P. edwinii* utilizes Csu type-pili to associate with and protect its fungal partner from PCA will further our knowledge of the mechanisms used by protective microbial partnerships to gain entry to AMC-abundant communities.

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Phytoalexins as host cues: convergent evolution of detoxification mechanisms in polyxenous pathogens *Botrytis cinerea* and *Colletotrichum tropicale*

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Throughout evolution, plants have acquired chemically diverse antimicrobial phytoalexins that limit the host range of fungal pathogens. In contrast, some fungi exhibit remarkably broad host ranges, such as *Botrytis cinerea*, which can infect more than 1,600 plant species, a trait referred to as polyxeny. However, how such polyxenous pathogens overcome diverse phytoalexins remains unclear. In this study, we investigated the mechanisms by which *B. cinerea* and the distantly related polyxenous fungus *Colletotrichum tropicale* cope with and detoxify host phytoalexins. Both fungi showed high tolerance to various phytoalexins, such as the sesquiterpenoids capsidiol (chili pepper) and rishitin (potato), the triterpenoid tormentic acid (strawberry), and the phenylpropanoid methyl trans-p-coumarate (cucumber). Each compound was metabolized into less toxic derivatives in both fungi, suggesting that their resistance is associated with active detoxification. RNA-seq analysis of both fungi after treatment with the four phytoalexins revealed that each compound induced a distinct set of genes. Notably, capsidiol induced specific ADH (alcohol dehydrogenase)-like genes in both species. Functional analyses showed that these ADHs are essential for the dehydration-mediated detoxification of capsidiol to the less toxic capsenone and for pathogenicity on capsidiol-producing hosts. Despite catalyzing the same detoxification reaction, phylogenetic analysis revealed that the ADHs in *B. cinerea* and *C. tropicale* are not orthologous but originated from completely different evolutionary lineages, supporting convergent evolution toward a common functional solution. This suggests that capsidiol can act as a strong selective pressure on fungal pathogens. Furthermore, the timing of the acquisition or lineage-specific expansion of capsidiol-detoxifying ADH in the *Colletotrichum* lineage is consistent with the emergence of capsidiol biosynthesis in Solanaceous plants. Taken together, these findings suggest that independently evolved polyxenous pathogens may have convergently established an infection strategy in which phytoalexins are perceived as host-derived chemical signals that in turn activate distinct gene sets in a host-dependent manner.

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The emerging fungal pathogen *Candida auris* induces IFNγ to colonize mammalian hair follicles

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Public health alarm concerning the emerging fungus *Candida auris* is fueled by its antifungal drug resistance and propensity to cause deadly outbreaks. Persistent skin colonization drives transmission and lethal sepsis although its basis remains mysterious. We compared the skin colonization dynamics of *C. auris* with its relative *C. albicans*, quantifying skin fungal persistence and distribution and immune composition and positioning. *C. auris* displayed a higher propensity to colonize hair follicles and avidly bound to human hair. While *C. albicans* triggered an effective sterilizing type 3/17 antifungal immune response driven by IL-17A/F-producing lymphocytes, *C. auris* triggered a type 1, IFNγ-driven immune response targeting hair follicles. Rather than promoting fungal clearance, IFNγ enhanced *C. auris* skin colonization by acting directly on keratinocytes impairing epithelial barrier integrity and repressing antifungal defense programs. *C. auris* exploits focal skin immune responses to create a niche for persistence in hair follicles.

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Identification and functional validation of avirulence effectors in wheat rusts

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The identification of avirulence genes (*Avr*) in plant pathogenic fungi remains a major bottleneck for understanding and ultimately mitigating the diseases these pathogens cause. Accurate effector annotation is particularly challenging as these genes often lack sequence homologs, reside in rapidly evolving

genomic regions, or represent single-exon genes, which leads to their exclusion by most annotation pipelines. Using EffectorGeneP, a self-training machine learning tool that leverages infection transcript data, we identify effector candidates and generate libraries to submit to a protoplast-based high-throughput screen that enables the characterisation of novel *Avr* genes. The power of this approach has been previously demonstrated with the increased number of isolated *Avr* genes in the wheat stem (black) rust fungus *Puccinia graminis* f. sp. *tritici*, contributing to the construction of an *Avr* gene atlas for the species. Here we report the identification of at least four *AvrSr26* loci through this approach, likely explaining why *Sr26* has yet to be overcome in the field. This not only constitutes a new *Avr* discovery for stem rust but also reveals a diverse and previously poorly annotated gene family. All *AvrSr26* variants were functionally validated by transient expression in wheat protoplasts, virus-mediated expression in intact wheat plants and agroinfiltration assays in *Nicotiana benthamiana*. In addition to this, we report our progress toward the identification of *Avr* effectors in *P. striiformis* f. sp. *tritici*, the causal agent of wheat stripe (yellow) rust. These advances mark an important milestone in dissecting stripe rust pathogenicity and demonstrate the broader applicability of our approach to other high-priority pathogens.

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Identification and characterization of *Cercospora beticola* effector candidates associated with adaptation to the sugarbeet resistance gene *BvCr4*

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Cercospora beticola, the causal organism of Cercospora leaf spot (CLS), is the most economically important disease of sugarbeet (*Beta vulgaris* subsp. *vulgaris*). Growers have recently utilized varieties that contain *BvCr4* that mediates CLS resistance. However, resistance breaking *C. beticola* populations have been found worldwide. A European study identified the gene, *AvrCr4*, associated with overcoming *BvCr4*-mediated resistance, but this association has not been examined in American populations. We conducted a population genomic analysis with *C. beticola* collected from American regions with and without *BvCr4* and identified 14 fungal genes putatively associated with breaking *BvCr4*, including *AvrCr4*. Functional validation of candidate genes indicated that *C. beticola* mutants lacking *AvrCr4* are more virulent on plants with *BvCr4* than the progenitor isolate. To explore how *C. beticola* populations are rapidly evolving to circumvent *BvCr4*-mediated resistance, we investigated how a single fungal isolate behaves under strict selective pressure. We conducted a passage experiment by inoculating a single *C. beticola* strain harboring *AvrCr4* onto three sets of 1500 sugarbeet plants. Whole genome sequencing showed that the locus containing *AvrCr4* is missing from later generations of isolates. The mechanism responsible for removing the locus is under investigation. Overall, this research aims to elucidate the interaction between *C. beticola* and the CLS resistance gene *BvCR4* to improve understanding of the *BvCr4* resistance phenotype and predict durability of similar resistance genes.

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Spp1-mediated processing of substrate proteins promotes plant defence suppression and virulence of fungal biotrophs

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In the smut fungus *Ustilago maydis*, the signal peptide peptidase (SPP) Spp1 is essential for plant defence suppression and establishment of a compatible biotrophic interaction with its host plant maize. SPPs are endoplasmic reticulum (ER)-resident intramembrane proteases, that cleave a subset of remnant signal peptides and transmembrane domain containing proteins, releasing them from the membrane for degradation or to exert a biological function. The conserved catalytic activity and generated cleavage products are crucial for the virulence function in *U. maydis*. Using a Co-IP/LC-MS approach from infected maize tissue, potential substrates of Spp1 were co-purified. Among these we identified the signal peptide (SP) of an effector protein, that is necessary for Spp1-dependent processing prior to effector secretion. Replacement of the Spp1-bound SP with an alternative SP abolished proper effector processing and significantly reduced virulence. In addition, expression of a predicted cleavage product is sufficient to partially rescue the virulence defect of the effector deletion strain. Another identified substrate is an ER membrane-anchored protein, that is required for virulence and depends on Spp1 for proper subcellular localisation. Direct transcriptional regulation via unfolded protein response elements (UPREs) links Spp1 function to biotrophic growth of *U. maydis*. In silico analyses suggest that the direct UPR-dependent regulation and its virulence function are specifically conserved in biotrophic fungi. Consistently, Spp1 is dispensable for virulence in the hemibiotroph *Verticillium dahliae* but essential for the biotroph *Sporisorium reilianum* to cause disease. Collectively, our data suggest that Spp1 mediates communication between biotrophic fungi and their host plants in a previously unknown manner.

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Investigating genetic and environmental factors driving *Starship*-mediated gene transfer in fungi

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Horizontal gene transfer (HGT) is the exchange of DNA between individuals outside of sexual reproduction and it plays a significant role in evolution through the introduction of novel genetic material that can facilitate adaptation to challenging environments. While HGT of transposable elements (TEs) is well documented in prokaryotes, its frequency and mechanisms in eukaryotes remain poorly understood. *Starships* are a newly discovered group of TEs widespread in Pezizomycotina. These TEs can be hundreds of kilobases in size and are able to mobilize numerous genes, including those conferring resistance to environmental stressors such as heavy metals. Previous research has demonstrated that *Starships* are able to move between individuals belonging to the same species, and recent experiments have shown they can also transpose between different fungal genera. Although we have clear evidence of *Starship* transposition, we have limited information on what drives their mobilization. We developed a high-throughput method that allows us to cross a "*Starship* donor" strain of *Aspergillus fumigatus* with virtually any wildtype strain and screen for transfer events. Through this design we can also test different stressors and environmental cues that may promote *Starship* mobilization. *Starship* movement is a cryptic process and we hypothesize that hyphal fusion is necessary for transfer between strains. Our crossing setup enables us to test whether the presence of heterokaryon incompatibility genes can prevent Starship transposition. I will discuss the recent progress into understanding the mechanistic basis of HGT in fungi.

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Comparative genomics using long-read sequencing of clinical *Candida auris* isolates reveals transmission dynamics and drug resistance mechanisms

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Candida auris is an emerging, nosocomial, multidrug-resistant fungal pathogen that has been responsible for healthcare associated outbreaks worldwide. The use of short-read sequencing has allowed outbreak tracing, yet it does not fully resolve genome structure, telomeres, and rearrangements that may drive adaptation and persistence. In this study, we utilized Oxford Nanopore long-read sequencing on outbreak-associated *C. auris* isolates from two U.S. hospital systems, Northwestern Memorial Hospital (NMH) and UC San Diego Health hospitals (UCSD), in addition to the bio-banked reference isolates from the Centers for Disease Control and Prevention. We generated telomere-to-telomere assemblies of 121 isolates in total. These de novo chromosome-level assemblies were used to perform phylogenetic analyses, comparisons of genomic architecture, and the detection of single-nucleotide polymorphisms (SNPs). Phylogenetic analyses highlighted overrepresentation of Clade IV isolates from NMH and Clade III isolates from UCSD, adding to the previously reported geographical distribution of *C. auris*. Comparisons of genome architectures and SNP analyses suggested low genomic variation within patients and hospital-specific SNP thresholds. We found that the mean SNP numbers were 109 for NMH and 34 for UCSD suggesting a hospital-system dependent accumulation of SNPs. In addition, we identified frequent mutations in the *ERG11* gene concurrent with the previous reports identifying *ERG11* mutations leading to the elevated azole minimum inhibitory concentration. In addition, we uncovered significant structural genome rearrangements including large chromosomal inversions and translocations between the isolates both within a given clade and across clades. These differences in genomic architecture were not apparent with the analyses using short-read sequencing data. Notably, major reorganization of chromosome 1 was observed in a group of isolates, suggesting a potential region that can be used for transmission tracing. Taken together, our study suggests comparative genomics approaches using de novo chromosome-level assemblies as a standard for outbreak surveillance, resistance prediction, and mechanistic insight into genome evolution in healthcare-associated pathogens.

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Selfish genetic element promotes mitotic stability of a dispensable chromosome in the fungal pathogen *Fusarium oxysporum*

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Many fungal pathogens harbour dispensable chromosomes (DCs) which are non-essential for life. DCs often confer a selective advantage in certain environments such as the presence of the host, but can be spontaneously lost under non-selective conditions. How then is long-term persistence of DCs in natural populations achieved? Here we tested the role of a selfish genetic element known as Spore Killer (*Spok*) in mitotic stability of DCs in *Fusarium oxysporum* (*Fo*). *Fo* chromosome 12 (Chr12) was previously shown to be dispensable during *in vitro* growth of this fungal pathogen and to carry genes involved in the utilization of non-conventional carbon sources. Here we performed serial passaging of a human cornea isolate, MRL8996, and observed a high spontaneous loss rate of Chr12 on solid media containing glucose as the sole carbon source, but not on ribose, pectin or polygalacturonic acid. Furthermore, we found that the spontaneous loss rate of Chr12 on glucose medium was significantly lower in the tomato pathogenic isolate *Fol*4287 compared to MRL8996. Comparative genomic analysis of Chr12 in the two strains revealed that the mitotically stable version from *Fol*4287 harbours a *Spok* gene located within a giant Starship transposon, both of which are absent in Chr12 of MRL8996. Transformation-mediated transfer of the *Fol4287 Spok* gene into Chr12 of MRL8996 led to a significant reduction in mitotic loss rate, whereas no such effect was observed with a *Spok* allele carrying a loss-of-function mutation in the killer domain. Conversely, targeted deletion of the *Spok* element from Chr12 in *Fol*4287 resulted in a significant increase of loss rate. These findings suggest that selfish genetic elements such as Spore Killers can benefit the host genome by counteracting spontaneous loss of DCs under non-selective conditions, thereby contributing to the long-term persistence of conditionally beneficial DCs in fungal populations.

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Phyletic Patterns of c-NHEJ Genes Are Reflected in Genomic Signatures in Fungal Genomes

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Double-strand break repair (DSBR) is a fundamental process that maintains genome stability through multiple pathways, including homologous recombination (HR) and classical non-homologous end joining (c-NHEJ). Phyletic pattern of DSBR pathways across hundreds of fungi revealed that a group of early-diverging fungi, Zoopagomycota, completely lacks the c-NHEJ pathway. In addition, a subdivision of Basidiomycota, the Ustilaginomycotina, lacks LIG4, a DNA ligase that is essential for c-NHEJ. We hypothesized that the absence of c-NHEJ would be reflected in in the genome as distinct genomic signatures. To test this hypothesis, we developed a comparative genomic pipeline that detects and classifies insertions and deletions as potential genomic signatures of different DSBR mechanisms. The analysis is based on pairs of genomes from different phylogenetic groups that either possess or lack c-NHEJ genes. We found that, genomes completely lacking c-NHEJ or missing only LIG4 exhibit a significantly higher proportion of variants that are consistent with unequal crossing over, single-strand annealing, replication slippage, and alternative end joining, whereas genomes predicted to retain c-NHEJ activity show more variants that are likely resulting from c-NHEJ. Furthermore, Genomes entirely lacking c-NHEJ tend to have the longest microhomologies, those missing only LIG4 show intermediate lengths, and genomes with a complete c-NHEJ pathway have the shortest. Together, these results suggest that the absence of c-NHEJ in fungal genomes is associated with distinct genomic signatures that are largely shaped by alternative DSBR mechanisms.

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Dissecting the fitness effects of copy number variants using CRISPR-activation across diverse strains and environments

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Changes in gene copy number are an extremely important source of variation over both long and short evolutionary timescales. The opportunistic pathogen *Candida albicans* is a diploid yeast that frequently adapts to antifungal drug via large copy number variants (CNVs) that amplify hundreds of genes simultaneously. It is not known how many genes within a CNV contribute to the fitness of a CNV-containing strain in antifungal drug. Furthermore, it is not known whether the same or different genes have strong effects on fitness across different environments. Finally, how the fitness effects of gene amplification are impacted by different genetic backgrounds is not well understood. The answers to these questions have major implications for how CNVs are expected to contribute to genome evolution over time. To address these questions, we identified more than 800 genes that have been amplified in evolution experiments via complex CNVs. We then systematically assayed the fitness effects of individually overexpressing each of these genes in 4 genetically diverse isolates across 8 physiologically relevant environments. We identified multiple individual genes with positive or negative fitness effects that are specific to a single environment, as well as individual genes with fitness effects in multiple environments. We also find that in some isolates, but not all, the additive fitness effects of individual gene overexpression are a significant predictor of the fitness effect of the entire CNV. CNVs exhibit fitness trade-offs between environments that can be attributed to different amplified genes with large fitness effects. This suggests that complex CNVs are likely to serve as transient sources of adaptation and be either refined or eliminated over time.

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Chromosomal translocation of a horizontally-acquired gene cluster and virus-like elements in a cosmopolitan yeast

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The instability and dynamic nature of subtelomeric regions in yeasts can facilitate dramatic genomic alterations**.** However, these regions can be difficult to characterize due to lower assembly quality and contiguity from short-read sequencing technologies. Here, we leverage long-read sequencing to resolve cryptic subtelomeric regions in a cosmopolitan yeast, *Torulaspora delbrueckii***.** This approach revealed the organization and varied chromosomal locations of a subtelomeric horizontally-transferred metabolic gene cluster for galactose consumption. Interestingly, we identified genetic signatures of double-strand break (DSB)-mediated repair of fragile subtelomeric regions flanking the cluster, offering insights into the mechanisms that facilitated its integration and chromosomal translocation. Strikingly, these subtelomeric gene clusters exhibited evidence of non-homologous repair with both endogenous and exogenous DNA sequences, most notably sequences derived from a killer toxin-encoding virus-like element (VLE) plasmid system. Additional searches of both short and long-read genomes further revealed extensive evidence of integration of VLE sequences in both subtelomeric and internal chromosomal locations. We furthermore identified several strains harboring active VLE plasmid systems. These results support recent evidence that VLE systems have ancient and broad associations with yeasts which can result in extensive genomic integration and can interact with other horizontally-acquired genomic features. Furthermore, we propose that the integration of such sequences in subtelomeric regions can exert influence on subtelomeric recombination and translocation dynamics. This work highlights the utility of long-read sequencing to resolve cryptic subtelomeric regions that can harbor diverse and complex signals of exogenous DNA integration.

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Comparative genomics of plant associated fungi to assess endophytic genome signatures

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Beyond the scope of Epichloë fungi, the global diversity of grass endophytes contains many fungi that exist as plant pathogens and other non-endophytic lifestyles. The host ranges and life history strategies of these fungi are broader, resulting in a wider range of selective pressures acting on them compared to the Clavicipitacean endophytes. Here we present a phylogenetic placement of a wide range of primarily Ascomycete fungi isolated from endophytic niches within pre-dispersal grass seeds collected from 76 locations in Madagascar over a series of decades, then preserved at the Millennium Seed Bank, UK. We provide a focussed look at isolated fungi that are regularly documented as pathogens of plants and other grasses: Fusarium, Aspergillus, Alternaria and others, to examine whether the endophytic strains are phylogenetically constrained within a wider setting of closely related non-endophytes, or whether they are distributed throughout their genera. We then conduct comparative genomic tests between these endophytic strains and non-endophytic relatives. We use dN/dS to test for positive selection, then genome enrichment and gene family expansion to look for genomic signatures that are unique to endophytic strains. The results explore the phylogenetic grouping of endophytic strains within their genera and evolutionary processes that direct diverse Ascomycete fungi towards endophytism.

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Population-Specific Transcriptomic Rewiring Underlies Secondary Metabolic Diversification in *Aspergillus flavus* and the Domestication of *Aspergillus oryzae*

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The genomic signatures of microbial domestication remain poorly understood within the context of natural population variation. Here, we demonstrate that *Aspergillus oryzae*, the filamentous fungus used in soy sauce production, shares more recent ancestry with a predominantly northern, largely non-aflatoxigenic population of *Aspergillus flavus* (population C). Strikingly, *A. oryzae* isolates also overlap with the recently described, clinically enriched *A. flavus* population D, suggesting the possibility of multiple domestication events. Although *A. oryzae* exhibits reduced virulence compared to *A. flavus*, all isolates tested retained pathogenicity in a zebrafish infection model. At the transcriptomic level, *Aspergillus* populations are significantly differentiated, with distinct responses to population density, indicating that population-specific transcriptomes adapt differently to ecological conditions. These differences

extend beyond gene content and are not always explained by phylogenetic relationships, suggesting that phenotypic diversification occurs through the rapid reorganization of transcriptomic architectures. For example, *A. oryzae* displays significantly elevated expression of a module enriched for carbohydrate metabolism. Population-specific variation is also evident among secondary metabolite (SM) gene clusters. While *A. oryzae* shows markedly reduced expression of specific SM genes, particularly those involved in aflatoxin biosynthesis, this trend does not extend across the entire secondary metabolome. Using machine- learning-based gene regulatory network inference, we identified population-specific transcriptomic differences linked to distinct transcription factors, with evidence for both cis- and trans-acting regulatory divergence, but no changes in global regulators such as *laeA*. Together, these findings provide new insights into the domestication of *A. oryzae*, its global significance, and the microevolution of fungal secondary metabolic pathways.

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Hi-C and HiFi Sequencing Unveils Genome Compartmentalization and RNA Operon Physical Interactions in *Gigaspora margarita*

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Arbuscular mycorrhizal fungi (AMF) are widespread symbionts that enhance nutrient uptake in most land plants. Chromosome-level assemblies of the model species *Rhizophagus irregularis* revealed that genomes can segregate into active (A) and repressed (B) compartments, which shape their biology and evolution. However, it remains unclear whether this genomic architecture is conserved across AMF lineages, particularly those with large, transposable element-rich genomes, and how these features influence topologically associating domains (TADs) in the presence or absence of bacterial endosymbionts. To address this, we combined PacBio HiFi sequencing with Hi-C to generate a chromosome-scale and 3D genome assembly of *Gigaspora margarita* and its endosymbiont *Candidatus Glomerobacter gigasporarum* (CaGg). The *G. margarita* genome comprises over 40 chromosomes totaling 778 Mb, organized into A/B compartments and conventional TAD-like structures that remain stable regardless of CaGg presence. The *G. margarita* genome carries 21 divergent rDNA copies distributed across six chromosomes, which physically interact within AMF nuclei and whose sequence divergence causes phylogenetic and taxonomic challenges. The CaGg genome harbors a reduced yet mobilome-rich multipartite genome with intact heme and vitamin B_{12} biosynthetic pathways, likely benefiting its fungal host. Overall, our findings provide new insights into genome biology of prominent symbionts, highlighting the plasticity and functional significance of their 3D genome architecture and its interactions with plant hosts and intracellular bacteria

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Integrative structural genomics of the secretome in a plant pathogenic fungus provides insights into effector evolution and functional diversification

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During host colonization, fungal plant pathogens secrete effector-like proteins that alter host cell physiology and target plant-associated microbes. However, rapid evolution and low sequence conservation often hinder the study and characterization of these proteins. The fungus *Zymoseptoria passerinii* infects *Hordeum* spp. and includes lineages adapted to wild and domesticated barley. We combined comparative structural genomics and heterologous protein expression to decipher evolution and functional divergence of secreted proteins produced by *Z. passerinii*. First, we applied deep learning methods to establish a robust baseline for structural protein analyses. Structure-based clustering unraveled 72 structurally similar clusters across sequence-divergent proteins in the secretome of *Z. passerinii*. Integration of functional annotations showed that effector-like proteins clustered into a limited number of folds linked to host modulation, whereas predicted antimicrobial effectors were broadly distributed across the network. This observation suggests that immune interfering and antimicrobial proteins share similar structural properties. We applied heterologous expression of selected proteins to validate predicted antimicrobial functions using a representative set of microorganisms from the barley microbiome. Focusing on one cluster of effector proteins, we furthermore confirm in-planta immune interfering properties for proteins with structural similarity. Finally, we conducted comparative analyses of physicochemical properties revealing how surface charge and electrostatics properties distinguish antimicrobial and immune-interfering proteins. Overall, we demonstrate how the secretome of *Z. passerinii* is organized around common structural folds that support diverse biological roles, including host manipulation and host-associated microbial interactions.

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Host Association and Secondary Metabolism in a Widespread Amphibian Gut Symbiont

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Fungal symbioses across the parasitism–mutualism spectrum emerge from the interplay of ecological context, host association, and genomic innovation. The early-diverging genus *Basidiobolus* exemplifies this complexity. Our multi-year sampling across Central Massachusetts shows that *Basidiobolus* is a ubiquitous amphibian gut symbiont, often represented by multiple co-occurring lineages within a single host, and exhibiting substantial cryptic diversity, including several novel clades. This ecological breadth is mirrored in its genomic architecture. Phylogenomic and genome-resolved analyses reveal an expanded secondary metabolite repertoire, including siderophores, terpenes, and putative antibiotic pathways. Many of these genes show strong signatures of horizontal gene transfer from gut-associated bacteria, suggesting that metabolic innovation may reinforce both host association and competitive success in microbiome-rich environments. Together, these results position *Basidiobolus* as a compelling model for understanding how early-diverging fungi integrate symbiosis, lifestyle flexibility, and accessory metabolism to thrive across distinct ecological settings.

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Transcriptomic variation explains *Suillus tometosus* zinc tolerance in the absence of population structure

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Soil zinc (Zn) excess, largely caused by anthropogenic activities such as mining and metal processing, can disrupt metabolism and reproduction of organisms inhabiting these sites, often leading to death. Some ectomycorrhizal fungi can tolerate elevated Zn concentrations and protect their plant

partners against metal toxicity. However, the mechanisms of Zn tolerance are still unclear. To fill this gap in knowledge, we used a combination of *in vitro* Zn fungal assays, whole genome resequencing, and transcriptomics to investigate Zn tolerance in the ectomycorrhizal *Suillus tomentosus*.

Whole genome comparison of 48 isolates found no evidence of population structure in *S. tomentosus* based on Zn tolerance, but Zn-tolerant and -sensitive isolates showed regions of genomic differentiation, including in genes related to zinc ion binding. Global gene expression profiling in one Zn-sensitive (ZnS) and one Zn-tolerant (ZnT) isolate revealed distinct baseline profiles in the absence of Zn, with differential expression of genes related to signaling, transport and antioxidant activity. This points to ZnT having constitutive Zn tolerance. When exposed to low Zn toxicity, ZnS mounted a broad stress response characterized by downregulation of essential cellular processes and upregulation of detoxification enzyme genes such as glutathione S-transferases. ZnT, on the other hand, showed no transcriptional change at low Zn toxicity, but at higher concentrations this isolate downregulated Zn transporters and upregulated antioxidant genes. The ZnT response at high Zn involved fewer genes than that of ZnS at low Zn.

These results show Zn tolerance in *S. tomentosus* is a constitutive and polygenic trait, less defined by genomic signatures, but related to the expression of metal homeostasis genes, providing mechanistic insight into the molecular mechanisms of ectomycorrhizal fungal tolerance in metal contamination sites.

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Genomic, transcriptomic and molecular study of lichenization in the model lichen *Xanthoria parietina*

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Lichens represent complex multipartite symbioses consisting of lichen fungal symbionts (LFS), photosynthetic partners (chlorophyte algae or cyanobacteria), and diverse microbial communities including bacteria and yeasts that colonize the thallus after its formation through lichenization. The common lichen species *Xanthoria parietina,* that associates with the alga *Trebouxia sp.*, has been extensively investigated using metagenomic and metatranscriptomic approaches, establishing it as a model system for understanding lichen symbiosis and lichenization. Comprehensive analyses revealed putative immunity-related proteins alongside lichen-specific, structurally conserved secreted proteins exhibiting similarity to fungal pathogen effectors. These findings suggest that effector-like proteins may play crucial roles in modulating host immunity during symbiosis establishment and maintenance. To explore the evolutionary conservation and functional mechanisms of these effectors, referred to as Xanthoria effector proteins (Xep), I investigated their evolution and distribution across fungi using comparative phylogenomics approaches. Among the candidate effectors identified, Xep4 encodes a thaumatin-like effector which is a class of pathogens effectors known to target plant chloroplast protein called rubisco activase (RCA) to modulate the host response and increase plant susceptibility. Heterologous expression of Xep4 in *Nicotiana benthamiana* demonstrated that this effector is computationally predicted and targeted to the chloroplast, and its localization depends on the protein's chloroplast targeting peptide (cTP). Additionally, yeast-2-hybrid and co-immunoprecipitation-mass spectrometry (IPMS) analyses revealed that Xep4 and *Trebouxia sp.* RCA interact, indicating that the protein-protein interactions might be crucial for lichenization. Finally, I will present further work conducted on *Chlamydomonas reinhardtii*, the model chlorophyte alga closely related to lichen algal symbionts, which deepens our understanding of the role of Xep in lichen symbiosis.

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Host-specific isocyanide natural product diversification in the pathogenic mold *Fusarium sporotrichioides*

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Fungi are renowned for their staggering array of natural products, ranging from harmful mycotoxins to life-saving antibiotics. Isocyanide natural products mediate competition with other microorganisms by chelating metals and binding to other metabolites, sometimes causing dangerous diseases in livestock that consume infected wild or cultivated grasses. Most Ascomycete genomes have recently been shown to encode two to three unique isocyanide synthase (ICS) genes; however, we have revealed that many plant-pathogenic lineages have expanded repertoires of these compounds. For example, the reference genome for *Fusarium sporotrichioides*, a common endophyte of wild grasses and a causal agent of Fusarium head blight on wheat and barley, encodes seven ICS genes. To understand how ICSs in *F. sporotrichioides* have genetically differentiated between hosts, we analyzed the pangenome of 151 *F. sporotrichioides sensu stricto* isolates collected from wild and domesticated grasses worldwide. We compared ICSs from our pangenome with 34 closely related isolates from the Sporotrichioides species complex and other closely related Sordariomycetes and found that the expansion in ICSs began prior to the diversification of the Sporotrichioides species complex. Most *F. sporotrichioides* isolates retain three ICS families that are widespread across genera, two Fusarium-specific ICSs, and up to two species-specific ICSs. One of these species-specific ICS families shows signatures of overrepresentation from wild-grass isolates, suggesting a potential role of its associated biosynthetic gene cluster in host-range specificity. This expanded cluster is restricted to our sampling of North America, where our sampling suggests two geographically isolated North American lineages and three populations with global distributions. Together, our work suggests that isocyanide expansion may contribute to host-range in an agriculturally important pathogen.

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The core and accessory gene repertoire of rust fungi (pucciniales): Genomic insights into their adaptive evolution

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The order Pucciniales (rust fungi) comprises an ecologically diverse group of obligate biotrophic plant pathogens that infect crops such as cereals, soybean, and coffee. These fungi typically undergo rapid evolutionary changes driven by host-selective pressures; however, the mechanisms underlying their genetic

adaptability remain poorly understood. In this study, we characterized core, accessory, and singleton gene repertoires of Pucciniales. Our analysis utilized publicly available high-quality genome assemblies from 12 Pucciniales species, including the soybean rust *Phakopsora pachyrhizi*. We observed striking variation in the distribution of core genes across Pucciniales genomes, with core gene frequencies ranging from 23.74% (*Puccinia graminis*) to 34.41% (*Puccinia triticina*). On average, accessory gene frequency (44.61%) was larger than core genes. The highest proportions of accessory genes (>55%) occurred in *Melampsora* spp and *P. pachyrhizi*. The myrtle rust fungus, *Austropuccinia psidii*, stood out for having 52% of its genes classified as singletons. In contrast, *Melampsora* species and *P. pachyrhizi* exhibited the lowest frequencies of singleton genes, ranging from 5.07% to 12.75%. A PCA analysis revealed that the accessory gene composition of Pucciniales formed three major clusters, reflecting distinct evolutionary trajectories among *Melampsora* spp, *P. pachyrhizi,* and *Puccinia* spp. We identified multiple expansions and contractions of genes within the 119 core and 365 accessory orthogroups across Pucciniales. The *P. pachyrhizi* genome, specifically, displayed 40 expanded core and 62 expanded accessory orthogroups. The expanded core and accessory orthogroups of *P. pachyrhizi* included primarily G25 CAZyme family, S8/S53 Peptidase, Homeobox transcription factors, Ca2+/cation antiporter (CaCA) family, and effector genes with unknown functions. As expected, transposable elements (TE) contributed intensively to the expansion of both core and accessory components in *P. pachyrhizi*, with 582 accessory genes localized within TEs. We conclude that rust fungi exhibit substantial variation in the size and composition of their core, accessory, and singleton gene repertoire. This highly variable repertoire was likely shaped by gene gains and losses through TE activity, particularly in *P. pachyrhizi*. Our study sheds light on the genomic and evolutionary processes influencing adaptation in Pucciniales.

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Among Us: Cheaters and transposons drive adaptations in experimental evolution of *Botrytis cinerea*

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We conducted experimental evolution to enhance the tolerance of the fungus *Botrytis cinerea* to the plant defense compound α-tomatine. While our initial goal was to select for physiological tolerance to α-tomatine, the evolutionary outcomes revealed complex trade-offs between survival, reproduction, and social behaviour. One out of 5 α-tomatine evolved lines exhibited a specific adaptive response: a three-fold increase in spore production only when only exposed to α-tomatine. To determine the genetic basis of this hyper-sporulation phenotype, we performed RNA-seq and whole-genome sequencing. We identified insertion of flipper transposable element (Fot-1) in the promoter region of a transcription factor that was significantly down-regulated in the evolved line. We are currently analysing the knock-out mutant of this transcription factor to confirm if its suppression is responsible for the adaption toward hyper-sporulation. Beyond individual adaptation, we observed a breakdown in social cooperation. In 6 out of 10 independent lines, "cheater" phenotypes emerged. These strains lost the ability to produce sufficient spores themselves but thrived by exploiting the resources provided by sporulating "cooperator" strains in mixed cultures. We were able to successfully separate the cheater and cooperator pairs from all six lines and sequenced their genomes to pinpoint the mutations driving this social exploitation. Later we investigated the consequences of this cheating on pathogenicity. In tomato infection assays, cheater strains were significantly less virulent than wild-type strains. Cooperator strains retained full pathogenicity even after 30 transfers. However, in mixed inoculations their virulence was reduced in the presence of cheaters. These findings suggest that cheaters act as a social burden, effectively dragging down the population's overall ability to cause disease. Overall, in our work we highlight laboratory experimental evolution can often see the emergence of cheater-like lines that can drive the outcome away from the anticipated traits and towards social diversification within populations.

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Starship transposons mediate horizontal gene transfer between fungi

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Horizontal gene transfer (HGT) is well established as a major evolutionary force in prokaryotes, but its significance in fungi has historically been controversial. Genomic studies are now uncovering growing evidence of gene movements between fungi. However, it remained unclear whether these events are merely isolated chance occurrences or if active mechanisms exist to mediate gene exchanges between eukaryotic species.

We recently demonstrated that giant transposable elements, which we call *Starships*, can mediate active gene transfer between distantly related fungal species. In simple co-culture experiments we observed repeated *Starship* transfers between *Paecilomyces variotii* and *Aspergillus fumigatus.* Using whole-genome sequencing, we confirmed that only *Starship* DNA is precisely transferred, thus excluding alternative hypotheses such as the random uptake of exogenous DNA or hybridisation. The transferred *Starship* elements total 500 kilobases in length (similar to the size of some fungal chromosomes) and mobilize approximately 150 genes with diverse biological functions as cargo. One transferred *Starship* carried genes for metal resistance. Following transfer, the recipient strain acquired a metal resistance phenotype. We are now using this established system to uncover how *Starships* mediate these extraordinary transfers.

Beyond our experimental results, bioinformatic analysis suggests that *Starship*-mediated HGT is a widespread and frequent natural phenomenon across filamentous fungi including a variety of plant pathogens. In a number of cases these HGT events carry known plant pathogenicity genes. These findings suggest that *Starships* are a key mechanism driving the transfer of pathogenicity-related genes between fungal pathogens.

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Starship-mediated horizontal transfer of transposable elements across fungi

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Horizontal transfer of transposable elements (TEs) is common in eukaryotes and can drive genome evolution by triggering sudden bursts of TE activity. Yet, the mechanisms that enable such transfers remain poorly understood. In this study, we compared the genomes of two strains of the insect-pathogenic fungus *Metarhizium anisopliae* and show that one strain displays a very recent TE expansion. By tracing the origin of these expanded TEs, we found that they were most likely introduced via a Starship. Starships are large transposons within the *Pezizomycotina* subphylum that often carry genes and can move horizontally. We show that a particular Starship in *M. anisopliae* was likely horizontally acquired and carried a total of 73 TEs, 43 of which expanded in the recipient genome, generating 508 perfect copies outside the Starship. This expansion was accompanied by extensive chromosomal restructuring and a loss of pathogenicity. Broadening our analysis to other fungal taxa, we found that TEs are frequently present on Starships, with many showing signatures of recent amplification outside the element. Notably, identical or highly similar TEs are also shared between Starships from different species, and several Starships in the *Metarhizium* and *Aspergillus* genera carry TEs that were likely horizontally transferred across species boundaries. Together, our findings identify Starships as vectors for the horizontal transfer of TEs and highlight their ability to drive structural and functional changes in the recipient genome.

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Experimental evolution reveals new mechanism of niche adaptation in *Fusarium oxysporum*

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Fusarium oxysporum is an ubiquitous fungus that provokes vascular wilt disease in more than 150 crop species and causes life-threatening infections in humans. The mechanisms underlying this striking adaptive versatility in the absence of a known sexual cycle remain poorly understood. A previous serial passage experiment with the tomato pathogenic isolate *Fol*4287 revealed that transposable elements (TEs) act as the main drivers of genetic variation . During selection on plates with complete medium, independently evolved lines carried recurrent TE insertions in the gene *FOXG_21009,* which is encoded in an accessory genomic region and encodes a hypothetical protein homologous to Fe(II) and 2-oxoglutarate-dependent oxygenases. *FOXG_21009* loss-of-function mutants displayed dramatic phenotypic changes such as increased hyphal growth and sporulation, absence of conidial quorum sensing, as well as impaired invasive growth and virulence on plant and animal hosts. Interestingly, serial passaging of these evolved lines under distinct selection conditions led to recovery of a near wild type phenotype, which was associated with the loss of a genomic region adjacent to *FOXG_21009*. Importantly, *FOXG_21009* loss-of-function leads to transcriptional deregulation of this adjacent gene cluster, Our results suggest the presence of an accessory genome region controlling core functions of fungal growth, development and pathogenicity.

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Evolution of supernumerary chromosomes in blast fungal pathogens

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The genomes of *Pyricularia oryzae* (synonym *Magnaporthe oryzae*), the fungal pathogen that causes blast disease on diverse grass species, have seven core-chromosomes and may contain supernumerary mini-chromosomes. We have explored mini-chromosomes using long-read genome sequencing and assembly, chromosomal karyotyping, and computational approaches. Our data and other reports indicate that mini-chromosomes are prevalent in *P. oryzae* isolates. The number and composition of mini-chromosomes vary significantly among isolates from different host-adapted pathotypes and among isolates from the same pathotype. Such mini-chromosomal variation can even be observed in different cells of an isolate. Analysis of the strains causing wheat blast, whose first outbreak was in 1985, revealed the presence of mini-chromosomes in all recent wheat strains but the absence in most of the strains collected before 1991. This suggests preferential selection for strains carrying mini-chromosomes in recent years. Surprisingly, the wheat blast strain T47 isolated in 1985 carried a mini-chromosome with two avirulence *PWT7* genes, while the 1986 strain T3 that was nearly identical to T47 lacked the mini-chromosome. Loss of mini-chromosomes in T3 might have enhanced female fertility, facilitating sexual crosses with strains of other pathotypes and significantly increasing genomic diversity soon after the initial outbreak. Additionally, our data strongly suggest horizontal mini-chromosome transfer between *P. oryzae* and other *Pyricularia* species. In summary, our genomic analysis depicts the evolution of mini-chromosomes in *P. oryzae*, indicating the important roles of mini-chromosomes in pathogen adaptation and pathogenicity.

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Transposable elements drive evolution and alternative virulence strategies in *Colletotrichum lupini*

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Colletotrichum lupini is the sole species within its clade capable of infecting lupin, exhibiting remarkable genomic plasticity that underpins its host specialization. Through comparative genomic analysis with non-pathogenic relatives, we reveal how transposable elements (TEs) architect a distinctive "two-speed genome" that facilitates rapid evolutionary innovation and pathogenic adaptation.

Our analysis reveals a diverse TE repertoire distributed throughout the genome. Notably, many lineage-specific regions (LSRs), genomic compartments unique to *C. lupini* that serve as evolutionary hotspots for gene gain and loss, are associated with Starship elements. These recently characterized large mobile elements play a pivotal role in reshaping the genomic landscape by harboring and potentially mobilizing entire gene clusters, often related to fitness.

A striking example of this TE-driven innovation is a hybrid polyketide synthase-nonribosomal peptide synthetase (PKS-NRPS) gene cluster that bears signatures of horizontal gene transfer and resides within a Starship element. This species-specific BGC, compartmentalized within an LSR, represents a departure from canonical effector-based pathogenicity strategies. Instead, *C. lupini* employs an alternative virulence mechanism centered on secondary metabolite production, a strategy that may confer selective advantages in its specialized lupin niche.

Through TE-mediated compartmentalization, mobile elements can serve as architects of pathogen evolution, creating genomic neighborhoods where acquired virulence factors are insulated from purifying selection in the stable core genome. Our findings illuminate how the interplay between TEs, horizontal gene transfer, and genome architecture shapes host range determination and pathogenic potential in fungal plant pathogens. Ongoing functional characterization of candidate BGC-encoded metabolites will reveal whether this compartmentalized architecture directly contributes to host-specific virulence, providing insight into how genomic organization translates to pathogenic outcomes.

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Roles of aneuploidy in *Aspergillus fumigatus* unstable adaptation and antifungal resistance

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Aneuploidy causes genome plasticity and enables adaptive responses that confer drug resistance in eukaryotes ranging from fungal pathogens to human cancer cells. *Aspergillus fumigatus* is a soil-resident fungus and the most common etiologic agent of invasive fungal infections globally. Aspergillosis has an alarmingly high mortality rate due in part to treatment failure resulting from widespread antimicrobial resistance and an ability of the fungus to dynamically adapt to changing environmental and host conditions. Our understanding of these treatment failures is hindered by gaps in knowledge about how this organism adapts to resist antimicrobial stress. We find that exposure to FK506, an antifungal and immunosuppressive compound produced by *Streptomyces*, selects for whole-chromosome aneuploidy in this fungus. These chromosome duplications also reduce susceptibility to clinically-used azole antifungals, and whole-chromosome and segmental aneuploidies arise independently directly in response to azole exposure and confer resistance. Transcriptomic analysis revealed that Chr7 disomy leads to marked induction of the normally-silent neosartoricin biosynthetic gene cluster in response to FK506 exposure, and we demonstrate that constitutive genetic induction of this cluster recapitulates in the euploid background the response to FK506 in the aneuploid state. We further show that this response is independent from the production of neosartoricin, suggesting a role for cross-pathway metabolic changes resulting from the activation of this gene cluster or from the aneuploid state. In the aneuploids that arose in response to voriconazole, we find that a single duplication of the left arm of Chr2 is sufficient to confer a high level of resistance, and that the expression of known azole resistance genes is not altered in this strain. In both FK506-resistant and voriconazole-resistant aneuploids, culture in the absence of drug leads to aneuploid chromosome loss and rapid reversion to a euploid, drug-susceptible state. These results uncover a novel mechanism of rapid and reversible antimicrobial stress adaptation in *A. fumigatus* and demonstrate the utility of aneuploid strains to identify novel genetic contributions to antifungal adaptation.

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Beyond the lab: The variable landscape of genomic instability in yeast isolates

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Previous work has shown that aneuploidy, a condition in which cells have an uneven chromosome content, may be favorable when growing cells under stressful conditions. In this work, we examine how the frequency of genetic instability events, focusing on aneuploidy and other large-scale structural variations, vary based on the genetic background of the budding yeast *Saccharomyces cerevisiae*. We designed an evolution experiment where diploid cells from a variety of wild and laboratory yeast backgrounds are passaged through strong bottlenecks on solid medium. Unlike liquid culture, solid medium minimizes selection generated by nutrient competition, thus allowing for nearly unbiased choosing of cells that move on to the next generation, regardless of their relative fitness. By using both long and short read sequencing data, we examined how aneuploidy and other structural variant frequencies change over time. In addition, when we measured genome instability in the ancestral and evolved strains, our results show a variable landscape of genetic instability frequencies dependent on the genetic background. Our work implies that studying laboratory yeasts alone provides a narrow understanding of how genetic instability emerges and evolves in nature. Understanding the effects of genetic instability is critical in the context of human disease, amidst its involvement in an increasingly dire landscape of treatment resistance acquisition in fungal pathogens and cancer cells.

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The genetics of speciation in *Saccharomyces*

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New species are formed when they become reproductively isolated, usually as a consequence of a combination of isolating barriers. We investigated the potential genetic causes of reproductive isolation between two closely related species of budding yeast, *Saccharomyces cerevisiae* and *S. paradoxus*. First generation hybrids between these species are viable but infertile, with 99% of gametes failing to survive. We find that the primary reproductive barrier is chromosomal divergence, which results in mis-segregation of chromosomes during meiosis due to the rejection of recombination. All chromosomes in *Saccharomyces* yeast are essential, so a gamete missing a single chromosome is inviable. In this system, a lack of recombination is the cause of hybrid sterility. We then break this chromosomal species barrier by restoring recombination in the hybrid, allowing us to uncover evidence of genic incompatibilities between diverged alleles from the two parental species.

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Inducing novel endosymbioses in the filamentous fungus *Rhizopus microsporus*

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Endosymbioses are widespread in nature, often giving rise to new metabolic capabilities that enable host cells to inhabit diverse environments or enhance resistance to predators. Yet, the processes of how these intricate partnerships are established remain poorly understood and are difficult to extrapolate from far-evolved endosymbiotic relationships. The mucoromycete *Rhizopus microsporus* is emerging as a model system for studying intracellular endosymbiosis, as it comprises both endobacteria-harbouring and endobacteria-free strains. Previous work has established a fluidic force microscopy (FluidFM) approach for implantation of bacteria into the cytosol of *R. microsporus*, bypassing the requirement for self-directed bacterial entry. The introduction of bacteria pre-adapted to an intrahyphal lifestyle led to a compatible interaction with their new fungal host. Both partners resumed growth and vertical inheritance of bacteria was achieved through asexual sporulation. In contrast, the implantation of free-living bacteria more distantly related to natural endobacterial strains led to varied interaction outcomes. While some strains failed to establish, others successfully multiplied and spread through the fungal hyphae without being vertically inherited. Our live-cell observations across a selection of bacterial strains suggest that fungal immune responses, dispersal within the growing hyphae, and growth rates of both partners each play a critical role in the initial encounter and potential for stabilization of the endosymbiosis. By revealing these early determinants of compatibility, we aim to establish synthetic endosymbioses as a tractable framework for identifying the requirements of stable intracellular partnerships.

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Polarized cell wall growth during exit tube assembly in the chytrid *Batrachochytrium dendrobatidis*

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Polarized growth is fundamental for fungal physiology and symbiosis. Unlike animal cells and amoebae that mediate polarized growth using an actin cortex to directly control cell shape, polarized growth in yeast, filamentous fungi, and other Dikarya is defined by spatially restricted cell wall remodeling mediated by directed exocytosis and endocytosis. A key transition in fungal evolution, therefore, was the shift from using the cytoskeleton to control cell shape by directly pushing on the plasma membrane to using the cytoskeleton to control cell shape by guiding vesicle traffic to mediate polarized cell wall growth. Understanding when and where this transition occurred is important for understanding the evolution of fungal morphogenesis. To this end we study chytrid fungi, which diverged before the radiation of dikaryotic fungi. Chytrids develop from a motile cell type that lacks a cell wall (the “zoospore”) that controls its cell shape using an actin cortex to a sessile cell type that controls its shape using a cell wall (the “sporangium”). During the sporangial phase of their lifecycle, chytrid fungi build several polarized structures. For example, the frog-killing chytrid *Batrachochytrium dendrobatidis* (*Bd)*, like many chytrids, builds one or more exit tubes that discharge the next generation of zoospores from the sporangium into the environment. To determine how *Bd* regulates polarized cell wall growth during discharge apparatus assembly, we developed an assay to induce synchronous assembly of exit tubes. Using this assay, upwards of 70% percent of cells initiate exit tube assembly within a three-hour window. These exit tubes appear similar in diameter to daughter zoospores, suggesting that sporangia may actively control tube widths. We also stained cells with phalloidin to highlight actin polymers and observed higher density of peripheral actin patches near sites of active exit tube development, consistent with vesicular trafficking of cell wall synthases, similar to polarized cell wall assembly in Dikarya. Currently, we are using small molecule inhibitors to test this hypothesis and probe other mechanisms driving exit tube development in *Bd* to explore the deep evolution of fungal morphogenesis.

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The dynein light chains differentially affect cargo movement in *Aspergillus nidulans*

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The proper transport of cargos such as organelles and protein complexes is critical for cellular functions such as cell division, cell signaling, and organelle health. Most cargos are transported by motor proteins along cytoskeletal tracks. The dynein motor complex is one such motor responsible for both the long-distance transport of cargos as well as other crucial cellular functions such as orienting the mitotic spindle. The dynein motor complex is composed of the motor protein dynein (6 different subunits), its co-factor dynactin (11 different subunits), and a cargo adaptor that links dynein-dynactin to cargo. The general dogma in the transport field is that an identical dynein-dynactin is used for all cellular roles by linking to different adaptor proteins associated with different cargos. On the other hand, we discovered extensive variation in the dynein-dynactin subunits encoded in genomes across the fungal kingdom. Furthermore, we found that the presence of certain dynein-dynactin subunits correlates with dynein-mediated functions in those species. We discovered a loss of dynein light chains *roadblock* and/or *tctex* in the *Saccharomycotina* and *Taphrinomycotina*, fungi that use dynein for “pulling” but not “walking” movement. Therefore, we hypothesized that Roadblock and/or Tctex are required for walking but not pulling movement. “Walking” is the canonical form of transport, where a cargo is directly linked to dynein for long-distance movement on microtubules. In “pulling” movement, dynein is attached to the plasma membrane and binds microtubules, indirectly moving microtubule-associated cargos. In *Aspergillus nidulans,* pulling movement is used to position nuclei while walking movement is used to transport early endosomes. To test the role of *roadblock* and *tctex* on pulling versus walking movement, we knocked out *roadblock* and *tctex* in *A. nidulans* and examined distribution of nuclei and early endosomes. We found that while knocking out *tctex* has little effect on pulling or walking, knocking out *roadblock* results in severe defects in early endosome transport (walking), but minor effects on distribution of nuclei (pulling), supporting our hypothesis. Follow-up work suggests that the light chains have differing effects on dynein/dynactin localization, microtubule organization, and motor activation. Together, our work suggests that rather than just stabilizing the dynein motor complex, the dynein light chains have differing roles in dynein motor complex assembly and regulation.

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Acyl-coA binding protein AcbdA regulates peroxisome movement via hitchhiking on early endosomes in Aspergillus nidulans

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Motor-driven transport on microtubules is critical for distributing organelles throughout the cell. Most commonly, organelle movement is mediated by cargo adaptors, proteins on the surface of an organelle that directly recruit microtubule-based motors. An alternative mechanism called hitchhiking was recently discovered: some organelles move, not by recruiting the motors directly, but instead by using membrane contact sites (MCS) to attach to motor-driven vesicles and hitchhike along microtubules. Organelle hitchhiking is observed across fungi and animals. In filamentous fungi, nearly all peroxisomes move by hitchhiking on early endosomes (EE). In the fungus Aspergillus nidulans, EE-associated linker proteins PxdA and DipA are critical for establishing EE-peroxisome MCS required for peroxisome movement. Whether peroxisome-membrane proteins exist that regulate peroxisome hitchhiking on EEs is not known. Through a forward mutagenesis screen, we discovered an acyl-CoA binding (ACB) domain-containing protein AcbdA/AN1062 that localizes to peroxisomes via its tail-anchored transmembrane domain (TMD). Deleting the AcbdA gene or only its N-terminal ACB domain perturbs the movement and distribution of peroxisomes. Importantly, AcbdA is not required for the movement of EEs or for the recruitment of PxdA and DipA on EEs. Fatty acid (FA)–induced increases in peroxisome movement require AcbdA, suggesting that peroxisome hitchhiking on EEs is coupled to FA metabolism. Mutating a conserved FFAT motif, predicted to interact with the endoplasmic reticulum (ER), has no effect on peroxisome movement. Taken together, our data indicate that AcbdA is a peroxisome-membrane protein required for peroxisome hitchhiking on EEs. AcbdA's involvement in peroxisome hitchhiking represents a divergence from known functions of Acbd4/5 proteins and adds layers to our understanding of the functionality of the Acbd4/5 family of proteins.

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A Bipartite Complex Coordinates Glucan Synthesis and Remodeling to Build the Fungal Cell Wall

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The fungal cell wall is an essential extracellular matrix that underpins growth, morphogenesis, and pathogenesis, and its assembly requires the coordinated action of poorly understood enzyme networks. In *Schizosaccharomyces pombe*, we find that Ghs2, a protein containing a glycoside hydrolase 16 (GH16) domain, functions in strict partnership with the β-1,3-glucan synthase Bgs3. Ghs2 and Bgs3 co-localize to sites of polarized growth and are mutually dependent for localization. They also physically associate in cells and structural modeling predicts a Ghs2-Bgs3 complex in which the Ghs2 catalytic domain is positioned proximal to the Bgs3 glucan extrusion pore. Using solid-state NMR we found that Ghs2 and Bgs3 are required for the β-1,6-glucan content of the cell wall. Thus, we propose a model in which Ghs2 acts directly on the nascent β-1,3-glucan chains produced by Bgs3 to introduce β-1,6-linked branches, identifying Ghs2 as the long-sought factor required for β-1,6-glucan synthesis in *S. pombe*. With the Ghs2-Bgs3 complex representing the first example of a glucan synthase physically coupled to a remodeling enzyme to generate branched glucan networks, these findings establish a new principle of fungal cell wall assembly in which synthase-modifier modules function as inseparable units and highlight an enzymatic activity essential for growth.

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Regulation of cell polarity in a multibudding fungus

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Cell polarity refers to the asymmetric distribution of cellular components. Establishing asymmetry is central to a wide range of processes in fungi, including budding, hyphal growth, and morphogenesis (e.g., shmoo formation, conidiogenesis). Polarity establishment has been most intensively studied in single-budding yeast, in which the master regulator Cdc42 concentrates at a single site, ensuring that each mother cell produces only one bud. However, other fungi are not limited to a single polarity site: filamentous fungi can branch to form multiple hyphae, and pathogens such as *Paracoccidioides brasiliensis* transition to a multibudding phenotype upon host invasion. How fungi integrate physiological and environmental cues to specify the correct number of polarity sites remains an open question. In the multinucleate budding yeast *Aureobasidium pullulans*, a mother cell can produce multiple buds simultaneously. Intriguingly, cells with more nuclei also tend to form more buds.

Here, we ask how *A. pullulans* "counts" its nuclei and uses that information to form different numbers of polarity sites. We find that doubling ploidy increases bud number, so that cells with fewer nuclei make too many buds. This suggests that *A. pullulans* senses DNA quantity to count nuclei. To identify the relevant "nuclear-counting" and polarity factors, we combine top-down (quantitative proteomics, genetic screening) and bottom-up (targeted deletions of polarity components) approaches. Our targeted deletion screen reveals two polarity subcircuits: a Cdc42-centric branch that favours the formation of more polarity sites and a Rac1-centric branch that favours fewer. Moreover, the Rac1 subcircuit inhibits the Cdc42 subcircuit via the p21-activated kinase (PAK) Pak1, yielding a tunable architecture for generating variable numbers of polarity sites. Together, our results provide a preliminary mechanistic framework for the regulation of multipolarity in *A. pullulans* and illustrate how evolution of the conserved polarity circuit can generate striking morphological diversity.

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Peroxisomal targeting via organellar transit

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Peroxisomes are vital eukaryotic organelles that are required for diverse oxidative metabolic pathways including the breakdown of fatty acids. The majority of peroxisomal matrix and membrane proteins can reach peroxisomes following their translation in the cytosol. Here, we discuss alternative pathways that involve an intermediate destination in mitochondria or in the endoplasmatic reticulum (ER) in the two fungal model systems *Saccharomyces cerevisiae* and

in *Ustilago maydis*. The protein phosphatase Ptc5 is first imported into mitochondria; a fraction is rerouted into peroxisomes at organellar contact sites thereby regulating peroxisomal redox homeostasis. The tail anchor protein Pex15 can enter peroxisomes directly and via ER. We have used the latter pathway to identify novel factors for ER to peroxisome protein sorting. In summery, we provide insight into alternative protein targeting pathways, which might be relevant also outside the fungal kingdom. We believe that direct targeting and indirect targeting are coexisting pathways that regulate peroxisome formation and function.

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The role of eisosomal proteins in pathogenesis of *Cryptococcus neoformans*

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Cryptococcus neoformans is a human fungal pathogen responsible for the death of ~20% of AIDS related patients.The cell wall and plasma membrane (PM) are critical for survival of a pathogen in the host and pathogenicity. Several protein complexes associated with the PM sense the unfavorable host environment and initiate compensatory mechanisms to cope with stress. Eisosome is one such protein complex important for nutrient acquisition, stress response and pathogenesis. Eisosome complex is well-studied in the model yeasts of the ascomycetes phylum; however, no study has been conducted yet to define eisosome's role in *C. neoformans* which belongs to basidiomycetes. In *Saccharomyces cerevisiae*, Pil1 and Lsp1 proteins are the core components of eisosomes that shape the curvature of the PM based on their conserved AH-BAR domains. We identified respective homologues of Pil1 and Lsp1 in *C. neoformans*. Our results revealed two localization patterns for Pil1 tagged with the GFP: the canonical punctate localization to the PM and a previously uncharacterized localization to the mother-bud neck. The *pil1*Δ and *lsp1*Δ mutants exhibited inverse sensitivity when grown under various stress conditions suggesting a novel mechanism of how Pil1 and Lsp1 cooperate in stress response. Cells lacking Lsp1 exhibited fewer punctate PM-associated signals of Pil1-GFP that were also notably brighter as compared to those found in the wild-type, suggesting a role of Lsp1 in eisosome formation and dynamics. Interestingly, elimination of Lsp1 had similar consequences on the localization of the septin Cdc12-GFP, which together with other septins, forms filamentous punctate assemblies at the PM specifically when cells are stressed with 37°C. As the well-described function of septins is at the mother-bud neck during cytokinesis, our results point to a novel connection between, septins and eisosome components that involves PM homeostasis and cytokinesis. Strikingly, the double mutant (*pil1*Δ*lsp1*Δ*)* revealed defects in two major virulence factors, polysaccharide capsule and secretion of urease, pointing to an important contribution of eisosomes to pathogenicity of *C. neoformans*. As eisosome components have no resemblance to human proteome, they constitute a promising therapeutic target.

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Evolution of form, function and cross-kingdom interactions in Pezizales

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Pezizales is among the most morphologically and ecologically diverse orders within the Ascomycota. Species of Pezizales produce a diversity of sexual fruiting body forms, including epigeous cup fungi, iconic morels and lorchels (i.e., *Morchella, Gyromitra*) and hypogeous truffles (e.g., *Tuber*), with many lineages also forming asexual mitospore mats. Ecologically, Pezizales encompass saprotrophic, copiotrophic, and pyrophytic life histories, as well as endophytic and ectomycorrhizal associations with plants. Recent hypotheses further suggest that some lineages may engage in bacterial "farming" to meet nutritional demands, highlighting complex cross-kingdom interactions.

Here, we aimed to resolve genus and family-level relationships across the Pezizales to test for phylogenetic patterns underlying genome evolution, ecological transitions, and functional diversification. Using shotgun sequencing of specimens derived from both field collections and herbarium materials, we generated a robust phylogenomic framework for the order. These data substantially improved family-level resolution and provided a basis for testing evolutionary hypotheses concerning morphological and ecological innovations. We also obtained genomic information of prokaryotic organisms associated with Pezizales fruiting bodies.

Our analyses reveal that ectomycorrhizal ecology and truffle-like morphologies have co-evolved independently multiple times across the Pezizales, indicating strong and repeated selective pressures favoring hypogeous fruiting in symbiotic contexts. Genomic signatures of the transition to ectomycorrhizal habit include an increased genome size, with an enrichment of transposable elements and reduction in carbohydrate active enzymes. In contrast, endophytism appears to be widespread among non-mycorrhizal clades, indicating that asymptomatic plant colonization may represent an ancestral or readily accessible ecological strategy. Additionally, volatile metabolite profiles and bacterial-associated community composition within Pezizales apothecia appear to be phylogenetically structured, supporting lineage-specific cross-kingdom interactions and functions. Together, these findings illuminate the repeated evolution of form and function in Pezizales and underscore the central role of phylogeny in shaping fungal ecological and symbiotic diversity.

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Trait tradeoffs drive survival of pyrophilous fungi after wildfires

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Fires are increasing in frequency and severity across the globe. While severe wildfires can reduce microbial richness by up to 80-90%, several "fire-loving" or pyrophilous taxa that were rare or absent pre-fire massively increase in abundance post-fire. Pyrophilous fungi play crucial roles in post-fire ecosystem recovery by metabolizing carbon (C) sequestered in pyrogenic organic matter (PyOM) and enhancing nitrogen (N) cycling. Plant ecologists have long identified traits that determine plant responses to fire, including adaptations such as thermotolerant structures, thick bark, seed banking, and rapid colonization. Pyrophilous fungi likely exhibit similar traits, including rapid growth or colonization and the ability to exploit post-fire resources, such as high N availability and ability to degrade PyOM. Importantly, due to energetic costs or evolutionary constraints, it is likely that traits trade-off such that a single organism does not simultaneously express traits for all strategies. Here, we tested for trait-tradeoffs through complementary genomics, transcriptomics after PyOM amendment, and biophysical assays including hyphal extension rates, thermotolerance, and N emissions from 20 pyrophilous

fungi isolated from burned soils and mushrooms after 7 California wildfires spanning five Ascomycota (Eurotiales, Pleosporales, Sordariales, Coniochaetales, Pezizales) and three Basidiomycota orders (Agaricales, Holtermanniales, Geminibasidiales). We found a dramatic trait trade-off between fast growth and nutrient acquisition, including strong N cycling via fungal denitrification and many genes responsible for PyOM degradation, implying burned environments select for metabolically costly genes despite their evolutionary cost. Adding in publicly available genomes, we used a comparative genomics framework to evaluate genomic signatures of evolution and found that either gene duplication and somatic mutation, or recombination via sexual reproduction, were the primary drivers of fungal genomic variation in PyOM breakdown and N acquisition genes. We also identified cross-kingdom bacterial to fungal horizontal gene transfer as a secondary strategy producing novel aromatic C degradation genes. We used genomics and bioassays to show how evolutionary trade-offs, like prioritizing aromatic C degradation at the expense of rapid growth, enable pyrophilous fungi to thrive post-fire, and uncovered fungal genomic adaptations that can be modeled to predict impacts on global C and N cycling.

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Microbial symbioses and their role in fungal biology

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Rhizopus microsporus is an early-diverging fungal species with major relevance in ecology, agriculture, food production, and public health. Pathogenic strains of *R. microsporus* harbor endosymbiotic bacteria from the genus *Mycetohabitans* (*Burkholderia* sensu lato). These bacterial symbionts are indispensable for the production of toxins, which are necessary for the pathogenicity and defense of *Rhizopus*, as well as for its reproduction. We have recently demonstrated that, in addition to bacteria, *R. microsporus* can also engage in symbiosis with two positive-strand RNA viruses that belong to the genus *Narnavirus.* These bacterial and viral partners are both vertically inherited and modulate the number of sporangiospores and zygospores produced by their fungal hosts.

Over the past years, our research has focused on addressing three significant inquiries:
1. How is fungal metabolism rewired to accommodate these bacterial and viral symbionts?
2. How does the symbiosis between *Mycetohabitans* and narnaviruses influence one another?
3. How do *Mycetohabitans* bacteria and narnaviruses contribute to sexual fungal reproduction in the species *Rhizopus microsporus*?

This seminar will provide some answers to these fascinating questions.

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What is the ecology of *Penicillium* species? Insights from Fleming, forests, and fromage

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Since the discovery of penicillin by Fleming and colleagues, a handful of well-studied *Penicillium* species have provided humans with a wealth of benefits and received considerable attention from the fungal biology research community. Very little is known about the vast majority of other *Penicillium* species that live in natural ecosystems. How many species of *Penicillium* exist in nature? Do *Penicillium* species have distinct biogeographic distributions? What phenotypic and genomic traits explain the distributions of *Penicillium* species around the world? What are the ecological roles of *Penicillium* in microbial communities? From wheels of cheese to forests of New England, this talk will highlight several different projects from our lab that are helping to build an ecological framework for the genus *Penicillium*. Studies in cheese microbiomes have revealed a variety of mechanisms by which *Penicillium* species shape the ecology and evolution of bacterial communities, from secretion of antimicrobial metabolites to altering resource availability. Long-term monitoring of *Penicillium* populations has revealed how labile genomes can cause the rapid evolution of ecologically-relevant traits. Ongoing studies of soil communities in forests and fields of New England are revealing how phenotypic and genomic traits can help predict the distributions of *Penicillium* species. To better harness the potential of *Penicillium* biodiversity, we need to work collectively as a global community of fungal biologists to fully illuminate the natural history and functional biodiversity of this iconic genus.

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Genome-scale model, *in vitro* experimentation, and stable cultures of algae, cyanobacteria, and fungi support a *s*ymbiotic exchange of amino acids for melanin

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Melanin is a complex biomolecule that confers broad resistance to abiotic and biotic stressors in fungi. Polyextremotolerant fungi (PEF) are the only group that constitutively produce melanin, incorporating it into their cell walls, allowing them to colonize rock surfaces, icebergs, and desert soils. However, these extreme oligotrophic ecosystems lack organic matter for consumption. Instead, it is hypothesized that PEF engage in lichen-like symbioses with photoautotrophs to obtain essential carbon and nitrogen for growth. We previously characterized a novel PEF named *Exophiala viscosa,* which we isolated from a biological soil crust (BSC) in the arid tundra of the Canadian Rockies. BSCs are microbial communities that form on barren soils and are rich in algae and cyanobacteria. *E. viscosa* exhibits a unique phenomenon of releasing melanin into its environment, which is documented in only two other organisms. In this study, we used *in silico* genome-scale modeling (GSM) and *in vitro* experimentation to identify metabolic and genetic triggers that induce melanin excretion.

We hypothesized that symbiotic algae and cyanobacteria induce melanin excretion in *E. viscosa* by providing organic carbon and nitrogen. Our previous work established the first GSM of the PEF *E. dermatitidis*, which was used as the foundation for *E. viscosa*'s GSM. With these models, we identified key metabolites, reactions, and regulatory mechanisms involved in melanin biosynthesis. Corroborating experimental results supported our findings, indicating

specific amino acids significantly influence melanin excretion in *E. viscosa.* We believe this is due to an increase in the pool of acetyl-CoA, the precursor molecule for DHN melanin. We propose that cyanobacteria specifically provide amino acids via the GOGAT pathway to *E. viscosa*, supplying carbon and nitrogen sources while also inducing melanin excretion for abiotic protection. In our previous work, we successfully established stable cultures of WT *E. viscosa, Coccomyxa simplex, and Anabaena inaequalis* in oligotrophic media lacking carbon and nitrogen for at least 49 days. These tri-culture results, amino acid drop-in tests, and our model's *in silico* predictions support our hypothesis that amino acids are exchanged for melanin. Moving forward, we aim to perform additional genetic modifications targeting predicted and logic-based melanin regulatory pathways, as well as mimicking melanin from amino acids using media drop-ins in place of algae and cyanobacteria.

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Microfluidic "Fungi-on-a-Chip" platforms for probing fungal interactions

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Soil is one of the most complex systems on Earth, governed by numerous physical, geochemical and biological processes, and provides the ecosystem services vital for all forms of terrestrial life. This 'material' supports a myriad of plants, microorganisms and microfauna and hosts a complex array of interactions taking place between these living elements at the cellular scale. Microbes play a crucial role in the ecosystem services provided by soils to humans and provide several important ecosystem functions that include nutrient cycling, the biocontrol of pathogens and regulation of greenhouse gas emissions. However, despite the importance of microbes in soil functioning, there exists a major knowledge gap concerning the function and dynamics of the soil microbiome and influence of the physio-chemical environment upon microbial interaction and communication at the cellular scale.

The ability to untangle microbial interaction and communication networks in soil, in particular with relation to bacterial-fungal interactions (BFIs), is central to gaining an enhanced understanding of soil microbiome and ecosystem function. In recent years, it has been demonstrated that microfluidic technology offers new opportunities to study whole living organisms and their interactions at a cellular level, affording precise environmental control, high-resolution imaging and the simulation of environmental complexity. The BFI microfluidic device was developed to probe interactions between fungi and bacteria, and has since been adapted to explore a multitude of interactions including fungal-nematode and fungal-fungal interactions. We are now utilising microfluidic technology to study liquid transport within hyphal networks, as well as hyphal-mediated dispersal of bacteria across air-filled regions via the so-called "fungal highway". Herein, the Fungal-Highways Device is presented, which affords quantitative assessment of fungal highway biology at a cellular level.

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Unravelling the Molecular Mechanisms of Bacterial–Fungal Endosymbiosis

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Fungal-bacterial interactions (BFIs) are critically important in agriculture, biotechnology, and medicine. An important feature of BFIs is the ability of bacteria to control and exploit their eukaryotic hosts. The association of the pathogenic fungus *Rhizopus microsporus* with the endosymbiont *Mycetohabitans rhizoxinica* exemplifies a remarkable example of bacteria influencing their host's physiology. We have identified various strategies and effectors used by endosymbiotic bacteria to control their fungal host. For example, we show that a transcriptional activator-like effector (TALE) released by endofungal bacteria is an essential symbiosis factor. By combining microfluidics with fluorescence microscopy, we observed enrichment of TALE-deficient mutants in lateral hyphae. High-resolution live imaging showed formation of septa at the base of infected hyphae, leading to compartmental inclusion of endobacteria. The trapped symbionts show reduced intracellular survival, suggesting a protective host response. Beyond revealing an unprecedented function of TALE proteins, this mechanism of host control illustrates an unusual survival strategy of endosymbionts in the host and provides deeper insights into the dynamic interactions between bacteria and eukaryotes.

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The increase in cell volume and nuclear number of the koji-fungus *Aspergillus oryzae* contributes to its high enzyme productivity.

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While the ratio of nuclei to cell volume is well regulated, it remains largely unexplored in multinucleate organisms. The koji-fungus *Aspergillus oryzae*, traditionally used in Japanese brewing and fermentation for over a thousand years, is now widely utilized in modern biotechnology as a host for enzyme production. We discovered that, over time in culture, hyphae become thicker, resulting in a tenfold increase in cell volume, and the number of nuclei in hyphal cells also increases tenfold, exceeding 200. The increase in cell volume and nuclear number is unique among the investigated *Aspergillus* species and correlates with its high enzyme production capabilities. Since nuclear number and cell volume are correlated, both must increase simultaneously for either to expand. Our analyses identified genetic factors and nutritional environmental signals involved in each of these increases. Increases in nuclear number and cell volume were also observed in other fungi bred for industrial use. This study not only deepens our understanding of the evolutionary processes that promote high enzyme productivity through fungal breeding, but also provides insights into the molecular mechanisms regulating cell volume and nuclear number in multinucleate organisms.

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Lichtheimia: An Emerging Fungal Pathogen in Human Mucormycosis – Insights from Lung-on-Chip and Invertebrate Models

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Lichtheimia is a thermotolerant fungal genus and a leading cause of mucormycosis, a life-threatening angioinvasive infection. Its importance is underscored by its high mortality, especially in immunocompromised hosts, and challenges in rapid diagnosis and treatment. Emerging as the second most prevalent agent in some regions, it presents a significant clinical burden. The genus currently consists of eight species, but this number is likely to increase as taxonomic efforts continue to define new species. Recent advancements in pathogenesis research have been propelled by innovative infection models. Invertebrate models, such as *Galleria mellonella* and *Drosophila melanogaster*, provide a high-throughput, ethically accessible system for virulence studies and antifungal screening. Complementarily, human lung-on-chip models offer a sophisticated, physiologically relevant platform to dissect host-pathogen interactions at the alveolar interface, mimicking critical aspects of invasive pulmonary disease. These models are crucial for elucidating the mechanisms of angioinvasion, immune evasion, and tissue damage, thereby accelerating therapeutic discovery. Together, they bridge the gap between traditional *in vitro* assays and complex mammalian studies, enhancing our understanding of *Lichtheimia* pathogenicity and informing strategies to combat this deadly infection. The pathogenicity of the entire *Lichtheimia* species complex was assessed through invertebrate infection models, and the invasive mechanisms of *Lichtheimia corymbifera* were further elucidated using exemplar lung-on-chip models.

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Fungal structure as inspiration for advanced engineering materials

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In recent years, there has been a greater interest in using fungal materials as a possible alternative for more sustainable engineered materials. While much of this work has investigated the direct implementation of fungal structures into materials, such as using mycelia as a natural binder in biocomposites, many of the characteristic macroscale structures formed by a variety of fungal taxa. This work explores how the structures and inherent mechanical and material properties of two basic types of structures: filamentous networking hyphal structures (such as those seen in the sporocarps of filamentous fungi) and filamentous-gel composites (such as those seen in sporocarps of jelly fungi). While different in microstructure and mechanical resistant, both of these structures provide inspiration for the creation of advanced materials. Porous filamentous networks provide a design that can maximize strength with less material, which can help for the creation of advanced ceramics that can maintain some of the functional properties of these sporocarps. Jelly fungi, on the other hand, provide an example of a natural hydrogel that can achieve greater resistance over many cycles of loading, providing insight to more robust hydrogels. These two examples demonstrate the potential of using fungi and their many forms and functions can be used to create engineered materials that can outperform or provide a more sustainable material option.

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Mycotechnology to the rescue: Biosustainable Production of secondary metabolites by filamentous fungi using carbon sourced from Polystyrene and Polyethylene Plastic Waste

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Plastic waste is an acute threat to the environment and human health. The complete elimination of plastics, however, is an inconceivable solution due to their revolutionary impact on daily life and immense economical contribution. Consequently, long-term plastic sustainability is an urgent matter that requires new approaches to achieve, such as the utilization of microorganisms. We previously have demonstrated the conversion of polystyrene (PS) and polyethylene (PE), two common plastic polymers with poor recycling rates, into metabolically relevant fungal substrates. Further, the sustainable impact of fungi can be broadened by their ability to create complex secondary metabolites that have the potential to replace our current environmentally hazardous synthetic processes like those used in dye manufacturing. In this talk I will present our groups efforts to produce various useful secondary metabolites using our hybrid chemical biological approach. The talk will demonstrate the power of mycotechnology to address issues in plastic waste and sustainability.

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Toward a platform for space mycotecture

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Long-duration spaceflight and off-world exploration will require materials that can be produced in situ while tolerating extreme thermal, vacuum, and radiation environments. Mycotecture—architectural composites grown from fungal mycelium and lignocellulosic or regolith-based feedstocks—offers a biologically regenerative route to such materials, with potential to reduce upmass and integrate with bioregenerative life support and ISRU architectures.

We are developing a modular “mycotecture for space” platform to systematically link fungal strain, substrate formulation, growth parameters, and post-processing to mechanical and environmental performance metrics relevant to space exploration. Using three basidiomycete strains (Ganoderma lucidum, Ganoderma spp., Pleurotus ostreatus) on a custom NASA Woody Substrate with wheat or flax supplements, plus a commercial Ecovative Inc. reference material (ERM), the system generated low-density foams whose elastic modulus and compressive strength approach those of expanded polystyrene. To probe flight-relevant robustness, baked mycomaterial coupons were treated in a planetary simulator under flight-relevant conditions. Ongoing work extends this platform toward flight certification, e.g., flammability and fire-response testing under terrestrial and spaceflight-relevant protocols. Together, these results position mycotecture as a tractable fungal biomaterials and biomaterials–systems biology topic for space exploration, and outline a generalizable platform for evolving genetically tractable fungal strains into space-rated materials.

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Engineering filamentous fungi for waste-to-food conversion

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A great tragedy of our time is that many people go hungry while massive amounts of food are wasted. In the U.S, close to 20% of households are food insecure due to poor access to high-quality nutrition. At the same time, over a third of all food is wasted along the supply chain, causing trillion-dollar economic losses and threatening food security and public health. This includes diverse edible byproducts that are generated in the transformation of crops into foods, including spent brewers’ grain from beer brewing, soybean residue from tofu production, and pomace from fruit and vegetable processing. While byproducts are produced at the million-ton scale, most end up in landfills, a process that ironically costs money to the producer and causes massive greenhouse gas emissions.

Edible filamentous fungi provide a tantalizing solution to this problem, as they have the potential to convert byproducts into nutritious human food. However, to fully leverage the powerful capabilities of fungi for waste-to-food conversion, we need to transition from merely observing what the organisms do in nature, to being able to program their biology as an engineerable solution for human and planetary health. In this talk, I will describe our lab’s efforts developing and deploying synthetic biology tools to engineer edible filamentous fungi for enhanced conversion of waste into food. I will share recent results bridging fundamental discoveries with synthetic biology tool development that now allow us to program fungal flavor and nutrition at the level of molecules and genes. I will also discuss how we are leveraging these tools and insights to develop new foods in collaboration with Michelin-star chefs, using our state-of-the art R & D kitchen at Stanford. By bridging bioengineering with gastronomy, this work promises to lay the foundation for a new generation of delicious and sustainable fungal foods – that happen to be made from abundant byproducts.

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Stress-driven emergence of heritable non-genetic drug resistance

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Drug resistance is the chief cause of treatment failure for therapies targeting chronic and infectious diseases. Whether the emergence of resistance is accelerated by environmental exposure to low levels of therapeutics remains controversial. Here, we report a non-genetic mechanism of stress adaptation that promotes heritable resistance to fluconazole and other widely used antifungal drugs. In the human pathogen *Candida albicans,* transient exposure to subtherapeutic fluconazole doses induces a protective response that we term para-resistance. Like conventional resistance mechanisms, para-resistance is heritable. However, it does not arise from genetic mutations and can revert spontaneously. Key regulators of this state and its associated multidrug resistance include the stress-activated MAP kinase Hog1, the histone deacetylase scaffold Snt1, the global chromatin regulator Rap1, the Sko1 transcription factor, and the Yck2 kinase. Molecules that disrupt biomolecular condensation and prion propagation block the induction of para-resistance, and its acquisition is associated with conformational shifts in these regulatory factors. Para-resistance is common in clinical isolates and, remarkably, passage through the mammalian gut triggers its acquisition, compromising fluconazole’s therapeutic efficacy. Our work defines a pervasive, prion-like epigenetic mechanism of stress adaptation and highlights potential strategies to mitigate the rapid emergence of drug resistance.

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Epimutations driven by RNAi or heterochromatin evoke transient antimicrobial drug resistance in fungi

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Antimicrobial resistance (AMR) is a global health threat emerging through microbe adaptation, driven by genetic variation, genome plasticity or epigenetic process. In this study, we investigated how the *Mucor circinelloides* species complex adapts to the antifungal natural product FK506, which binds to FKBP12 and inhibits calcineurin-dependent hyphal growth. In *Mucor bainieri*, most FK506-resistant isolates (90%) were found to be unstable and transient, readily reverting to drug sensitivity when passaged without drug, and with no associated DNA mutations. In half of the isolates (50%), FK506-resistance was conferred by RNAi-dependent epimutation in which small interfering RNAs (siRNAs) silenced the *fkbA* encoding FKBP12 post-transcriptionally. In contrast, most of the remaining FK506-resistant isolates (40%) were found to have undergone heterochromatin-mediated silencing via H3K9 dimethylation, transcriptionally repressing *fkbA and* neighboring genes. In these heterochromatic epimutants, only minimal enrichment of siRNA to the *fkbA* locus was observed, but in three of the four examples, siRNA was significantly enriched at a locus distant from *fkbA*. A similar mechanism operates in *Mucor atramentarius*, where FK506 resistance was mediated by ectopic heterochromatin silencing *fkbA* and associated genes with siRNA spreading across the region. Heterochromatin-mediated *fkbA* epimutants exhibited stability during *in vivo* infection, suggesting epimutation could impact pathogenesis. These findings reveal that antifungal resistance arising through distinct, transient epimutation pathways involving RNAi or heterochromatin, highlighting adaptive AMR strategies employed by ubiquitous eukaryotic microbes.

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Death or disomy: perturbing spindle checkpoint kinases in the fungal pathogen *Cryptococcus neoformans*

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Fungi change shape and reconfigure their growth & division processes in response to environmental stresses. Under severe stress, such as during human infection, individual fungal cells adapt rapidly. Adaptation is often accompanied by large-scale genomic alterations including polyploidy and aneuploidy (an abnormal number of chromosomes). Aneuploidy produces genetic diversity within the fungal population and selection will favour those cells best suited to growth in the stressful conditions. Aneuploidy can also generate drug-resistance, which is a serious problem in clinics world-wide.

During an infection, Cryptococcus cells become large and polyploid and this protects them from immune cells. These polyploid cells then produce haploid daughters, through a fascinating reductive division. We study cell division, chromosome segregation and aneuploidy in *Cryptococcus neoformans,* including mechanisms of error correction and spindle checkpoint signalling. This checkpoint acts as a surveillance system: only when all chromosomes are attached appropriately is the checkpoint satisfied, allowing high fidelity chromosome segregation to take place.

We will report our analysis of *Cryptococcus mad*, *bub1* and *mps1* mutants, the use of SAC-specific protein kinase inhibitors and perturbation of PP1 and PP2A mitotic phosphatases. Certain perturbations lead to increased rates of chromosome mis-segregation and aneuploidy. Our recent analysis of *mps1* and *bub1* deletion mutants demonstrates that both are very defective in the formation of viable, polyploid titan cells. In addition, both null mutants appear to be kept alive by extra copies of chromosome 6. On the hunt for specific suppressors, we are evolving these strains in the lab. We are also studying, through quantitative whole cell proteomics, how these mutants cope with the proteotoxic stress caused by their aneuploidy.

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Functional dissection of the RNAi pathway in the oomycete *Phytophthora sojae*

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The oomycete *Phytophthora sojae* is a devastating pathogen of soybean, causing multi-billion US dollar losses every year across the world. Due to its economic importance and well-established toolkit, *P. sojae* has been developed as a model system to study oomycete biology, genetics, and pathology. Epigenetic regulation enables organisms to rapidly adjust gene activity without altering their underlying DNA sequence, and gene silencing is one of the most important epigenetic mechanisms for protecting genome integrity and fine-tuning gene expression. Although multiple pathways contribute to gene silencing, they are generally grouped into two major categories: Transcriptional Gene Silencing (TGS), which involves DNA methylation and histone modifications, and Post-Transcriptional Gene Silencing (PTGS), which is mainly mediated by RNA interference (RNAi) via small interfering RNA (siRNA). In previous studies, we identified the core RNAi components across *Phytophthora* species and uncovered their diverse evolutionary trajectories. We also observed that TGS-associated histone marks, including H3K9me3 and H3K27me3, strongly coincide with siRNA hotspots across the *P. sojae* genome, especially at effector loci encoding small secreted virulence proteins. These findings suggested a potential link between RNAi, heterochromatin, and effector regulation, yet the RNAi pathway itself has not been functionally characterized in *P. sojae*. In this study, we generated deletion mutants of three core RNAi components, namely RDP1, *DCL1*, and *DCL2*, and systematically investigated their biological functions. Deletion of *rdp1* did not noticeably affect siRNA production or virulence, suggesting that additional RNA-dependent RNA polymerases participate in RNAi. In contrast, deletion of either *dcl1* or *dcl2* disrupted siRNA profiles and led to impaired growth and reduced virulence. Together, these results demonstrate that the Dicer-mediated RNAi pathway is critical for *P. sojae* development and infection. In addition, comparative analysis of siRNA profiles revealed pronounced differences in siRNA enrichment between *P. sojae* and *P. infestans*, suggesting that RNAi functions have diverged considerably during *Phytophthora* evolution. We are now investigating how RNAi interacts with heterochromatin and how this relationship shapes effector gene regulation.

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Paralogs of the *Candida albicans TLO* (telomere-associated) gene family form interconnected functional networks through incomplete redundancy

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Gene families form through a process of repeated gene duplications. Although most cells containing duplicated genes are culled from the population, paralogs that enhance organismal fitness may be retained in a population. Retained paralogs are often thought to increase gene expression, encode a specialized function, or acquire novel function. While the function of gene duplicates has been studied in detail, little work has explored how repeated duplication impacts paralog redundancy and may restrict the emergence of new paralogs or novel function. Here, we constructed a panel of single deletion mutants for each of the 14 members of the *Candida albicans* telomere-associated (*TLO*) gene family to determine the degree of molecular and biological redundancy among paralogs from a lineage-specific expansion. Tlo proteins function as interchangeable subunits of the Mediator transcriptional regulatory complex and have the potential to alter gene expression and an array of cellular responses. Approximately 80% of the phenotypic assays performed with single gene deletion strains displayed redundancy. However, mutants for all 14 paralogs displayed non-redundant functions ranging from carbon utilization to *in vivo* virulence. Loss of single *TLO* genes produced similar trends in redundant and non-redundant changes in gene expression that disproportionately affected cell cycle regulation, morphology, and the response to stress. Importantly, sequence divergence between paralogs correlated with the frequency

of altered phenotypes in single *TLO* mutants, indicating the acquisition of non-redundant function with increased evolutionary distance. Double mutants lacking two *TLO* genes produced both positive and negative synergistic phenotypes, suggesting that crosstalk or coordinated regulation is common among paralogs. Together, this study demonstrates that recently emergent paralogs acquire non-redundant functions despite often retaining redundancy with other gene family members to create a highly interconnected functional network.

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Epigenetic regulation of *Starship* activity in *Pyricularia oryzae*

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Pyricularia oryzae (synonym *Magnaporthe oryzae*) is the causative agent of blast disease and a multi-crop threat to global food security. Many *P. oryzae* strains reproduce asexually and therefore require the creation of genetic variation without a sexual cycle. For instance, strains of *P. oryzae* have an association between genomic regions with increased genetic variation and specific histone modifications, but the mechanism and causality driving the association remains unknown. *Starships* are a recently discovered group of giant fungal transposons that mobilize genes as cargo and can horizontally transfer within and between species, thus making *Starships* a potential source of genetic diversity in asexual pathogens. To understand if *Starships* impact *P. oryzae* evolution, *Starships* were annotated and characterized in 13 diverse *P. oryzae* strains. Two distinct families of *Starships* and related *Starship*-like regions were identified that differed in their activity, sequence evolution, and epigenetic modifications. While many elements were enriched for heterochromatic histone modifications, one family largely lacked such epigenetic marks. *Starships* from this family have repeatedly transferred across the *Pyricularia* genus, exhibit higher rates of structural variation, and tend to be depleted of repeat-induced point mutations. However, contrasting histone modification profiles within this family suggests that *P. oryzae* genomes may be targeting *Starships* to repress their activity. Our findings demonstrate that the epigenetic modifications associated with *Starships* underpin the impacts of these giant transposons on fungal genome evolution and biology.

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Strain-specific redox balancing for *Aspergillus fumigatus* pathoadaptation and antifungal drug susceptibility

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Significant genotypic and phenotypic diversity exists in the *Aspergillus fumigatus* population whose significance for pathogenesis and drug susceptibility is only beginning to emerge. The complexity of this diversity poses both challenges and opportunities to define mechanisms of pathogenicity, virulence, and antifungal drug susceptibility. Previously, we identified *A. fumigatus* isolates with an increased ability to grow in low oxygen conditions found at sites of infection and with enhanced virulence in murine models of invasive pulmonary aspergillosis. However, the underlying mechanism(s) for increased low oxygen fitness and virulence remain ill-defined. An experimental evolution experiment with the reference strain AF293 revealed a novel gene cluster located on a moveable Starship element necessary and sufficient for low oxygen fitness and virulence. These strains provide a basis for defining mechanisms of low oxygen adaptation in a host-relevant context. With these strains, we discover that an enhanced ability to reduce nitrate and catabolize ethanol provides increased biomass generation in low oxygen conditions and the ability to form more robust biofilms. In contrast to strains less fit in low oxygen conditions, the ability to reduce nitrate and catabolize ethanol is necessary for the low oxygen growth of these biofilm-fit strains. Loss of this ability impacts pathogenesis and significantly impacts biofilm antifungal drug susceptibility. These data suggest important redox balancing metabolism that also provides key substrates for cell wall and ergosterol biosynthesis under oxygen limited conditions. These observations have potential implications for the emergence of antifungal drug resistance in the environment and the ability of a given strain to persist and progress disease in the dynamic lung infection microenvironment. The extent to which this metabolic rewiring and flexibility is observed across the *A. fumigatus* phylogeny is under further investigation.

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DNA adenine methylation as a key epigenetic switch in non-Dikarya fungi

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Epigenetic modifications and the resulting changes in chromatin structure play a crucial role in regulating gene expression. Unlike most eukaryotes, where 5-methylcytosine (5mC) is the predominant DNA epigenetic mark, the main form of DNA modification in non-dikarya fungi is N6-methyldeoxyadenine (6mA), a feature shared with ciliates and green algae. In these fungi, 6mA accumulates in promoter regions, forming MACs (methylated adenine clusters) that are associated with active transcription. This epigenetic modification is essential in *Rhizopus microsporus*, and likely in other non-dikarya fungi, due to the large number of genes whose expression depends directly or indirectly on 6mA. Consistently, *in vivo* inhibition of adenine methylation leads to reduced virulence in a mouse infection model.

To investigate how 6mA regulates transcription, we generated conditional mutants to explore its interplay with other epigenetic marks. Our analyses reveal that 6mA correlates with strong nucleosome positioning and with an enrichment of activating chromatin features, such as H3K4 methylation and the histone variant H2A.Z, suggesting that 6mA contributes to the establishment of open euchromatin regions conducive to transcription initiation.

To gain insights into the 6mA-mediated chromatin modification and transcriptional activation, we generated mutants in genes encoding H3K4 methyltranferases Set1 and the H3 acetyltransferases Gnc5 to dissect the interplay between 6mA and other epigenetic modifications in shaping the transcriptional activity. In addition, we identified two candidate 6mA-binding proteins that may link 6mA with chromatin-modifying enzymes to establish the chromatin environment required for transcription. Together, these findings deepen our understanding of the regulatory the role of DNA adenine methylation in eukaryotes.

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From Environmental Microbiomes to Human Health: Fungal Pathogen Ecology Across Scales

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Microbial community research has historically emphasized bacteria, even though fungi play essential ecological roles and profoundly impact human health. Through large-scale analyses of global microbiomes, we identified bacterial and fungal taxa that are both prevalent and abundant across diverse biomes and environmental conditions. These generalists share common ecological and genomic traits, including larger genomes enriched in secondary metabolism genes, and the ability to influence community structure by promoting higher alpha diversity. Building on these insights, we are extending similar approaches to investigate the global distribution and ecology of human fungal pathogens that primarily reside in the environment. Our findings reveal that pathogen abundance in the environment frequently does not a mirror clinical epidemiology. Furthermore, climate metadata reveal that solar radiation and average temperature are strongly associated with the abundance of certain human fungal pathogens, while precipitation exerts a stronger influence on others. Together, this work advances our understanding of the global ecology of fungi by linking generalist strategies with the environmental dynamics of pathogenic species. These results have important implications for assessing exposure risk, anticipating the impacts of climate change, and addressing the One Health dimensions of antifungal resistance.

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Fitness effects of multi-fungicide resistances in *Aspergillus fumigatus*

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Aspergillus fumigatus is an opportunistic human pathogenic fungus mainly found in plant-degrading material. Although not a plant pathogenic fungus, within this substrate it is exposed to multiple classes of agricultural fungicides. It has been shown that exposure to agricultural triazoles leads to cross-resistance of clinical triazoles, but the evolutionary impact of exposure to other fungicide classes is unknown. Recent genomic surveys have shown correlations of variants assumed to lead to resistance between DMI, QOI, SDHI, and MBC fungicides, suggesting ongoing adaptation to current agricultural practices. To identify the full diversity of genetic resistances, we used literature to identify target genes and screened for common (>0.3%) non-synonymous variants across 1500 available A. fumigatus genomes. We identified 12 resistance variants, spread across seven genes, with five resistant variants being shown for the first time. Natural isolates that contain one resistance likely contain multiple, resulting in multi-fungicide resistance phenotypes. On the other hand, certain resistance variants like sdhB H270Y and sdhC S105I were never found together, suggesting negative fitness costs of some combinations, particularly alleles of the same gene or in members of the same protein complex.

To test the impact of these resistance variants, we introduced point mutations with CRISPR/Cas9 using co-transformation of a transient hygromycin resistance plasmid. All predicted resistance variants caused resistance, including newly described alleles to the aminopyrimidines class of fungicides. We did not observe a fitness cost for single mutants or naturally co-existing combinations. However, by constructing combinations not observed to occur in nature, we show significant fitness reductions. For instance, multiple variants in the SDH complex are not tolerated (more than 40% growth reduction), and likewise within beta-tubulin, the target of MBCs (more than 70% growth reduction). Our results indicate that natural selection is shaping a combination of resistance variants in different genes and for different fungicide classes and provides additional evidence that the continued use of non-triazole fungicides are indirectly selecting for triazole resistance.

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Genomics insights into the *Bipolaris gigantea* host range expansion from grasses to hemp

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The ascomycete *Bipolaris gigantea* has recently emerged as a significant pathogen of hemp (*Cannabis sativa* containing <0.3% THC), triggering severe outbreaks following the legalization and resurgence of hemp production. Since its first report in Kentucky in 2014, *B. gigantea* has caused leaf spot epidemics on hemp in 15 eastern states, with crop losses reaching up to 100%. Widespread epidemics on the invasive grass *Microstegium vimineum* in the same region led to the hypothesis that grass-infecting populations served as sources of inoculum driving emergence on hemp. To investigate gene flow and reproductive dynamics of *B. gigantea* among hosts, we conducted a field survey in September 2024, collecting over 250 samples from six locations across Kentucky. From these, we selected ten representative isolates from hemp and adjacent grass hosts for PacBio HiFi long-read sequencing and *de novo* genome assembly. Assembly sizes spanned 30–32 Mb, with scaffold N50 values of 2.8–3.7 Mb, L50 of 3–6, and GC content near 50–52%. Multiple scaffolds terminated in telomeric repeats, indicating near chromosome-scale contiguity. BUSCO completeness for Ascomycota database was consistently high (~98–99%), indicating nearly complete assemblies with low fragmentation. Average pairwise nucleotide identity between hemp- and grass-derived isolates exceeded 99%, indicating a shared population across hosts and supporting *Bipolaris gigantea*'s recent host range expansion from grasses to hemp. Mating-type searches identified both idiomorphs (MAT1-1 and MAT1-2), each present in isolates from hemp and *M. vimineum*, revealing the coexistence of complementary mating types in the landscape. Additionally, RNA-seq data from a hemp isolate (A4-1) were generated and used as transcript evidence to support structural gene prediction and functional annotation of a representative reference genome, with annotations subsequently transferred to the remaining assemblies. Comparative genomic analyses revealed high genome collinearity and shared gene content between isolates from hemp and grass, which together with ecological data, indicate ongoing cross-host gene flow. Overall, these genomic resources provide a valuable foundation for investigating the genomic basis of host expansion and have important implications for the management of *B. gigantea* in hemp production.

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Re-parameterising wheat stem rust (*Puccinia graminis* f. sp. *tritici*) epidemiological models to explore disease dynamics in a changing climate

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Climate change is driving fluctuations in environmental conditions that are reshaping the distribution, biology, and aggressiveness of many plant pathogens, increasing the threat of disease-related yield losses in major crops. One concerning consequence is the potential return of historically devastating diseases as climate change alters their distribution [1].

In the United Kingdom (UK), reports of wheat stem rust caused by *Puccinia graminis* f. sp. *tritici* (*Pgt*), a basidiomycete rust fungus, have risen in recent years, suggesting a potential resurgence of this disease that was once vanquished in the region [2, 3]. Because *Pgt* exhibits relatively high thermal requirements for infection and development, ongoing climatic warming is expected to modulate epidemic risk. Yet, most epidemiological models for wheat stem rust are still parameterised using data from North American *Pgt* isolates collected in the mid-20th century, limiting their relevance under current climatic and pathogen population conditions [3].

To generate updated, regionally relevant epidemiological parameters, we performed controlled-environment experiments using contemporary UK *Pgt* isolates to quantify the effects of temperature and leaf wetness duration on key stages of the pathogen life cycle. We measured epiphytic fungal development leading to infection, the *in planta* latent period, and sporulation dynamics across a range of environmental regimes. These experiments yielded revised estimates of cardinal temperatures and leaf wetness thresholds for UK *Pgt* isolates fungal development.

These new parameters are being integrated into climate-driven epidemiological models of infection and disease development to refine projections of future wheat stem rust risk in the UK. Our findings advance understanding of infection biology in modern *Pgt* populations and strengthen preparedness of the UK for the potential re-establishment of this high-impact wheat disease.

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The last of cats: Zoonotic spillover, evolution, and the continental expansion of *Sporothrix brasiliensis*

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The rise of *Sporothrix brasiliensis* in the Americas echoes the premise of HBO's The Last of Us, where a fungal pathogen breaches thermal and host barriers to unleash a continental epidemic. Unlike the fictional *Cordyceps*, however, this threat is real: *S. brasiliensis* has evolved into a hypervirulent, distinct species that exploits domestic cats (*Felis catus*) as superspreaders. This emergence is driven by a critical evolutionary jump in thermal tolerance and virulence, transforming an ancestral saprophyte into a formidable threat capable of direct, bite-mediated transmission from cat-to-cat and cat-to-human, causing disease ranging from subcutaneous to severe disseminated sporotrichosis in warm-blooded vertebrate hosts.

We present a comprehensive molecular and genomic survey that integrates the population genetics of 519 *Sporothrix* isolates (408 *S. brasiliensis*, 76 *S. schenckii*, and 35 *S. globosa*) with novel insights into wildlife reservoirs. Population genetic analysis identified Rio de Janeiro (Southeast) as the center of diversity, harboring complex population structures with both *MAT1-1* and *MAT1-2* idiomorphs, indicating the potential for sexual recombination. Conversely, the rapid expansion of *S. brasiliensis* into northern and northeastern Brazil is driven by founder effects (usually *MAT1-2*), where low-diversity lineages are established via the introduction of infected animals. This dual pattern highlights a highly adaptable pathogen capable of both local diversification and rapid, clonal expansion.

Furthermore, we provide molecular evidence of spillover beyond the urban domestic cycle. The screening of roadkilled vertebrates in the Atlantic Forest revealed *S. schenckii*, *S. brasiliensis*, and *S. globosa* in the visceral organs of wild felids (*Leopardus guttulus*), opossums (*Didelphis albiventris*), agoutis (*Dasyprocta* spp.), birds (*Columbina picui*), and reptiles (*Oxyrhopus* spp.). These findings challenge the strict delineation between synanthropic and sylvatic cycles, suggesting that wildlife serve not as accidental dead-end hosts but as competent reservoirs capable of sustaining fungal populations. This sylvatic establishment creates a novel evolutionary arena for the emergence of genotypes that may eventually re-enter the urban sphere.

Therefore, the convergence of founder-driven expansion, zoonotic spillover, and rapid evolution underscores the urgency of establishing a One Health genomic surveillance framework to address the emergence of human and animal sporotrichosis.

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Human-mediated hybrid zones between amphibian-killing chytrid lineages produce second generation recombination

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Global biodiversity is under threat from introductions of non-native fungal disease. The pathogenic chytrid, *Batrachochytrium dendrobatidis* (Bd), causes chytridiomycosis – the infectious disease implicated in frog, toad, and salamander population declines worldwide. Where this fungus has been introduced, a single hypervirulent strain (Bd-GPL) proliferates through host populations. In the southern Atlantic Forest of Brazil, recent human introduction brought the globally invasive Bd-GPL strain into secondary contact with a distantly related, endemic strain, Bd-Brazil. In most anthropogenically mediated secondary contact scenarios such as this one, the epidemiological and evolutionary consequences of strain interaction remain unknown. We found that Bd, long considered obligately asexual, is capable of second-generation hybridization following the human induced contact of divergent lineages. Using whole-genome sequencing of fungal isolates cultured from wild-infected Brazilian frogs, we characterize the hereditary relationships among pathogen populations in this contact zone. Our analyses reveal regions of the Bd genome that are potentially driving adaptive variation among invasive and endemic strains. The patterns of hybrid inheritance we observe offer new insights into the genetic underpinnings of fungal reproductive isolation, the process which ultimately results in speciation of emerging fungal diseases. Finally, we compare our hybrid sequences to a newly discovered zone of Bd hybridization in South Africa and reveal parallel patterns of second-generation introgression. These newly observed patterns of hybridization we describe are of particular ecological and evolutionary concern because they demonstrate the ability of anthropogenic change to drive novel recombinant genetic variation in a deadly pathogen. These findings show how humans are actively creating new evolutionary trajectories for emerging diseases, such as chytridiomycosis, by creating novel mating opportunities between previously allopatric strains in different regions of the globe.

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Contrasting environmental and clinical isolates of *Coccidioides*, the causative agent of Valley Fever

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Coccidioidomycosis (Valley fever) is an emerging, potentially life-threatening fungal disease with an estimated burden of over 200,000 cases in the U.S. each year. Humans become infected by inhaling wind-dispersed spores of *Coccidioides*—a soil-dwelling fungus frequently associated with desert mammals. Reducing exposure to environmental sources remains the primary means of disease prevention, as there is no vaccine for coccidioidomycosis and few therapeutic options. Yet whether all environmental sources of *Coccidioides* pose equal disease risk to humans remains unknown. In one possible scenario, colonizing the human lung could represent a selective barrier that only *Coccidioides* of certain phenotypic profiles are able to breach. Alternatively, the human host could be selectively indistinguishable from *Coccidioides*' native rodent niche, such that any strain would be capable of human infection.

A powerful approach to address this question is to compare isolates of *Coccidioides* derived from soil vs human tissue, yet the former are exceedingly rare owing to the practical difficulties of surveying for a microscopic fungus across thousands of acres of desert soil. Here, we overcome this challenge by leveraging a novel resource: 17 genomes of *C. immitis* from its endemic environment (California's San Joaquin Valley), generated through extensive soil sampling over the last decade. Combining these with previously sequenced genomes of *C. immitis* from infected patients in the same region, we conducted comparative genomic analyses to identify loci differentiated between these two groups and to detect signatures of differing selective pressures between soil and human infection settings. To date, we have identified 29 genes with significantly elevated FST between soil and host-infecting strains. These candidate genes include cell well protein PhiA (involved in conidia formation), two NADH-ubiquinone oxidoreductase subunits (linked to oxidative stress responses and pathogenicity in other fungal species), and a multidrug resistance protein. Ongoing analyses using McDonald-Kreitman and Hudson-Kreitman-Aguadé (HKA) tests will evaluate whether these candidate genes likely arose due to neutral processes or selection associated with host infection. These FST outliers represent compelling targets for functional analyses, potential drug development, and expanded mechanistic understanding of virulence in this emerging pathogen.

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Reductive evolution in *Paracoccidioides lobogeorgii*, causing Jorge Lobo's disease

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Jorge Lobo's disease (JLD) is a chronic, mutilating cutaneous mycosis restricted to the Amazon basin and caused by the uncultivable fungal pathogen *Paracoccidioides lobogeorgii* (former *Lacazia loboi*). Despite more than a century of clinical aspects, the basic biology, virulence mechanisms, and evolutionary history of this pathogen remain largely unknown due to the inability to culture it in vitro. Here, we establish the first standardized culture-independent workflow for the isolation of viable *P. lobogeorgii* yeast cells directly from infected BALB/c mouse footpads using combined mechanical dissociation, enzymatic digestion using trypsin and colagenase, and selective purification. Isolated cells retained structural integrity, metabolic activity, and

distinctive chain-like morphology, enabling direct experimental investigation. Using this material, we generated and annotated the first draft genome of *P. lobogeorgii* (30.9 Mb, 249 contigs, contig N50/N90 of 233/61 kb), which revealed a markedly reduced gene repertoire relative to its dimorphic relatives (*P. brasiliensis*, *P. lutzii*, and *P. americana*). BUSCO analyses revealed markedly reduced genome completeness in *P. lobogeorgii* (75%) compared to 93–95% in other *Paracoccidioides* species, suggesting extensive gene loss and/or high sequence divergence associated with host-restricted evolution. Comparative genomic analyses demonstrated pervasive contraction of metabolic pathways, secretory proteins, CAZymes, peptidases and transcription factors consistent with extreme host dependence and long-term reductive evolution. Despite this contraction, the species retains a fully conserved Onygenales core genome and exhibits lineage-specific expansions linked to stress response, cell wall remodeling, and cofactor-dependent enzymatic processes. Molecular evolution analyses across 6,771 orthogroups revealed overwhelming purifying selection, with a small subset of genes under positive selection enriched for host–pathogen interaction and cell surface functions. Macrophage co-culture assays further showed limited phagocytic internalization and long-term extracellular persistence, supporting a model of immune evasion and chronic granulomatous survival. Together, our results provide the first integrated experimental and genomic framework for *P. lobogeorgii*, positioning it as a highly specialized, host-restricted lineage within *Paracoccidioides*. This work unravels fundamental aspects of the biology and evolution of one of the most enigmatic human fungal pathogens and establishes a foundation for future studies on pathogenesis, immunity, and therapeutic vulnerability.

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Environmental triggers, including immune responses, enhance morphotype formation and dissemination in a CD4+ T Cell-Deficient model of cryptococcosis

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Cryptococcus neoformans is an opportunistic fungal pathogen that disproportionately affects immunocompromised individuals, particularly those with $CD4^+$ T cell deficiencies. Since the disseminated brain infection is both the driver of mortality and the presenting illness, understanding the mechanisms of dissemination and pathogenesis in these vulnerable populations is crucial for developing effective therapies. Our goals here are to elucidate the mechanisms underlying cryptococcal dissemination and how they change in $CD4^+$ T cell-deficient mice compared to wild-type mice. $CD4^{-/-}$ mice exhibited increased susceptibility to *C. neoformans* infection, including higher fungal burdens in extrapulmonary organs and more rapid time-to-endpoint compared to wild-type mice. Interestingly, infected $CD4^{-/-}$ mice displayed a compensatory increase in neutrophils, both quantitatively and functionally. Furthermore, these mice showed a significant increase in the proportion of *C. neoformans* 'seed cells' in the lungs. This dissemination-prone cryptococcal morphotype is a small, yeast-form cell that forms in the lungs over the course of infection and exhibits enhanced ability to enter extrapulmonary organs. Seed cells are induced by phosphate, including by DNA, and their formation is regulated by a network of environmentally responsive transcriptional regulators. This network includes regulators involved in the yeast-to-hyphal in other human fungal pathogens. However, *C. neoformans* grows primarily as a budding yeast, so this conserved network has been repurposed to regulate a yeast-to-yeast switch. Moreover, we hypothesize that host tissue damage and cell death creates a phosphate pool that could be a key driver of seed cell formation. Neutrophil extracellular trap (NET) formation and pyroptosis both release DNA into the tissue environment, and DNA induces seed cells. Our findings indicate that $CD4^+$ T cell deficiency leads to increased susceptibility to *C. neoformans* and the host immune system compensates with an elevated neutrophilic response. However, this compensatory mechanism appears to be detrimental, as the neutrophils inadvertently promotes the formation of *C. neoformans* seed cells, thereby enhancing fungal dissemination and exacerbating disease severity.

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On the origins and evolution of the necrotrophic effector *ToxA* within giant Starship transposons

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Our work over the last several years has sought to understand how transposons drive adaptive evolution in plant pathogenic fungi, namely the necrotrophic wheat pathogens causing spot blotch, tan spot and Septoria nodorum blotch. Fungal genomes are highly variable in their transposon content and contain many novel transposons that have yet to be functionally characterised. We now have very detailed examples of how transposons drive the duplication, pseudogenisation, and horizontal movement of effector genes, all of which can have real world impacts on the incidence and severity of disease in the field. This talk will explore the evolutionary history of the capture and horizontal transfer of a novel Class II DNA transposon, ToxTA, by different giant *Starship* transposons. ToxTA carries the well-characterised necrotrophic effector *ToxA,* whose origins have remained a source of debate since the first horizontal transfer was reported in 2006. Here, we explored the diversity of the two best characterised *Starships,* Sanctuary and Horizon, within the *Bipolaris, Parastagonospora* and *Pyrenophora* genera in order to better define the direction and timing of horizontal gene transfer between these the species carrying the *ToxA* effector. I will also present some preliminary work towards establishing novel experimental systems to study the mechanisms of *Starship* transposon mobilisation and horizontal transfer outside of their native fungal hosts.

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Pikobodies: What does it take to bioengineer NLR immune receptor-nanobody fusions

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Recurrent epidemics caused by plant pathogens pose a significant threat to global food security. While modifying natural components has been successful to retool the plant immune system, this approach can be rapidly rendered ineffective by the emergence of new pathogen strains. Made-to-order synthetic plant immune receptors, however, offer a promising solution for tailored resistance to pathogen genotypes present in the field. Here we define four technical and conceptual advances which enabled successful engineering of NLR immune receptor-nanobody fusions, termed Pikobodies, for this purpose. These advances include **1)**evolutionary analysis to identify domain boundaries, **2)** structural modelling to predict nanobody integration boundaries, **3)** rapid screening using transient immune and disease assays, and **4)** guided mutagenesis to abolish autoactivity triggered by some nanobody integrations. We show that plant immune receptors can serve as effective scaffolds for nanobodies that bind fluorescent proteins (FPs), and that the resulting immune receptor-nanobody fusions trigger immune responses and confer resistance against plant viruses expressing FPs. Since nanobodies can be raised against

most molecules, immune receptor-nanobody fusions have the potential to generate resistance against most plant pathogens and pests delivering effectors inside host cells.

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Intracellular metabolism of the fungal pathogen Histoplasma capsulatum

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The fungal pathogen Histoplasma capsulatum infects and proliferates within host phagocytes, turning these normally fungicidal immune cells into permissive host environments. Within the phagosomal compartment of host cells, Histoplasma must acquire suitable resources for proliferation. However, the host molecules used by Histoplasma for energy and biosynthesis are largely undefined. Initial genetic studies implicated amino acids as the primary source of nutritional carbon used by Histoplasma within the phagosome. Histoplasma is unable to grow on proteins unless they are first digested by proteinases suggesting that amino acids are acquired either as peptides or free amino acids. To fully define the intracellular metabolism of Histoplasma, we integrated genetic and transcriptional studies with targeted metabolomic profiling of macrophages and intracellular Histoplasma yeasts. Our results reveal that not all amino acids are sufficiently present within the phagosome, indicating that Histoplasma uses select metabolic pathways for intracellular proliferation. Metabolite analysis of intracellular yeasts shows cysteine, leucine, and phenylalanine are consumed by Histoplasma within the phagosome. 13C-metabolic flux analysis showed significant carbon flux through the mannitol pathway and the methylcitrate cycle, which may facilitate NADPH generation and detoxification of branched chain amino acid intermediates, respectively during amino acid catabolism. Together these results provide a framework for understanding how Histoplasma acquires and metabolizes nutritional molecules to proliferate within the phagosome of host cells during infection.

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Understanding how Candida albicans, a fungal species, is adapted for survival in the human gut microbiome

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The World Health Organization (WHO) has classified fungal pathogens like *Candida albicans* and *Candida auris* as critical priority pathogens due to their capacity to cause severe, often lethal medical complications. Understanding how fungal pathogens evolve is essential to reveal how they differ from their close relatives and to anticipate emerging threats. I aim to address a simple yet important question: why does *C. albicans* successfully colonize the human gut, while its close relatives including *Candida dubliniensis* cannot? To identify the adaptations that enable *C. albicans* to dominate this niche, I performed a systematic screen of 70 diverse fungal species under conditions that mimic the gut. This revealed a key discovery: the ability to utilize host-derived sulfur compounds acts as a powerful ecological filter. The metabolism of these compounds in fungi is entirely unexplored.

Comparative analysis across 1,000 fungal genomes spanning 500 million years suggests that this metabolic capability arose through small modifications of existing genes and regulatory elements, highlighting a lineage-specific adaptation that enables *C. albicans* to thrive in the gut. These findings provide new insight into the molecular and evolutionary mechanisms by which fungi colonize host niches and interact with microbial communities, establishing a framework for understanding gut-specific adaptations in fungal pathogens and informing strategies to combat fungal infections.

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Dissecting appressorium-mediated plant infection by FIB-SEM Volumetric EM and Cryogenic Electron Tomography

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Fungal pathogens pose a significant threat to global agriculture and understanding their virulence mechanisms is essential for developing durable disease control strategies. Many of the most destructive plant infecting fungi form specialized infection cells called appressoria. Appressoria develop on the host leaf surface, undergo rapid cytoskeletal reaorganisation, and generate enormous turgor pressure that breaches the plant cell wall and enable pathogen penetration. Disruption of appressorium development either by genetic mutation or by chemical inhibition disrupts pathogenicity, making this process an attractive target for innovative crop protection approaches. However, the specialized and highly dynamic nature of appressoria have largely prevented the resolution of their underlying mechanisms using conventional structural biology approaches. Here, we present a method to germinate fungal spores and induce appressorium formation directly on cryo-EM grids. Appressoria formed on grids display normal cytoskeletal rearrangements, septin aggregation and turgor generation, effectively recapitulating their invasion mechanism and exerting forces on the grid film. This breakthrough allows us, for the first time, to use techniques such as FIB-Milling, FIB-SEM Volume EM and Electron Tomography in cryogenic conditions to study the cellular architecture of appressoria and the establishment of fungal disease at unprecedented resolution. These findings offer a robust methodology to resolve the biological processes driving appressorium-mediated plant infection, including repolarization events that initiate host penetration. Furthermore, this approach could lead to the development of novel strategies to combat some of the most devastating plant diseases in agriculture.

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IL-17 derepresses cytokine translation to induce immunity against candidiasis

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Candida albicans asymptomatically colonizes the oral cavity in most healthy individuals but can transition to a pathogenic state upon local or systemic immune compromise, leading to oropharyngeal candidiasis (OPC). During OPC, *C. albicans* invades oropharyngeal epithelial cells, which respond by secreting proinflammatory mediators that recruit and activate neutrophils. This response is amplified by IL-17, produced by various lymphocyte subsets. Human and animal studies confirm the central role of IL-17 signaling in host defense against OPC.

To elucidate oral epithelial responses to *C. albicans*, we performed bulk RNA-seq on infected human oral epithelial cells. Infection induced marked upregulation of transcripts encoding transcription factors such as c-FOS, c-JUN, NFκB1, and NFκB2, with immunoblot analysis confirming their phospho-activation. Consequently, genes encoding pro-inflammatory cytokines such as *IL-6*, *CXCL8*, *CSF2*, and *CSF3* exhibited massive mRNA induction—increasing by several hundred- to thousand-fold. However, their corresponding protein levels rose only modestly (2–4 fold), indicating substantial post-transcriptional regulation.

Using polysome profiling and SUrface SEnsing of Translation (SUnSET) assays combined with RNA-seq analysis of polysome-associated RNA, we found that *C. albicans* infection induces a broad translational repression in oral epithelial cells in vitro. This inhibition depended on the fungal adhesin/invasin Als3 and the toxin candidalysin. Mechanistically, infection activated host eukaryotic elongation factor 2 (EEF2) kinase, leading to EEF2 phosphorylation and subsequent arrest of translation elongation. This repression occurred independently of integrated stress response activation or formation of p-bodies/stress granules.

Notably, although the addition of IL-17 cytokine to infected cells did not reverse the global translation inhibition observed in polysome profiles, it acted synergistically to enhance the translation of a small subset of mRNAs including *NFKBIZ*, *MCPIP1*, *CXCL8*, and *CSF2*. Addition of IL-17 to *C. albicans* infected cells results in sustained activation of transcription factors, as well as selective de-repression of translation inhibition of the subset transcripts. Virtually all of the products of these transcripts are known to be required for the normal host defense against OPC. *C. albicans*-induced translational repression was verified in a murine OPC model.

In summary, we propose a model wherein *C. albicans* infection induces strong transcriptional activation in oral epithelial cells, counterbalanced by translational repression. IL-17 releases this repression for key immune genes, enabling a targeted inflammatory response. These results elucidate a pivotal mechanism by which the host response to an oral commensal is modulated until the fungus becomes pathogenic.

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Translational Control of *Cryptococcus neoformans*: Uncovering Mechanisms of Latency, Reactivation, and Pathogenesis in the Immunocompromised Host

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Cryptococcus neoformans (*Cn*) is an opportunistic human pathogen found ubiquitously in the environment, making exposure widespread. There are two major routes for the development of cryptococcosis, one being progressive disease following exposure, second is dissemination following reactivation of latent *Cn*. Primary exposure often occurs in childhood with the collaborative efforts of endothermy and the immune system sufficient to clear the pathogen or keep an infection at bay in a latent state. A majority of cases arise following reactivation following immunosuppression. Reactivation and dissemination can lead to meningoencephalitis. Treatment options are limited, costly and implicated in anti-fungal resistance. With the climate crisis, the geographic range of fungi is changing, as is their ability to adapt to new environmental conditions. In addition, advancements in modern medicine have left a population of individuals living longer, healthier but in an immunosuppressed state and thus vulnerable for opportunistic pathogens. We are facing a unique public health threat, and the development of antifungals is necessary.

To rapidly adapt to the host, *Cn* must reprogram its translatome to produce tools to survive. Our lab has shown this is a concerted ribosome reallocation process that frees up ribosomes to prioritize the translation of stress responsive transcripts, with Ccr4 (mRNA decay) and Gcn2 (regulation of translation initiation) being key players in this acute process. Historically, we have parsed out the effects of acute individual stressors on *Cn* translation, having found that the response to temperature is driven by Ccr4-dependent mRNA decay, while the response to oxidative stress is more reliant on the activation of Gcn2. However, following this acute reprogramming, the translational mechanisms that promote growth and disease in the host, as well as entry, maintenance and exit from latency have yet to be described.

My data shows when *Cn* is grown at human body temperature, 37°C, there is a reduction in translational capacity. This is evidenced by a reduced abundance of translational machinery and translational output compared to cells grown at 30°C. Despite this, wild type retains the ability to reach a saturated liquid culture in 24 hours. In addition, 37°C growth results in a dampened response to the drug 3-AT that induces Gcn2 to repress translation initiation. This suggests that stress inputs to the ribosome have a distinct outcome in the context of compounded thermal stress. Beginning investigation into dormancy and latency has led to preliminary data that surprisingly suggests our *gcn2*Δ mutant is resistant to hypoxic growth at 37˚C. Continuing the

investigation into the regulation of translation prioritization via Ccr4 and Gcn2 during hypoxic and latency-like conditions is critical to uncover its importance in stress adaptation and virulence.

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Bioprospecting marine and freshwater fungi from Mexico

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Wetlands are habitats with unique physical, chemical, and biological features. Here, the chemical, biological, and metabolomic studies of fungi isolated from sinkholes (Cenotes), mangroves, and marine areas of the unexplored Mexican biodiversity are discussed. Examples of this work include the studies on the marine-facultative *Aspergillus* sp. MEXU 27854 isolated from Caleta Bay in Acapulco, Guerrero, Mexico. This fungus produced a series of new dioxomorpholines with inhibitory activity against the drug transporter Pgp. Additionally, its genome annotation predicted 10,822 putative genes, of which 67 BGCs were identified (~60% belong to the NRPS and NRPS-like classes), and a putative BGCs for the dioxomorpholines and other metabolites were proposed. In another study, a series of cultivable fungi (*Aspergillus* spp. NCA257, NCA264, and NCA276; *Stachybotrys* sp. NCA252; and *Cladosporium* sp. NCA273) isolated from the cenote Tza Itzá were subjected to chemical, coculture, and metabolomic analyses. From these, nineteen compounds were obtained and tested for their antimicrobial potential against ESKAPE pathogens, *Mycobacterium tuberculosis*, and nontuberculous mycobacteria. Finally, from a freshwater unidentified fungus isolated from submerged wood in the waterfall “El Caracol”, Nuevo Leon, at the Sierra Madre Oriental mountain system, three new cyclodepsipeptides and two known compounds were elucidated by spectroscopic and spectrometric methods. Some of these compounds showed antimicrobial activity against Gram-positive strains and the Gram-negative *Acinetobacter baumannii* ATCC 17978, including multidrug-resistant clinical strain A564. Overall, our over-the-years’ work highlights the unique taxonomic, genomic, and chemical features of Mexican aquatic fungal biodiversity in the search for potential drug candidates.

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Benthic and beyond: Genomic, biogeographic, and experimental insights into the aquatic and terrestrial lifestyles of *Tetracladium*

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Most *Tetracladium* species are quintessential ‘aquatic hyphomycetes’, the dominant microbial decomposers of leaves in streams, **and** are abundantly reported as endophytes and from soils around the globe. Although the ‘terrestrial’ components of *Tetracladium* ecology have been known to exist for more than 50 years, and some species have been described as endophytes or from soil, extremely little is known about their life/ecology on land. Using a combination of phylogenomics, comparative genomics, and experimental approaches, I am working to understand the evolution of the multi-niche and multifunctional ecology of *Tetracladium*, the distributions of diversity in the genus, how these fungi differentially regulate their genomes as saprotrophs and biotrophs, and how they interact with living hosts (plant-fungal interactions). The genomes of twenty-five strains, representing all species in the genus, were sequenced and used to produce a fully resolved phylogeny. With this phylogeny as a scaffold to explore the distributions and relationships among the *Tetracladium* sequences from metabarcoding studies globally, I find that nearly all known species in *Tetracladium* are Aquatic hyphomycete, Soil, and Endophytic fungi (ASE, pronounced ‘ace’). The ASEs appear to have diverged from an endophytic and soil-dwelling lineage about 70 million years ago. The capacity to be ASE likely impacts population sizes, intraspecific diversity, and dispersal for these fungi. Further, I identify geographically restricted and cold-adapted lineages, both findings have important implications for the conservation of fungal biodiversity and raise exciting questions about adaptation to extreme temperatures. To produce the first insights into differential genome regulation in *T. marchalianum* during saprotrophic vs biotrophic lifestyles and plant-*Tetracladium* interactions, I grew *T. marchalianum* with live or dead *Arabidopsis thaliana* (*A. thaliana* without fungi as a control) in custom designed sterile hydroponics chambers (with replication). ‘Hot off the press’ results from the ongoing transcriptome analyses will be presented.

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Genomic and phenotypic comparison of Terrestrial and Aquatic Rhodotorula yeasts

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Aquatic and marine environments can harbor specialist and generalist fungi exhibiting filamentous and yeast morphologies. Rhodotorula yeasts are red and orange pigmented basidiomycetes found in a broad range of environments including terrestrial biomes, marine and freshwater aquatic environments. Their near ubiquity in most biomes is intriguing as to whether it is a generally resilient fungus or is adept at rapid and specific adaptation to new environments. Isolation of these fungi from environments is straightforward with semi-selective media and easy to identify pigmented colonies. We have curated a set of strains for genomic and phenotypic exploration benefiting from extensive culture collections of Rhodotorula strains from at least 10 described species. These isolates have origins spanning food spoilage, soils, plants, rocks, and aquatic environments. Particular efforts were performed to isolate strains from brackish, mangrove, and tidal flat areas in China to identify species diversity and distribution among several similar environments. We have sequenced genomes of these strains to produce a set of 300 draft genomes and 10 reference quality genomes for type strains for most species. These efforts are intended to test how environmental origin, genomic composition and allelic diversity relate to phenotypes including carotenoid production, sensitivity to osmotic, UV, and heavy metal stresses. We used automated robotic approaches to image colony growth on solid media in the UCR ExFAB BioFoundry to test whether phenotype traits of aquatic isolates differ from those collected in terrestrial environments. Genome comparative approaches included population genomics of strains within a species complex and gene content and synteny between species. Our approaches to isolate these fungi from aquatic and terrestrial environments have produced a rich collection of fungal diversity and the comparisons will help identify adaptations to aquatic or marine environments and further test the hypothesis about these fungi resilience and adaptability.

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Extremely halotolerant black yeast Hortaea werneckii in the Arabian Gulf: Novel Thermotolerant Genotypes from One of the World's Most Extreme Marine Environments

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Hortaea werneckii, the sole species of the genus *Hortaea*, is an extremely halotolerant black yeast capable of growth from 0% NaCl to salt saturation and is widely recognized as a key model organism for studying physiological, morphological, and genomic adaptations to hypersaline environments. Population genomic analyses have revealed that this species exhibits "stable parasexuality," a unique reproductive mode in which highly heterozygous diploids persist without meiotic recombination—an evolutionary strategy thought to facilitate survival in fluctuating extreme habitats. Ecologically, *H. werneckii* occurs in diverse saline and hypersaline environments, including solar salterns, bitterns, deep-sea water, beach soils, coastal rocks, microbial mats, marine sponges, and corals. Earlier studies indicated that growth at 37 °C is possible only with added NaCl, suggesting a temperature–salinity dependency in its ecology.

The Arabian Gulf, however, presents a distinct marine system characterized by exceptional salinity, intense evaporation, and some of the highest seawater temperatures recorded globally. In a two-year survey of Qatari coastal waters, 911 yeasts were isolated, including 36 (4%) black yeast-like fungi. DNA sequence analyses of the ITS region and LSU rDNA identified 20 isolates as *H. werneckii*. Remarkably, 15 represent previously unreported genotypes capable of growth at 37 °C across a broad salinity range (0–15% NaCl), demonstrating thermotolerance exceeding that known from global populations.

The discovery of these new thermotolerant genotypes expands the recognized ecological and genetic diversity of *H. werneckii* and suggests adaptive responses to the Gulf's increasingly warm and saline conditions. Such adaptations may contribute to the emergence of strains with enhanced environmental resilience and pathogenic potential. These findings underscore the value of marine extremophiles as indicators of ecosystem change and as model systems for understanding fungal adaptation under accelerating climatic and anthropogenic stress.

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Fungal diversity and their involvement in the decomposition of deep-sea submerged plants

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Fungi are the main decomposers of plants on land, and they are presumed to play an important role in the degradation of sunken plant materials in the deep sea. However, the diversity, distribution, and ecological functions of fungi associated with deep-sea sunken plant substrates remain largely unknown.

To investigate fungal diversity and their involvement in the decomposition of deep-sea sunken plant debris, we performed ITS amplicon sequencing on 20 samples collected from four sites (~700–5,800 m) in the northwestern Pacific. Amplicon analyses indicated that the marine fungal lineage Halosphaeriaceae (Sordariomycetes, Ascomycota) was dominant and widespread in deep-sea plant debris, and some samples showed distinct community patterns. To further assess functional roles, metagenomic analyses were conducted on four representative samples, including seawater- and sediment-facing branches, a heavily decayed camphor wood, and a relatively fresh pine branch. Metatranscriptomics was additionally applied to two samples (decayed camphor and fresh pine) to identify actively expressed genes.

Metagenomic analyses showed that bacteria contributed the majority of total gene abundance, whereas fungi, especially from Ascomycota, were also abundant in both the decayed camphor and fresh pine samples. Two fungal metagenome-assembled genomes (MAGs) were recovered. MAG1, a Sordariomycete lineage related to the marine wood-decomposer *Zalerion maritima,* was highly abundant in the decayed camphor, and MAG2, a Chaetothyriales-related genome, was mainly present in pine. MAG1 contained a variety of carbohydrate-active enzymes (CAZymes) involved in degrading plant biomass, including cellulose, hemicellulose, pectin, and lignin.

Metatranscriptomic data revealed that Ascomycota were likely the major active decomposers in the decayed camphor sample, expressing multiple lignocellulolytic and oxidative CAZymes (e.g., AA7, GH3, GH11, CE8, and CBM1). In contrast, fungal transcription was limited in the fresh pine sample, implying that this difference may be related to either the decomposition stage or tree species, which requires broader sampling for clarification.

Overall, our comprehensive analyses indicate that Halosphaeriaceae dominate deep-sea submerged plants and that undescribed Ascomycete lineages actively contribute to wood degradation in the deep sea. Continuing investigations into their diversity, physiology, and genomic traits will help to elucidate the adaptation, evolution, and ecological significance of deep-sea fungi.

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Genomic insights into the melanized fungus *Exophiala pacifica* ETNP2018 reveals adaptation to the open ocean

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Despite exhibiting significant diversity and impact in marine biogeochemical cycling, few fungal genomes isolated from the open ocean have been published and characterized. Fungi in the open ocean must adapt to limited organic carbon availability in the water column and intense competition with other microorganisms. To investigate potential fungal adaptations to the open ocean, we isolated a novel species of black yeast-like fungus from the eastern

tropical North Pacific (ETNP) oxygen minimum zone, named *Exophiala pacifica* ETNP2018. The genus *Exophiala* consists of pleomorphic, extremotolerant fungi characterized by their melanized cell walls. Here we present and analyze the draft genome of *E. pacifica* ETNP2018 and compare it with 26 representative *Exophiala* genomes spanning terrestrial, host-associated, polluted, and aquatic environments. *E. pacifica* ETNP2018 possesses a 40.3 Mb genome, placing it among the largest in the genus and significantly exceeding the average *Exophiala* genome size. Together with the only other aquatic species, *E. aquamarina*, it forms a distinct clade characterized by expanded gene content. The genome encodes an extensive repertoire of 406 CAZymes, including numerous extracellular glycoside hydrolases and auxiliary enzymes that enable degradation of both terrestrial polymers (e.g., β-1,4-xylan) and marine algal polysaccharides (e.g., laminarin, fucoidan, chitin), linking this fungus to multiple organic carbon pools. Secondary metabolic capacity is similarly enriched, with diverse biosynthetic gene clusters for siderophores, melanin, and carotenoids that likely underpin polyextremotolerance and protection from oxidative and radiative stress. *E. pacifica* ETNP2018 additionally encodes assimilatory nitrate reduction, nitrilase, and a fungal nitric oxide reductase (P450nor), indicating potential contributions to nitrogen cycling and N_2O production in oxygen-depleted waters. The presence of phosphoenolpyruvate mutase and a putative PhnZ-like oxygenase further suggests access to 2-aminoethylphosphonate, implicating this species in marine phosphonate and phosphorus cycling. Collectively, these features reveal an unexpectedly expanded, metabolically versatile genome in an oligotrophic oceanic fungus, contrasting with the streamlining often observed in marine microbes and highlighting a previously underappreciated role for black yeasts in open ocean biogeochemistry.

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Actin-based cell crawling in chytrid zoospores illuminates potential mechanisms of ancestral fungal locomotion

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Understanding the nature of the last universal fungal ancestor (LUFA) provides essential insight for how fungi evolved and diversified into the millions of species we see today. To explore what the biology of this ancestor may have looked like, we study chytrids, a fungal lineage that diverged before the radiation of Dikarya–the group that includes commonly studied yeasts and multicellular fungi–and that share many features with animals that have been lost in other fungal lineages. Chytrids are characterized by a biphasic life cycle, alternating between sessile walled sporangia and motile zoospores capable of swimming through the water and crawling across surfaces like an amoeba–behaviors lost in Dikarya but inferred to have been present in the LUFA. Although the flagellar-based motility of zoospores appears well conserved with that of diverse lineages, the mechanisms that drive crawling motility vary greatly across eukaryotes, leaving it unclear what mechanisms chytrids and their fungal ancestors may have used to migrate across solid surfaces. By studying the motility of chytrid zoospores, we may infer how the fungal ancestor locomoted.

Using the “frog-killing” chytrid Batrachochytrium dendrobatidis, we show the crawling motility of zoospores involves highly dynamic pseudopod fronts, relying on pushing forces generated by branched actin networks and actomyosin contractility. These mechanisms are similar to that of amoeboid migration seen in other parts of the Eukaryotic tree of life, such as animal cells, the Amoebozoan Dictyostelium and and the Heterolobosean Naegleria. We also show that the flagellum is immobile during crawling, indicating this migration is not dependent on forces arising from flagellar beating. Remarkably, retention of the flagellum and its associated basal body are important for stable polarity of migrating cells; the flagellum is located at the rear of the cell during migration and loss of the flagellum results in erratic and multiple competing pseudopod fronts. This behavior is consistent with the ability of the fungal ancestor to utilize machinery for both flagellar swimming and cell crawling, as well as the presence of cross talk between these two networks.

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Quantitative Multi-Omics Reveal Functional Diversity and Carbohydrate-active Enzyme Activity of Marine Fungi in the Eastern Tropical South Pacific OMZ

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Fungi are active yet understudied members of the marine microbiome, with potentially important roles in biogeochemical cycling in the open ocean. This study combines metagenomic, metatranscriptomic, and amplicon-based approaches with quantitative internal RNA standards and quantitative PCR (qPCR) to investigate the abundance, diversity, function, and activity of marine fungi across the eastern tropical South Pacific (ETSP) oxygen minimum zone (OMZ) during the 2023 El Niño event. Sixty seawater samples were collected from the surface to 4,700 m at four stations, spanning coastal to offshore. Ribosomal RNA–depleted and polyA-enriched RNA libraries were sequenced alongside metagenomes, and internal standards were used to perform quantitative metatranscriptomics, enabling the determination of absolute transcript abundance. To complement metatranscriptomic profiles, fungal 28S LSU regions were amplified and sequenced. QPCR of the 28S rRNA gene was performed to estimate copy-normalized genome equivalents, providing depth-resolved measurements of fungal abundance. The highest absolute fungal transcript abundances were detected in surface waters (~2.0×10^5 fungal transcripts per mL at the surface), reflecting elevated biomass and activity, while fungal transcript abundances declined at depth and in anoxic conditions (down to ~0.07×10^5 fungal transcripts per mL at depth in the coastal station). Among extracellular carbohydrate-active enzymes (CAZymes), glycoside hydrolase family 7 (GH7) dominated expression (~76% of extracellular CAZyme activity), suggesting a major role in cellulose and chitosan degradation. GH16, targeting β-glucans and galactans, was most active at anoxic depths, and multicopper oxidases were expressed under oxygen-depleted conditions, indicating potential alternative oxidative or respiratory pathways. Fungal community composition varied with depth: Basidiomycota dominated surface waters, whereas Ascomycota, Chytridiomycota, Mucoromycota, and Zoopagomycota were active throughout the water column, underscoring the ecological importance of early-diverging lineages. Offshore stations exhibited lower enzyme diversity, consistent with substrate limitation. These results demonstrate that marine fungi are metabolically versatile members of OMZ ecosystems, possessing extracellular enzymatic capacities that contribute to organic matter degradation and biogeochemical cycling in nutrient-poor, oxygen-deficient ocean regions.

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Mechanisms of RNA interference in Cryptococcus

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RNA interference (RNAi) is a conserved mechanism of genome regulation in eukaryotes. It is mediated by small RNAs, which are typically generated from long double-stranded RNA by Dicer enzymes, and associate with Argonaute effector proteins to direct down-regulation of target RNAs based on sequence

complementarity. In the human fungal pathogen *Cryptococcus*, RNAi plays an important role in silencing transposable elements, with loss of RNAi resulting in elevated rates of transposon-induced mutation that can contribute to adaptation and drug resistance. However, beyond the involvement of core pathway components (Dicer, Argonaute and RNA-dependent RNA polymerase), how RNAi operates in *Cryptococcus* remains poorly understood.

We are interested in dissecting the molecular mechanisms of RNAi in *Cryptococcus*, with a focus on *C. deneoformans*, which retains the full ancestral complement of core RNAi machinery including two Dicer and two Argonaute proteins. Our initial assessments of the relative contributions of these core factors revealed that while Dcr1 and Dcr2 function largely redundantly to one another in endogenous sRNA production, Ago1 is the principle Argonaute protein required to support sRNA accumulation and transposon silencing. To further understand mechanisms of silencing, we analysed Ago1-associated proteins by IP-MS. From this we identified several novel interactors with potential roles in facilitating/modulating RNAi activity, including putative RNA helicase and methyltransferase enzymes. We are currently further characterising the functions of these factors to understand their potential roles in RNAi function and/or regulation.

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RNAi restriction of a novel mycovirus prevents viral induced alterations in transcription and virulence in a critical human fungal pathogen

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RNA interference (RNAi) is a widely conserved genome-defense mechanism that protects eukaryotes against viruses and transposon genome parasites. We previously showed that RNAi loss leads to hypermutation and anti-fungal drug resistance in the human fungal pathogen *Cryptococcus neoformans*, illuminating the potential clinical relevance of this occurrence. In this study, we identify mycovirus restriction as another clinically relevant RNAi function in *C. neoformans*. Through investigation of two RNAi-deficient clinical isolates of *C. neoformans*, we identify a novel dsRNA mycovirus of the Totiviridae family we have named CnTV1. Interestingly, RNAi deficiency in these isolates results from the insertion of a nuclear mitochondrial DNA segment (NUMT) into the key RNAi component Ago1. We find that restoration of RNAi in sexually produced zygotes eliminates CnTV1. To further test the role of RNAi in CnTV1 persistence, we performed CRISPR-mediated allele exchange at the mutated RNAi loci which similarly led to CnTV1 elimination in vegetative cells. Through genetic mutations and chemical treatments, we identified several RNAi-independent factors involved in CnTV1 regulation, including a core RNAi component that unexpectedly promotes viral accumulation in the absence of functional RNAi. To assess the functional impact of CnTV1, we developed a cytoplasmic-mixing approach to generate isogenic strain pairs in an RNAi-deficient background that differ only in viral infection status. With these strains, we show that CnTV1 causes altered gene expression and moderately increased virulence in a mouse infection model. Our findings reveal that RNAi opposes infection of *C. neoformans* with a virulence-influencing mycovirus, highlighting another clinically relevant consequence of RNAi loss in this WHO priority fungal pathogen.

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Mitochondrial translation elongation speed controls OXPHOS biogenesis by coordinating the synthesis and folding of mitochondrially encoded proteins

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Mitochondria generate ATP through oxidative phosphorylation (OXPHOS), with core structural subunits encoded by mitochondrial DNA (mtDNA) and translated on mitochondrial ribosomes. However, how mitochondrial translation elongation influences OXPHOS biogenesis remains unclear. Here, we show that in *Neurospora crassa*, the mitochondrial rRNA methyltransferase MRM1 promotes OXPHOS biogenesis by repressing translation elongation independently of its catalytic activity. The N-terminal intrinsically disordered region (IDR) of MRM1 binds simultaneously to mitochondrial ribosomes and mRNAs. Disrupting either interaction accelerates elongation and enhances the synthesis of mtDNA-encoded subunits, but impairs their co-translational folding and membrane insertion. These defects can be partially rescued by pharmacologically slowing mitochondrial translation elongation. The IDR of MRM1 is conserved in Ascomycete fungi and is essential for plant infection by *Magnaporthe oryzae*. Our findings underscore the importance of mitochondrial translation elongation dynamics in coordinating protein synthesis and folding during OXPHOS biogenesis, and identify MRM1 as a potential target for broad-spectrum antifungal strategies.

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A long non-coding RNA mediates crosstalk between fungal thermal adaptation and azole drug response in *Aspergillus fumigatus*

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Fungal pathogens are remarkable in their ability to adapt to mammalian (37°C) and avian physiological temperatures (38-42°C) to cause infection. Saprophytic mold *Aspergillus fumigatus* (AF) is unique in its ability to adapt to temperature and can grow above 50°C. Here, we show that AF adaptation to increased temperature results in morphological changes and, more importantly, increased fungal growth in presence of azoles. Importantly, this is a reversible phenotype and is mediated by a long non-coding RNA whose RNA levels negatively correlate with temperature. Our data show that lncRNA levels differ during saprophytic phase (25°C), mammalian infection phase (37°C) and increased temperature exposure (42°C), thus acting as a link between temperature adaptation and fungal azole response. Furthermore, the overexpression of lncRNA in azole-resistant isolates increases fungal azole susceptibility and, importantly, improves the azole treatment outcomes in a murine model of infection, clearly showing the role of lncRNA in infection outcomes for azole drug-resistant isolates. Through our adaptation experiment, we also show that fungal adaptation to sub-MIC voriconazole concentration is a precursor to the development of a non-reversible novel drug resistance mechanism that is lncRNA dependent. Taken together, these data show that AF adapts to increased temperature by downregulating lncRNA levels that impacts fungal azole response and the subsequent exposure to azoles results in evolution of non-CYP51-dependent azole resistance.

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Extracellular vesicle-mediated RNA transfer across cell-wall barriers in plant–fungal pathogen communication

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Cross-kingdom RNA trafficking between plants and fungal pathogens plays a pivotal role in determining infection outcomes. Our work has demonstrated that plant extracellular vesicles (EVs) deliver regulatory sRNAs and mRNAs into the fungal pathogen Botrytis cinerea to suppress virulence, while fungal EVs transport sRNAs that suppress host immunity. Despite these advances, the fundamental question of how EVs cross the rigid plant and fungal cell walls remains unanswered. We are currently investigating the mechanisms enabling plant EVs to cross these cell-wall barriers, with the goal of defining the structural and molecular determinants that govern EV mobility. Understanding this process will reveal critical principles of cross-kingdom communication and may uncover new targets for controlling fungal diseases.

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tRNA modification and codon usage underlie phytopathogen effector evolution and secretion

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Magnaporthe oryzae causes blast, the most devastating disease of cultivated rice, and now poses a significant threat to global wheat production. To manipulate host immunity and establish infection, *M. oryzae* deploys apoplastic effectors into the space between invasive hyphae and the plant membrane-derived extra invasive hyphal membrane (EIHM) matrix via the conventional ER-Golgi pathway, and cytoplasmic effectors into the plant membrane-rich biotrophic interfacial complex (BIC) via poorly understood unconventional protein secretion (UPS) pathway, where they are translocated into host cells by host clathrin-mediated endocytosis. Despite extensive chemical modification of transfer RNA (tRNA) anticodon across taxa, which contributes to codon decoding and translation fidelity, few physiological roles are known. Using reverse genetics and live-cell imaging, we previously showed that, in the destructive blast fungus *M. oryzae,* the translation of cytoplasmic (but not apoplastic) effector mRNAs relies on efficient decoding of AA-ending codons by the Uba4-dependent thiolation of wobble uridine-34 (s^2U_{34}), a universally conserved modification, in cognate tRNAs. Loss of *UBA4* abolished secretion of AA-ending codon enriched cytoplasmic effectors (but not AA-ending codon depleted apoplastic effectors). Synonymous AA- to AG-ending codon changes in *PWL2* mRNA restored cytoplasmic effector production and secretion in *Δuba4* strains. In the *M. oryzae* WT strain, expressing recoded *PWL2* or *AVR-Pik* caused massive protein production and super-secretion into the BIC, which destabilized the host-fungus interface and abolishing infection. We show here that Uba4-dependent decoding of AA-ending codon-enriched cytoplasmic effector mRNAs is widespread across *M. oryzae* host-infecting lineages and across taxa. Furthermore, based on the AA-ending usage rates *of M. oryzae* secretome genes, we identify two new unconventionally secreted cytoplasmic effectors. In *PWL2 and AVR-Pik*, mutational analysis coupled with FRAP (Fluorescence Recovery After Photobleaching) experiments reveals that a single conserved AA-ending codon can optimize mRNA translation speed and effector production, maintaining BIC integrity and facilitating host infection. These findings emphasize how codon usage choice on fine scales underpins the success of the *M. oryzae*-host rice plant interaction indicating it is a conserved, fundamental principle of host infection driving effector evolution.

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The role of RNA interference in the human fungal pathogen Candida albicans

Elise Iracane, Khetile Mhlongo, Alessia Buscaino Quadram Institute

Candida albicans is a commensal organism that colonises the gastrointestinal tract of most people, but it is also a dangerous human fungal pathogen that causes life-threatening systemic infections. *C. albicans* is such a successful pathogen because it can adapt rapidly and reversibly to hostile host-environments. RNA interference is a conserved regulatory mechanism that modulates environmental adaptation and pathogenesis across eukaryotes. It was long assumed that *C. albicans* lacks a functional RNAi pathway. Contrary to this assumption, we have discovered that *C. albicans* possesses an active RNAi system that silences gene expression of the telomere-proximal TLO gene family (1).

Building on these findings, we are now asking whether RNAi plays a broader role in regulating C. albicans biology and whether it targets additional transcripts. Using genetic and transcriptomic approaches, we show that RNAi has targets beyond TLO genes. In particular, we identify a non-coding RNA that is silenced by RNAi and is required for the yeast-to-hypha transition, a key developmental switch linked to pathogenicity.

In this talk, I will highlight the results of this ongoing and unpublished analysis.

1. Iracane E, Arias-Sarda C, Maufrais C, Ene IV, d'Enfert C, Buscaino A. Identification of an active RNAi pathway in Candida albicans. Proc Natl Acad Sci U S A. 2024;121(17):e2315926121.

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Fungi-wide explorations show unique paradigms found in sRNA processing and function

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Small RNAs (sRNAs) are an ancient and important system of genomic regulation found throughout eukaryotic organisms. Numerous classes sRNAs have been described over decades of research, drawing clear links between genomic phenotypes and functional roles of sRNA genes. However, the majority of our knowledge is focused on plant and animal data, with comparatively little known in fungi. For example, milRNAs are perhaps the best described class of sRNA in fungi and, despite many published loci across the clade, a universal description of this class remains elusive. These gaps have motivated a push to analyze these genes across all available species and data, to understand the meta-level trends that govern classes of fungal sRNAs.

To answer these fundamental questions, we mined public repositories for sRNA-data, identifying over 1,200 libraries across 86 species. We annotated all sRNA genes in all conditions using our published software pipeline optimized for fungal sRNAs. By merging condition-level annotations into metaloci, we could filter for loci which are experimentally replicated. This ultimately found around 3,000 sRNA-genes across the 14 fungi with the strongest evidence. This is dramatically fewer loci than we generally find in other eukaryotes, averaging less than 600 genes in the most supported organisms.

We find that many sRNA-genes are derived from antisense strands of mRNA, implying unusual processing and a cis-regulatory role for many sRNAs. Examining hairpin-derived sRNAs shows that fungal miRNAs rare and less-precisely processed than in other eukaryotes, with intriguing signs of miRNA-related classes novel to fungi. These results hint at unique processing by Dicer proteins. Utilizing the vast proteomic resource for fungi, we have also searched and characterized Dicer proteins, revealing broad divergence among fungi and sub-clades, possibly providing an explanation for our sRNA result.

By using clade-wide approaches, we have built a community resource for fungal sRNAs and sRNA-relate proteins. This helps to define a broad understanding of fungal sRNAs and shows the power of meta-level analyses to find hidden characteristics of a topic which has been explored only in more-specific scales.

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Dissection of the epoxyjanthitrem pathway in *Epichloë* sp. *Lp*TG-3 strain AR37 by CRISPR gene editing to deliver improved SDN-1 type endophytes for grasses

Linda J Johnson, Natasha Forester, Debbie Hudson, Taryn Miller, Wade Mace, David Hume, Pranav Chettri, Sarah Finch, Christine Voisey, Richard Johnson Bioeconomy Science Institute

Epichloë festucae var. *lolii* and *Epichloë* sp. *Lp*TG-3 are filamentous fungal endophyte species of perennial ryegrass (*Lolium perenne*) and several strains of these species have and continue to have a substantial impact on New Zealand's agricultural economy by conferring biotic advantages to the host grass. *Epichloë* endophyte strain AR37, for example, provides ryegrass with improved agronomic performance, insect protection and plant persistence. Indole diterpenes are one class of well characterised secondary metabolite produced by *Epichloë* endophytes, with the AR37 strain expressing epoxyjanthitrems, a group of decorated indole diterpenes. Epoxyjanthitrems have been associated with the observed effects of AR37 on livestock and insect pests. Here we used gene inactivation by CRISPR-Cas9 to deconvolute the genetic basis for epoxyjanthitrem biosynthesis and manipulate this secondary metabolite pathway to reduce or remove endophyte-induced mammalian toxicity whilst retaining activity against some important agricultural insect pests. We also show that gene editing of *Epichloë* can be achieved without off-target events or introduction of foreign DNA (footprint-less) and that these gene edits can be classified as site-directed nuclease 1 (SDN-1), which are not regulated as genetically modified organisms in selected jurisdictions, including Australia. These SDN-1 technology edits have been successfully grown out of containment in the field in Australia for seed multiplication, followed by agronomy trials, which are now being evaluated. This technology has the potential to provide a step change in the future use of animal safe *Epichloë* strains in pastures within New Zealand and overseas, which will significantly reduce chemical inputs and increase animal welfare.

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Potential roles of volatile metabolite-mediated plant-fungal interactions in plant growth and health and custom-designed tools for investigating such interactions below ground

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Through evolutionary arms races and strategic alliances with other organisms, all organisms have developed diverse interaction strategies. A major strategy is the secretion of a wide variety of proteins, RNAs, and metabolites to facilitate or deter specific interactions. Chemical ecology explores how individual organisms and communities interact and function by producing and responding to specific metabolites, how such interactions drive organismal/community behavior, and the underlying mechanisms of these interactions. This presentation covers belowground chemical ecology, focusing on volatile organic compound (VOC)-mediated plant-fungus and fungus-fungus interactions that likely affect plant growth and health. As water is not constantly present in terrestrial ecosystems, plants and fungi do not exclusively rely on water to deliver various molecules needed to manipulate or communicate with neighboring organisms. However, compared to waterborne compounds, knowledge of bioactive VOCs, except those involved in insect-plant interactions, is limited. This disparity is partly due to an unintentional confirmation bias. The typically employed experimental approach of inoculating plants with microbial cells suspended in water to study their interactions tends to direct attention to waterborne molecules as the main drivers of interactions, unintentionally limiting efforts to check the involvement of VOCs. In addition, inquiries into belowground VOC-mediated interactions face a technical hurdle: the requirement for experimental designs/tools that allow the assessment of VOC-mediated effects in the absence of other molecules and without physically disrupting roots and surrounding soils. A brief overview of our work on VOC-mediated interactions, including the development of various customized tools to support belowground chemical ecology, is presented.

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Gas-mediated plant-microbial interactions: impacts to plant germination and growth

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Soil fungi can profoundly influence plants through the production of volatile organic compounds (VOCs), which act as signaling molecules that alter plant physiology, growth, and stress responses. Yet, the roles of these interactions in drylands remain poorly understood, despite the ecological importance and vast global extent of these ecosystems, which occupy roughly 40% of Earth's terrestrial surface. The Chihuahuan Desert, with its extreme temperature and moisture limitations, provides a unique system to study how desert fungi communicate with plants through gas-mediated pathways. Understanding these interactions may reveal mechanisms that influence vegetation patterns and restoration potential in drylands, where shrub encroachment and grassland loss are major ecological challenges. To investigate these dynamics, we isolated ten fungal strains from the northern Chihuahuan Desert and

examined their effects on the model plant *Arabidopsis thaliana* through volatile-mediated confrontations. We hypothesized that most fungi would emit VOCs that would inhibit plant growth. Our rationale was that this could help explain seed germination and plant establishment difficulties commonly observed in restoration efforts across the area. Contrary to our expectations, we found that fungal VOCs had mostly positive effects on *A. thaliana* with some exceptions where effects were negative or not discernible. We also found that the different plant structures of *A. thaliana* responded differently to fungal VOCs: when exposed, *A. thaliana* produced significantly more leaves and longer roots compared to control, while stem length and leaf size remained unaffected. These findings indicate that desert fungi emit diverse VOCs capable of either stimulating or inhibiting plant growth, highlighting their potential influence on plant performance even in difficult environments. It also establishes a foundational framework for investigating the ecological and genetic bases of fungal volatilomes in desert environments. Together, these findings advance our understanding of fungal–plant communication in drylands and its implications for ecosystem resilience under global change.

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Genome-wide association of Specialized metabolism in *Botrytis cinerea*

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Botrytis cinerea produces an extensive chemical arsenal that shapes its ecological strategies and interactions with plant hosts. While several core biosynthetic genes for major metabolites are already well characterized, the broader genomic landscape influencing quantitative metabolomic diversity remains poorly understood. We profiled both volatile and non-volatile metabolites from a genetically diverse panel of 96 *B. cinerea* isolates using HPLC and GC methods. Genome-wide association recovered expected associations with known biosynthetic genes, but more importantly revealed multiple novel loci and linkage patterns associated with metabolomic variaiton. Many of these newly associated regions encode uncharacterized enzymes, transporters, or regulatory elements not previously implicated in *B. cinerea* specialized metabolism. By identifying the extended genetic architecture underlying *B. cinerea* chemical diversity, this study provides new targets for functional validation and enables downstream applications including the development of metabolite-informed pathogen diagnostics, improved prediction of isolate pathogenicity, and integration of fungal chemotypes into crop resistance breeding pipelines.

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MERCK complex and downstream regulatory genes operate development and secondary metabolism in *Aspergillus nidulans*

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Aspergillus spp. contribute significantly to annual food and feed losses through the production of mycotoxins such as aflatoxin B1 (AFB1) and Sterigmatocystin (ST). These mycotoxins, part of a broader group of secondary metabolites (SMs) produced by *Aspergillus spp.,* are regulated by molecular mechanisms driven by post-translational modifications. A five-protein complex known as the 'MERCK' complex was identified and associated with secondary metabolism in both *Aspergillus nidulans* and *Aspergillus flavus*. The absence of this complex results in defects in sexual fruit body formation, sporulation and ST production. To uncover the MERCK complex's role in the regulation of SM biosynthesis in *A. nidulans*, RNA-Seq and ChIP-Seq were used to identify key direct targets of the MERCK complex at the primary metabolism (PM) stage (20 h of growth) and the SM stage (48 h of growth) in deletion mutants and wild type (WT). Comparison of deletion mutants with the wild type identified 214 genes commonly downregulated at the SM stage across all mutants. The top five most differentially regulated genes with regulatory functions (e.g., transcription factors, chromatin-binding proteins, and kinases) were selected for further functional characterization. The bZIP transcription factor mdrC, which is consistently downregulated in all MERCK mutants and is directly bound by RstB (R from MERCK), is required for normal growth, epigenetic regulation, transcriptional control of regulatory genes, stress response, sporulation and spore germination, sexual fruiting-body formation and maturation, as well as secondary-metabolite production. Protein pull-down experiments using MdrC-GFP and MdrC-3xHA tagged strains revealed that it binds with proteins involved in the synthesis of SMs antibiotic 2,4-dihydroxy-3-methyl-6-(2-oxopropyl)benzaldehyde (DHMBA) and Aspercryptins along with key proteins involved in amino acid metabolism. Collectively, the data indicate that the MERCK complex and its downstream regulatory network integrate developmental pathways with the regulation of secondary-metabolite production in *A. nidulans*.

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A biosynthetic survey and a heterologous production platform for molecules from biocontrol fungi

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Pests are responsible for the loss of up to 40% of global crops. Widespread resistance to pesticides has emerged due to the overuse of a limited set of compounds and targets. Many fungal species form symbiotic relationships with plants while antagonizing insects, nematodes, and bacteria, making them valuable sources of sustainable biocontrol agents and biopesticides. However, despite their potential, only a few species are currently used in biocontrol, and their biosynthetic capabilities remain poorly understood.

Here, we used metabologenomic approaches to catalog the biosynthetic repertoire of 82 plant- and insect-associated species within the Hypocreales order. We annotated over 5,000 biosynthetic gene clusters (BGCs), assigned putative products to more than 1,000 of them, and experimentally linked over 100 BGCs to molecules. In parallel, we established black yeasts as novel heterologous hosts for fungal natural products. These hosts combine the genetic tractability of conventional yeasts with the metabolic robustness of filamentous fungi. We developed genetic engineering tools and automation protocols enabling cheap, efficient, and precise gene integration and genome editing, allowing us to heterologously produce several natural products from biocontrol species.

Our work highlights the vast and underexplored chemical repertoire of biocontrol fungi and demonstrates the potential of black yeasts for systematic discovery, characterization, and production of natural products with pest-controlling activities. We believe this provides a strong foundation for developing sustainable pest control strategies in agriculture.

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GATA-type regulator NsdD-mediated species-specific rewiring of gene regulatory networks in *Aspergillus*

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NsdD is a conserved GATA-type transcription factor that governs development and secondary metabolism in *Aspergillus*, yet its species-specific regulatory mechanisms remain unclear. Using network-based multiomics analyses in *Aspergillus nidulans* and *Aspergillus flavus*, we show that NsdD functions in a cell type– and species-dependent manner. Cross-complementation of *A. flavus* Δ*nsdD* with the *A. nidulans nsdD* gene partially rescued developmental and transcriptional defects, indicating evolutionary divergence of function. Genome-wide ChIP-seq identified 502 direct NsdD targets in *A. nidulans* and 674 in *A. flavus*, including key developmental and metabolic regulators. A conserved binding motif (5′-GATCT-3′) was defined as the NsdD response element. Network reconstruction revealed conserved core modules but extensive regulatory rewiring between species, contributing to differences in asexual/sexual morphology and sterigmatocystin/aflatoxin biosynthesis. These findings provide the first comparative map of NsdD-mediated gene regulatory networks in filamentous fungi and highlight how evolutionary changes enable a conserved TF to acquire species-specific roles.

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Biosynthetic pathway of the volatile toxin 3-octanone in the oyster mushroom *Pleurotus ostreatus*

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Fungal carnivory often relies on secreted toxins that immobilize nematodes before hyphal invasion. In the oyster mushroom, *Pleurotus ostreatus*, specialized lollipop-shaped structures called “toxocysts” deliver such toxins, rapidly paralyzing and killing nematodes. We found that toxocysts concentrate the volatile ketone 3-octanone, which alone is sufficient to trigger paralysis, calcium influx, and neuronal cell death in the nematode *Caenorhabditis elegans*, fully reproducing the physiological effects of direct toxocyst contact. Despite its potent bioactivity, the enzymatic pathway that produces 3-octanone has remained unknown, and its structural similarity to certain fungal oxylipins suggests it may arise from oxylipin-generating enzymatic pathways. Oxylipins in fungi are typically synthesized through the oxidation of polyunsaturated fatty acids by lipoxygenases (*LOXs*) and dioxygenases (*DOXs*). The *P. ostreatus* genome encodes three *LOXs* and four *DOXs*, suggesting a diversified oxylipin biosynthetic capacity. Although *DOX4* is the lowest-expressed dioxygenase under toxocyst-producing conditions, our genetic and biochemical analyses indicate that it plays a role in 3-octanone biosynthesis. Deletion of *DOX4* significantly reduces toxocyst formation and strongly impairs 3-octanone production, as confirmed by GC-MS and hydroxylamine-HCl assays. These findings identify Dox4 as a key enzyme in the 3-octanone biosynthetic pathway and suggest that oxylipin metabolism is coupled to toxocyst biogenesis. Comparative analyses of the nematicidal mechanisms of other *Pleurotus* revealed that *P. cystidiosus* lacks 3-octanone and is less effective than *P. ostreatus* at killing nematodes, implying reliance on alternative, less potent toxins with distinct biosynthetic pathways. Future work will further explore the evolution and diversification of fungal nematicidal strategies through comparative genomics across *Pleurotus* species.

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The DEAD Box RNA Helicase PRD-1 Links Glucose Availability to Circadian Period and Nutritional Compensation in Neurospora crassa

Christina Kelliher[1], Sharon Veron Akisa[2] [1]Biology, University of Massachusetts Boston, [2]University of Massachusetts Boston

The circadian clock maintains ~24-hour rhythms across a range of physiologically relevant conditions including nutrient availability and temperature. This stability, known as circadian compensation, ensures the core clock machinery remains robust across different environments. The molecular mechanisms underlying circadian compensation have been primarily studied using Neurospora crassa as a model system. prd-1 (NCU07839) encodes an ATP-dependent DEAD Box RNA helicase and is implicated in modulating circadian timing.

Using race tube assays, we show that a mutant allele of prd-1 disrupts nutritional compensation: the wild-type control circadian period is ~ 21.5 hours at high and zero glucose, whereas the prd-1 mutants exhibited longer periods of 27+ hours across a range of high glucose levels and ~23 hours in the absence of glucose. These results show that PRD-1 normally acts to link glucose levels to circadian timing. Previous studies have shown that PRD-1 protein localizes to the nucleus under high glucose conditions and rapidly disappears when the glucose is depleted, indicating dynamic metabolic control by protein localization and by transcriptional control of the prd-1 gene. We are exploring protein-protein interactions of PRD-1 under high glucose conditions to identify which core clock factor(s) may be regulated by PRD-1 to maintain nutritional compensation.

Our findings identify PRD-1 as a critical mediator of glucose-dependent period stability, providing insights into the molecular interface between sensing and circadian clock in fungi, with broader implications for understanding circadian-metabolic interactions in humans, where the homolog of PRD-1 has been shown to physically interact with core clock proteins.

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Unravelling the biosynthetic pathway and bioactivity of pleurotin

Jack Weaver[1], Michaël Tadesse[1], Panward Prasongpholchai[1], Duha Alkhder[2], Nala Ali[1], Martha White[1], Emmanuel de los Santos[3], Lijiang Song[1], Christophe Corre[1], Antonia Sagona[1], Fabrizio Alberti[1] [1]University of Warwick, [2]University of Leicester, [3]UCB Biopharma

Pleurotin is a meroterpenoid natural product made by *Hohenbuehelia grisea* and related Basidiomycota fungi. It suppresses the growth of Gram-positive bacteria and certain fungi, while also exhibiting antitumor activity through the inhibition of thioredoxin reductase. In order to elucidate pleurotin biosynthesis, we assembled and annotated the full genome of *H. grisea* using a combination of long- and short-read sequencing. We then used comparative transcriptomic analyses to shortlist putative biosynthetic genes that are upregulated during pleurotin production. Using a combination of isotope-labelled precursor feeding and heterologous gene expression in *Aspergillus oryzae*, we elucidated the early biosynthetic steps of pleurotin and are now working towards characterisation of late biosynthetic steps. Besides performing studies on pleurotin biosynthesis, we also investigated its antimicrobial properties. Specifically, we focused on combining pleurotin with bacteriophages to control the growth of *Staphylococcus aureus*. A synergistic interaction between pleurotin and phage K was observed, leading to a reduced MIC of pleurotin for *S. aureus*, including on antimicrobial resistant clinical isolates. The cytotoxicity of pleurotin in human cells was also tested and shown to be comparable to clinically used antibiotics, such as vancomycin. Altogether, our studies have advanced the understanding of pleurotin biosynthesis and revealed its potential as a viable antistaphylococcal agent.

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Transition to an oxygen-limited phase in stirred-tank fermentation causes a transient increase in intracellular ROS and triggers respiratory rebalancing including an alternative oxidase-mediated bypass in *Aspergillus oryzae*

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During fermentation in stirred-tank bioreactors (STBR), filamentous fungi are frequently exposed to oxygen-limited conditions. However, the response to such conditions, particularly during short-term exposure (≤6 h), remains poorly understood. To address this gap, we conducted short-term multi-omics profiling of *Aspergillus oryzae* hyphal dispersion strain grown under the condition in which dissolved oxygen (DO) concentration was transiently controlled towards 0% in the 4 L STBR.

Transcriptome analysis revealed that genes encoding respiratory chain complexes I–III were temporarily down-regulated within 1 h after DO depletion (DO < 1%: hypoxia) followed by enhanced expression at later phases. In contrast, complex IV transcripts were immediately up-regulated under hypoxia. This rapid induction of complex IV genes likely reflects an enhancement in oxygen utilization capacity, thereby increasing the likelihood of capturing scarce oxygen under hypoxic conditions. However, biological interpretations of the observed transient down-regulation of complexes I–III genes were unclear. Based on a known phenomenon that the levels of reactive oxygen species (ROS) increases in respiratory chain under hypoxia, we hypothesized that elevated ROS may suppress the expression of complexes I–III genes. As a result of a ROS assay, a transient rise in intracellular ROS was observed under hypoxia. Furthermore, metabolome analysis showed a decrease in the reduced/oxidized-glutathione ratio under hypoxia, aligning with the ROS assay results.

To further investigate the relationship between respiratory chain and intracellular ROS, we focused on an alternative oxidase (AOX) of which gene expression showed immediate up-regulation under hypoxia. Generally, AOXs bypass complexes III–IV and can limit the generation of ROS, but their functions in *A. oryzae* remain unclear. Interestingly, a deletion of an AOX gene prolonged the ROS elevation. Consistent with the prolonged ROS elevation, complexes I–III genes remained lower expression levels compared to pre-hypoxia, further supporting our hypothesis. These results suggest a biological function of AOX in *A. oryzae* in mitigating the elevated ROS caused by hypoxia in the STBR fermentation.

Overall, our results provide new insights showing that early hypoxia triggers temporarily increases intracellular ROS levels, and the respiratory rebalancing including a strong AOX induction buffers the oxidative stress in *A. oryzae* in the STBR fermentation.

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Circadian clock control of translation fidelity in Neurospora crassa

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tRNA synthetases (RS) charge cognate tRNAs with the correct amino acids are key regulators of translation accuracy (translation fidelity), and we discovered that the levels of several tRNA synthetases, including the methionyl-tRNA synthetase (MetRS), are regulated by the clock in *Neurospora crassa* and peak during the subjective night. In mammalian cells and *Saccharomyces cerevisiae*, MetRS is phosphorylated by the mitogen-activated protein kinases, ERK1 and ERK2, when cells are exposed to oxidative stress. MetRS phosphorylation reduces the specificity of MetRS for tRNAMet leading to the charging of methionines (Met) to non-cognate tRNAs (Met-misacylation) and subsequent Met-misincorporation into polypeptides. These non-coded Met residues in polypeptides sequester reactive oxygen species (ROS) to help cells overcome oxidative stress. Because ERK1/2, as well as ROS levels, are clock-controlled in *N. crassa*, we hypothesized that the clock regulates the fidelity of translation through Met-misacylation, in part, to help cells overcome daily rhythms in ROS levels. To test this idea, we used a GFP-mCherry dual reporter to monitor the rates of Met misincorporation in *N. crassa*. We discovered that Met-misincorporation is clock-controlled with a peak during the night which is the time of day when internal ROS levels are high in cells. We found that rhythmic Met misincorporation is mediated by rhythmic P-MetRS and MAK1. Additionally, we are interested in understanding if Met misincorporation rhythms alter proteome function. To investigate this, we mined a rhythmic proteomics dataset to identify proteins with rhythmic Met misincorporation and identified 237 proteins with rhythmic Met misincorporation and are following up on these candidates. Taken together, these data supported that clock-controlled Met-misincorporation in polypeptides helps to not only protect the organism from circadian oscillations in ROS, but also to potentially extend the functional coding capacity of the genome providing an exciting mechanism of clock-regulation of the proteome.

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Secreted family of proteins required for high levels of mycotoxin synthesis and corn disease

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Contamination of corn with fumonisin mycotoxins is a problem worldwide and is caused primarily by the common soil fungus *Fusarium verticillioides*. This fungus is found frequently in healthy corn as well as in diseased corn at every life stage. How *F. verticillioides* establishes growth as an endophyte or a pathogen is unknown. Fungal hydrophobins are a large family of small, highly hydrophobic secreted proteins that play important roles in conidiation, mycelial morphology, resistance to stress and plant disease. Here we describe a new *F. verticillioides* class I hydrophobin, *HYD9*, and compare its function to several previously identified *F. verticillioides* hydrophobins. Deletion and complementation studies show that *HYD9*, *HYD7* (class I) and *HYD3* (class I) are required for corn disease and production of fumonisin mycotoxins on cracked corn, while neither *HYD6* (class I) nor *HYD4* (class II) are required for either. Transcription analysis of *F. verticillioides* over three weeks after planting in corn seedling roots found that *HYD9*, *HYD7* and *HYD3* were highly expressed at day five, with expression decreasing by 3- to 15-fold at 21 days. In contrast, expression of *HYD5* (class II) increased by 3-fold between day 5 and 21. Growth studies of *HYD9*, *HYD7* and *HYD3* mutants indicated significant differences in micro and macroconidia production, conidial desiccation resistance, mycelial hydrophobicity, and growth morphology on agar media and corn kernels and leaves, with *HYD7* deletion causing the greatest phenotypic differences. The identification of new targets to limit fungal growth and mycotoxin production will save farmers millions of dollars yearly and improve the safety of our food and feed supply.

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Investigation of pathways that operate downstream of G proteins to regulate cellulase production in *Neurospora crassa*

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Neurospora crassa is able to utilize lignocellulolytic biomass by secreting cellulases into the environment. Previous research from our lab has shown that several heterotrimeric G-protein subunits influence cAMP signaling to regulate cellulase production. However, the addition of cAMP is unable to completely rescue all mutants, which has led us to theorize that an alternative pathway controlled by heterotrimeric G-protein signaling regulates cellulase activity. Mitogen Activated Protein Kinase (MAPK) pathways are known to act downstream of G protein signaling systems in other fungi. MAPK cascades involve three sequentially activated kinases that phosphorylate the next kinase in the chain to ultimately regulate a transcription factor or other downstream signaling molecule. Previous work in our group has found that these pathways are involved in numerous cellular processes, including conidiation, sexual development, fungicide resistance, and protoperithecial development. Huberman et. al. 2017 found that the osmolarity-sensing (OS) MAPK pathway is used as a proxy for sensing glucose in *N. crassa*. We are currently investigating the possibility that cellulase production is regulated by MAPK signaling in a G protein-dependent manner in *N. crassa* through the scaffolds and upstream signaling molecules in the MAK-1 and MAK-2 pathways. Experiments are also underway to explore the potential for crosstalk interactions between the two ERK family MAPKK and MAPKKK genes that could impact cellulase activity. Our results will expand understanding of the role that environmental sensing plays in regulating cellulase activity in *N. crassa*.

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Genome-wide association of Specialized metabolism in *Botrytis cinerea*

Jordan Dowell[1], Kailey Gilliam[2], Justin Gambill[2] [1]Louisiana State University, [2]Biological Sciences, Louisiana State University

Botrytis cinerea produces an extensive chemical arsenal that shapes its ecological strategies and interactions with plant hosts. While several core biosynthetic genes for major metabolites are already well characterized, the broader genomic landscape influencing quantitative metabolomic diversity remains poorly understood. We profiled both volatile and non-volatile metabolites from a genetically diverse panel of 96 *B. cinerea* isolates using HPLC and GC methods. Genome-wide association recovered expected associations with known biosynthetic genes, but more importantly revealed multiple novel loci and linkage patterns associated with metabolomic variaiton. Many of these newly associated regions encode uncharacterized enzymes, transporters, or regulatory elements not previously implicated in *B. cinerea* specialized metabolism. By identifying the extended genetic architecture underlying *B. cinerea* chemical diversity, this study provides new targets for functional validation and enables downstream applications including the development of metabolite-informed pathogen diagnostics, improved prediction of isolate pathogenicity, and integration of fungal chemotypes into crop resistance breeding pipelines.

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Cryo-ET reveals the structural cell biology microsporidian parasites

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Microsporidia are a fascinating group of single-celled eukaryotes that infect a wide range of animal species, including humans. These divergent fungal pathogens employ a unique harpoon-like apparatus called the Polar Tube to invade host cells. The Polar Tube—nearly 50 microns long but just 100 nm in diameter—is fired out of the microsporidian spore over the course of just a few hundred milliseconds. Once fired, the Polar Tube is thought to pierce the plasma membrane of a target cell and act as a conduit for the transfer of the parasite cell, from the spore to the inside of the host cell, which initiates infection. The Polar Tube architecture and its association with neighboring organelles within the parasite cell remain poorly understood. In addition, microsporidia are highly diverged from other extant eukaryotes, lack mitochondria, and represent a minimal eukaryotic cell. Here, we will present our latest work using cryoelectron tomography to investigate the structural cell biology of dormant spores from the human-infecting microsporidian species, Encephalitozoon intestinalis. Segmentation and subtomogram averaging of the Polar Tube reveal at least four concentric layers: two protein-based layers surrounded by a membrane layer and filled with a dense core. Regularly spaced protein filaments form the structural skeleton of the Polar Tube. Combining cryoelectron tomography with cellular modeling, we propose a model for the three-dimensional organization of the polaroplast, a membranous organelle that surrounds the Polar Tube and is continuous with the outermost, membranous layer of the Polar Tube. Our results reveal the ultrastructure of the microsporidian cell and the Polar Tube invasion apparatus in situ, shedding light on the infection mechanism employed by this neglected human pathogen.

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An *Aspergillus fumigatus* homeobox transcription factor provides resistance to copper stress and alters lipid metabolism through oxylipin signaling

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Fungal pathogens like *Aspergillus fumigatus* must maintain copper homeostasis during infection, balancing copper's essential role as an enzyme cofactor against its toxicity when weaponized by host immune cells. While copper transporters have been well characterized, the regulatory mechanisms coordinating copper stress responses with broader metabolic adaptations remain poorly understood. We identified HbxH, a previously uncharacterized homeobox transcription factor, which when overexpressed (OE::*hbxH*) increases both germination and biomass in copper starvation and toxicity up to fourfold. To investigate the mechanisms underlying this phenotype, we performed untargeted proteomics under copper starvation, identifying 522 differentially expressed proteins between the deletion (Δ*hbxH*) and OE::*hbxH*. Multiple predicted lipid biosynthetic and regulatory proteins were significantly altered in OE::*hbxH*, prompting lipid visualization using Nile Red fluorescence microscopy. OE::*hbxH* displayed pronounced lipid accumulation and increased lipid droplet abundance across all tested conditions. Additionally, metabolomics revealed the oxylipin 5,8-diHODE was increased by approximately 30-fold in the overexpression. Strikingly, exogenous addition of 5,8-diHODE to wild type and Δ*hbxH* phenocopies the increased growth and lipid accumulation of OE::*hbxH* in copper stress. These findings reveal an unexpected link between copper homeostasis and lipid metabolism mediated by oxylipin signaling. Future investigation aims to determine if lipid accumulation itself confers copper stress resistance and understand these effects using *in vivo* and *in vitro* host-pathogen interaction models.

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Ergot Alkaloid Biosynthesis in Aspergillus aspearensis

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Ergot alkaloids are pharmaceutically important chemicals produced by fungi and are used to treat dementia, migraines, and other ailments. The recently described fungus Aspergillus aspearensis is the closest relative to Aspergillus leporis, a fungus that produces abundant ergot alkaloids and has significant pathogenic potential. Considering the relatedness of A. aspearensis to A. leporis, we tested whether it could produce ergot alkaloids and parasitize the model insect Galleria mellonella. High-performance liquid chromatography (HPLC) analysis demonstrated that A. aspearensis accumulates lysergic-derived ergot alkaloids including lysergic acid alpha-hydroxyethylamide (LAH) and lysergic acid, making it the fourth Aspergillus species demonstrated to produce lysergic acid-derived ergot alkaloids. Its ergot alkaloid profile was similar to A. leporis but not identical. Additionally, there were minor differences in their ergot alkaloid biosynthetic gene clusters A. aspearensis had one identical cluster with A. leporis and a similar second cluster with a functional copy of easD. Accumulation of LAH by A. aspearensis varied when the fungus was cultured on 15 different media; malt extract supported high concentrations. When spores of A. aspearensis were injected into larvae of the insect model, larvae died at a significantly faster rate than control larvae injected with buffer. The median time to 50% mortality was similar to that observed in experiments with A. leporis. The fungus produced ergot alkaloids during insect pathogenesis; later it produced sclerotia and spores on corpses, indicating that it can complete its life cycle in an insect. Aspergillus aspearensis was not an effective pathogen when exposed to insects topically, however. This observation suggests the pathogenesis observed in injected insects represents a pre-adaptation to animal virulence. Lysergic acid-producing Aspergillus species are rare and are recent discoveries; A. aspearensis is a new source of ergot alkaloids and may be useful for studying and producing these important chemicals.

254A

Rhythmic interaction between ZUOTIN and ribosomes may promote daily rhythms in protein folding and activity

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Several ribosome-associated chaperones bind nascent polypeptide chains to mediate co-translational folding. Zuotin (ZUO) is a part of the ribosome-associated complex (RAC) that functions as a ribosome co-chaperone. In yeast cells, ~30% of ribosomes associate with the RAC, suggesting some level of specificity. Quantitative mass spectrometry across circadian time in *Neurospora crassa* revealed that the interaction between ZUO and ribosomes is clock-controlled. Thus, we hypothesized that the rhythmic ZUO/ribosome interaction may lead to rhythms in the folding of specific protein targets. Protein aggregation assays confirmed that ZUO promotes proper protein folding in *N. crassa*. Identification of misfolded proteins by mass spectrometry revealed that ZUO-dependent folding targets included copper transporters, and proteins involved in snRNA processing and mRNA catabolic process. Protein aggregates are more likely to form when the ZUO-ribosome interaction is low during the day under control of the clock, supporting that protein folding of select targets is clock-controlled and dependent on ZUO. Interestingly, while the interaction of ZUO with ribosomes is clock-controlled, ZUO protein levels do not cycle. Thus, the daily rhythm in folding of specific protein targets, potentially including ZUO itself, likely contributes to protein activity rhythms in the absence of rhythmic abundance.

255A

Reconstructing the evolution of a widespread fungal isocyanide megasynthase using genomes of lichenized fungi

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The products of isocyanide synthase (ICS) biosynthetic gene clusters (BGCs) have been implicated in microbial interactions, pathogenesis, and metal homeostasis. While several ICS BGCs have been described as mediating metal-associated ecologies, the evolutionary history of this recently described class of specialized metabolites is unexplored among Lecanoromycetes, a clade comprised predominantly of symbiotic lichen-forming fungi (LFF), known to thrive in metal-contaminated environments (e.g., mine spills). Analyzing nearly 4,000 fungal genomes, including many Lecanoromycetes, we identified six unique LFF-associated ICS clades. Reconstructing the evolutionary history of LFF ICSs uncovered a widespread "split" variant of the copper-responsive (*crmA*) pathway, where the ICS and NRPS-like components are encoded on separate genes, contrasting the canonical "fused" *crmA* megasynthase. Metabolic characterization and genetic deletions in *Fusarium graminearum* confirmed that this split architecture is functionally equivalent to the fused form. Phylogenetic reconstruction indicated the fused *crmA* arose from a split ancestor, whose NRPS-like component evolved from a canonical thioester reductase architecture likely via domain replacement. Redefinition of the *crmA* pathway to include the "split" ICS/NRPS-like variant reveals that *crmA* is the most prevalent ICS in the fungal kingdom. Our work demonstrates how exploring understudied fungal lineages can define new specialized metabolism lineages and reshape our understanding of biosynthetic pathways across the fungal kingdom.

256B

Natural products from aquatic fungi as novel treatments for neurodegenerative disease.

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Alzheimer's Disease is a neurodegenerative disorder, characterized by reduced levels of acetylcholine, a neurotransmitter essential for brain function. Acetylcholinesterase (AChE), the enzyme responsible for breaking down acetylcholine, is a primary therapeutic target, and current drugs like Donepezil inhibit AChE to slow symptom progression. However, these treatments can be costly and associated with adverse effects, underscoring the need for alternative inhibitors.

Fungi represent a promising but underexplored source of bioactive compounds with potential AChE inhibitory activity. While drug discovery has historically focused on terrestrial species, both coral-associated marine fungi and freshwater fungi are adapted to unique ecological pressures that may drive the production of novel secondary metabolites. We therefore screened a collection fungi isolated from marine and freshwater habitats. We identified fungi by amplifying and sequencing ITS regions. Thus far, fifteen fungi from the library have been identified. To screen for the production of AChE inhibitors, we prepared fungal extracts and tested them for their ability to inhibit human AChE using a colorimetric assay. So far, thirteen freshwater and six saltwater fungi have shown early signs of inhibition. One isolate inhibited 72.8% of AChE activity *in vitro,* comparable to the 93.9% inhibition observed with Donepezil. Future work will identify the bioactive compounds in these extracts. This could potentially lead novel AChE inhibitors for treatment of neurodegenerative diseases.

257B

The High Cost of Assimilation: How Wood-Decaying Fungi Balance Transcriptional Investment Between Extracellular Deconstruction and Intracellular Metabolism

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Wood degradation by basidiomycete fungi requires coordination between extracellular enzymes and intracellular metabolism. Yet, how these systems integrate and how transcriptional resources are globally allocated during lignocellulose decomposition remain unclear. This study applies a standardized comparative transcriptomics framework to five Agaricomycete fungi—three white-rot (WRF: *Pleurotus ostreatus*, *Phanerochaete chrysosporium*, *Heterobasidion irregulare*) and two brown-rot (BRF: *Fomitopsis schrenkii*, *Rhodonia placenta*)—grown on glucose or washed poplar wood. The goal was to compare transcriptomic effort and differential expression of carbohydrate-active enzymes (CAZymes) between WRF and BRF and to contextualize these investments using central carbon metabolism (CCM), a conserved metabolic reference.

The transcriptomes showed a highly uneven distribution of expression, with a small set of genes dominating in all species, especially in BRF. Within this high-priority set, core CCM enzymes such as GAPDH and enolase matched or exceeded the expression levels of the most induced CAZymes, indicating that intracellular carbon processing demands remain high even during intense extracellular degradation. CCM profiles revealed a metabolic continuum: WRF displayed coordinated upregulation of oxidative pathways (glycolysis, PPP, TCA cycle), whereas BRF exhibited constrained oxidative metabolism and a stronger transcriptional bias toward fermentative NAD^+ regeneration via alcohol dehydrogenase.

CAZyme comparisons highlighted contrasting decomposition strategies. WRF deployed a broad and diverse enzymatic arsenal (e.g., GH7, AA9, PLs), while BRF invested in a narrower set of activities. Despite these differences, four enzyme classes—expansins, AA3_3, GH16, and GH128—were consistently prioritized across all fungi. The exceptionally high expression of central glycolytic enzymes across species suggests possible moonlighting roles beyond metabolism, potentially involving redox sensing or transcriptional coordination.

Overall, the study shows that fungal adaptation to lignocellulose involves an integrated transcriptional strategy: species-specific investment in extracellular enzymatic repertoires is coupled with a conserved, high-priority rewiring of central carbon metabolism. These findings refine our understanding of ecological niche differentiation and support the development of fungal platforms for biorefinery applications.

258B

A glucose-dependent defects in a CHV1-, sectorization-associated mutant of *Cryphonectria parasitica*

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CHV1 infection and sectorization in *Cryphonectria parasitica* are accompanied by distinct transcriptional changes, from which we identified 12 core candidate genes and generated targeted mutants. Mutants displayed severe growth inhibition, enlarged cell walls, and globose hyphae specifically under standard glucose-rich conditions (PDAmb including 2 % glucose and minimal media including 1 % glucose), while maintaining normal hyphal morphology on nutrient-depleted water agar. Growth of mutant strains was fully restored in minimal media supplemented with 1 M sorbitol, indicating defects in osmotic regulation. qPCR analysis under restricted growth conditions showed that transcription of RPL3 and ATG8 was accumulation as is the case of TOR, marker genes for TOR pathway suppression. Furthermore, complete recovery of colonial growth was observed on skim milk supplemented media regardless of the presence of glucose suggests alternative activation of TOR signaling through amino acid inputs. These results suggest that mutations of selected target genes affect the coupling between glucose sensing, TOR signaling, and osmotic stress responses. The mutant provides a useful model for studying how carbon source–dependent and nutrient signaling converge to regulate fungal growth.

259B

Biosynthetic pathway of the volatile toxin 3-octanone in the oyster mushroom *Pleurotus ostreatus*

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Fungal carnivory often relies on secreted toxins that immobilize nematodes before hyphal invasion. In the oyster mushroom, *Pleurotus ostreatus*, specialized lollipop-shaped structures called “toxocysts” deliver such toxins, rapidly paralyzing and killing nematodes. We found that toxocysts concentrate the volatile ketone 3-octanone, which alone is sufficient to trigger paralysis, calcium influx, and neuronal cell death in the nematode *Caenorhabditis elegans*, fully reproducing the physiological effects of direct toxocyst contact. Despite its potent bioactivity, the enzymatic pathway that produces 3-octanone has remained unknown, and its structural similarity to certain fungal oxylipins suggests it may arise from oxylipin-generating enzymatic pathways. Oxylipins in fungi are typically synthesized through the oxidation of polyunsaturated fatty acids by lipoxygenases (*LOXs*) and dioxygenases (*DOXs*). The *P. ostreatus* genome encodes three *LOXs* and four *DOXs*, suggesting a diversified oxylipin biosynthetic capacity. Although *DOX4* is the lowest-expressed dioxygenase under toxocyst-producing conditions, our genetic and biochemical analyses indicate that it plays a role in 3-octanone biosynthesis. Deletion of *DOX4* significantly reduces toxocyst formation and strongly impairs 3-octanone production, as confirmed by GC-MS and hydroxylamine-HCl assays. These findings identify Dox4 as a key enzyme in the 3-octanone biosynthetic pathway and suggest that oxylipin metabolism is coupled to toxocyst biogenesis. Comparative analyses of the nematicidal mechanisms of other *Pleurotus* revealed that *P. cystidiosus* lacks 3-octanone and is less effective than *P. ostreatus* at killing nematodes, implying reliance on alternative, less potent toxins with distinct biosynthetic pathways. Future work will further explore the evolution and diversification of fungal nematicidal strategies through comparative genomics across *Pleurotus* species.

260B

Translational Control of *Cryptococcus neoformans*: Uncovering Mechanisms of Latency, Reactivation, and Pathogenesis in the Immunocompromised Host

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Cryptococcus neoformans (*Cn*) is an opportunistic human pathogen found ubiquitously in the environment, making exposure widespread. There are two major routes for the development of cryptococcosis, one being progressive disease following exposure, second is dissemination following reactivation of latent *Cn*. Primary exposure often occurs in childhood with the collaborative efforts of endothermy and the immune system sufficient to clear the pathogen or keep an infection at bay in a latent state. A majority of cases arise following reactivation following immunosuppression. Reactivation and dissemination can lead to meningoencephalitis. Treatment options are limited, costly and implicated in anti-fungal resistance. With the climate crisis, the geographic range of fungi is changing, as is their ability to adapt to new environmental conditions. In addition, advancements in modern medicine have left a population of individuals living longer, healthier but in an immunosuppressed state and thus vulnerable for opportunistic pathogens. We are facing a unique public health threat, and the development of antifungals is necessary.

To rapidly adapt to the host, *Cn* must reprogram its translatome to produce tools to survive. Our lab has shown this is a concerted ribosome reallocation process that frees up ribosomes to prioritize the translation of stress responsive transcripts, with Ccr4 (mRNA decay) and Gcn2 (regulation of translation initiation) being key players in this acute process. Historically, we have parsed out the effects of acute individual stressors on *Cn* translation, having found that the response to temperature is driven by Ccr4-dependent mRNA decay, while the response to oxidative stress is more reliant on the activation of Gcn2. However, following this acute reprogramming, the translational mechanisms that promote growth and disease in the host, as well as entry, maintenance and exit from latency have yet to be described.

My data shows when *Cn* is grown at human body temperature, 37°C, there is a reduction in translational capacity. This is evidenced by a reduced abundance of translational machinery and translational output compared to cells grown at 30°C. Despite this, wild type retains the ability to reach a saturated liquid culture in 24 hours. In addition, 37°C growth results in a dampened response to the drug 3-AT that induces Gcn2 to repress translation initiation. This suggests that stress inputs to the ribosome have a distinct outcome in the context of compounded thermal stress. Beginning investigation

into dormancy and latency has led to preliminary data that surprisingly suggests our *gcn2*Δ mutant is resistant to hypoxic growth at 37˚C. Continuing the investigation into the regulation of translation prioritization via Ccr4 and Gcn2 during hypoxic and latency-like conditions is critical to uncover its importance in stress adaptation and virulence.

261B

Subcellular deep proteomics reveals extensive circadian clock regulation of the proteome, phosphoproteome, and translation fidelity in *Neurospora crassa*

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The circadian clock profoundly shapes cellular physiology by imposing daily rhythms on gene expression, yet most work has focused on transcription, leaving circadian regulation of the proteome far less understood. Recent studies in mammals suggest that protein compartmentalization is rhythmic, but how the clock coordinates protein abundance, localization, modification, and translational fidelity across distinct cellular compartments remains largely unknown.

To address this gap, we are applying deep subcellular proteomics using fractionated nuclei, mitochondria, and cytoplasm followed by mass spectrometry in wild-type and clock-deficient (Δ*frq*) *Neurospora crassa*. In the cytoplasmic fraction alone, the clock regulates 20% of proteins and 26% of phosphoproteins, revealing rhythmic control of unexpected pathways including vesicular trafficking and ubiquitin–proteasome function. We also identified proteins showing circadian cycling in amino acid misincorporation, suggesting a previously unrecognized mechanism by which the clock expands proteome diversity. These targets will be validated, and mutants analyzed to determine if temporal control of translation fidelity enhances proteome function and confers time-of-day fitness advantages.

Analyses of nuclear and mitochondrial fractions are underway. Together, this work will provide the most comprehensive view to date of how the circadian clock sculpts the proteome across cellular compartments to optimize physiology and drive daily rhythms in organismal fitness.

262B

Interplay between mitochondrial dysfunction and metabolic adaptation to non glucose carbon source in engineered yeast for improved biofuel production

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Renewable energy capacity must nearly triple by 2030 to meet global climate goals, and liquid biofuels remain indispensable for hard-to-electrify sectors like aviation. While current Sustainable Aviation Fuel production largely depends on lipid- and waste-derived feedstocks, their limited availability has intensified interest in scalable, low-carbon lignocellulosic alternatives. Bioenergy *Sorghum bicolor*, a diploid C4 grass with high biomass potential, drought resilience, and suitability for marginal lands, presents a promising feedstock for biofuels. Process of lignocellulosic biomass releases fermentable sugars, primarily glucose and xylose, along with microbial inhibitors. To achieve efficient bioconversion, the Great Lakes Bioenergy Research Center has developed xylose-fermenting and inhibitor-resistant strains capable of converting both sugars in the presence of inhibitors. However, xylose utilization still remains less efficient than glucose.

To identify genes associated with xylose use, we implemented adaptive laboratory evolution and genome-wide gene perturbation using CRISPR interference. These approaches consistently highlight genes associated with mitochondrial Fe-S cluster assembly, a pathway seemingly unrelated to sugar fermentation. When these genes were disrupted, xylose fermentation accelerated by up to 40 percent. To investigate how loss of Fe-S cluster related genes enhances xylose utilization, we performed transcriptomic and proteomic profiling on deletion mutants that exhibit similar improvements. Preliminary analyses reveal that genes commonly up-regulated across them are associated with the entry and maintenance of stationary phase even during exponential growth as well as general stress response pathways, suggesting substantial metabolic rewiring. Further integration of these profiles, supported by machine learning, aims to uncover regulatory networks that shed light on a path from functional loss of Fe-S cluster to improved xylose utilization.

We then evaluated their industrial applicability. Fermentation with the Fe-S cluster impaired *S. cerevisiae* strain GLBRCY1455 yielded up to 76.2 g L^{-1} ethanol from juice and 48.1 g L^{-1} from hydrolysates. A techno-economic analysis showed that a previous study of a similar process produced a minimum fuel selling price of $14.40 per gasoline gallon equivalent (GGE), whereas our pipeline reduces this to $6.39 per GGE, bringing the cost much closer to industrially competitive levels.

263B

Genetic variation in health and aging in spores isolated from *Schizosaccharomyces pombe*

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To survive in hostile environments, many organisms have evolved the ability to greatly decrease their metabolism and enter states of dormancy (e.g. diapause, hibernation). Interestingly, some aspects of aging are slowed during dormancy, and in multiple species, hibernators tend to live longer than non-hibernators. Many human cell types, such as neuronal stem cells, enter dormant states that are protective during injury and extend lifespan. Despite this prevalence, much remains to be learned about the molecular mechanisms of cellular dormancy.

Fungal spores represent a model to investigate cellular dormancy, as they are long-lived, metabolically inactive, and experimentally tractable. Here, we characterize spores produced by *Schizosaccharomyces pombe* and find that longevity and health of *S. pombe* spores declines over time and in response to heat stress. We characterize several traits associated with stressed and aged spores and use multiple assays (saturation mutagenesis screen, proteomics, and RNA-sequencing) to identify genes that either limit or promote spore longevity. We reveal parallels to aging cells in animals and important insights into a pervasive, yet understudied, cellular survival mechanism.

264B

Fungal Foundries: Differential Nutrient Uptake Across the Fungal Kingdom

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Within the fungal kingdom, differential lifestyles, genetics, morphotypes, and environmental conditions confer different nutrient uptake strategies for survival. While much has been studied on fungal nutrient exchange for applications in agriculture, little has been done to understand the overall differential elemental uptake across the fungal kingdom. To help address this knowledge gap, we assessed elemental uptake in more than thirty fungi from across the fungal kingdom in three common growth media formulations, one defined and two undefined, representing a gradient of growth conditions. To investigate the fungal elemental uptake, laser-induced breakdown spectroscopy (LIBS) was employed to determine the elemental profiles of these fungi, for comparison across media, species, lifestyle, morphotype, and taxonomy. A “core ionome” was found to be composed of Na, P, K, Fe, H, and C based on PCA biplot analysis, however, these elements had little ability to provide clustering or classification of traits. While there is no defining characteristic to explain all fungal nutrient profiles, certain fungal lifestyles (mutualist, pathogen, saprotrophs) take up some elements in higher relative quantities. This data provides evidence for different nutrient affinities by different lifestyles that cannot be easily explained by traditional evolutionary classifications, and no one fungal trait has high predictive power of elemental patterns. Future experiments will be needed to further investigate the roles of the elemental partitioning patterns may play within their traditional ecological or evolutionary niches as well as how different growth conditions may affect their patterns. Our results show mutualist fungi having a lower elemental uptake trend when compared to pathogens and saprotrophs. More research is needed to further investigate the role that different nutrients play within fungal systems, as well as how different conditions may influence these profiles. Finally, with the crucial symbioses that fungi form with plants, understanding how differential nutrient uptake and accumulation strategies may provide some insights into the exchange of nutrients between fungi and their host plants.

265B

Global Proteomic and Phosphoproteomic Analysis Reveals Potential Calcineurin-Dependent Effectors in Cell Wall and Membrane Organization in *Aspergillus fumigatus*

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Calcineurin phosphatase (CN) orchestrates growth and virulence of *Aspergillus fumigatus*, a critical human fungal pathogen responsible for life-threatening infections in immunocompromised patients making it an attractive antifungal target. Understanding the CN network of proteins in *A. fumigatus* and the mechanism of how this phosphatase regulates important fungal-specific effectors involved in cell wall and membrane biosynthesis and organization will lead to designing novel therapeutic approaches for the treatment of invasive aspergillosis. Here we utilized whole proteomic/phosphoproteomic and lipidomic approaches to define CN-dependent regulation of various cell wall- and cell membrane-related effectors. Our two-pronged proteomic approach included: (1) Quantitative LC-MS/MS analysis to identify CN interactors via CN-tagged GFP using GFP-Trap® affinity purification; and (2) Comparative whole proteomic/phosphoproteomic analysis between the wild-type versus the CN-deletion mutant to identify CN-dependent effectors. Additionally, we systematically assessed the influence of CN on membrane organization via quantitative lipidome profiling by comparing the wild-type versus the CN mutant to define CN-dependent regulation. Our whole proteomic and lipidomic results from this holistic approach will provide a mechanistic understanding of how the CN network drives cell wall and membrane organization during growth and antifungal response in *A. fumigatus*.

266B

Strain-specific redox balancing for *Aspergillus fumigatus* pathoadaptation and antifungal drug susceptibility

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Significant genotypic and phenotypic diversity exists in the *Aspergillus fumigatus* population whose significance for pathogenesis and drug susceptibility is only beginning to emerge. The complexity of this diversity poses both challenges and opportunities to define mechanisms of pathogenicity, virulence, and antifungal drug susceptibility. Previously, we identified *A. fumigatus* isolates with an increased ability to grow in low oxygen conditions found at sites of infection and with enhanced virulence in murine models of invasive pulmonary aspergillosis. However, the underlying mechanism(s) for increased low oxygen fitness and virulence remain ill-defined. An experimental evolution experiment with the reference strain AF293 revealed a novel gene cluster located on a moveable Starship element necessary and sufficient for low oxygen fitness and virulence. These strains provide a basis for defining mechanisms of low oxygen adaptation in a host-relevant context. With these strains, we discover that an enhanced ability to reduce nitrate and catabolize ethanol provides increased biomass generation in low oxygen conditions and the ability to form more robust biofilms. In contrast to strains less fit in low oxygen conditions, the ability to reduce nitrate and catabolize ethanol is necessary for the low oxygen growth of these biofilm-fit strains. Loss of this ability impacts pathogenesis and significantly impacts biofilm antifungal drug susceptibility. These data suggest important redox balancing metabolism that also provides key substrates for cell wall and ergosterol biosynthesis under oxygen limited conditions. These observations have potential implications for the emergence of antifungal drug resistance in the environment and the ability of a given strain to persist and progress disease in the dynamic lung infection microenvironment. The extent to which this metabolic rewiring and flexibility is observed across the *A. fumigatus* phylogeny is under further investigation.

267C

Delineation of the sterigmatocystin biosynthetic gene cluster in *Aspergillus nidulans* through functional analysis of *stcX*

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Production of sterigmatocystin (ST) in *Aspergillus nidulans* involves the ST biosynthesis gene cluster, which has been considered to comprise 26 genes. However, functions of several genes within this cluster remain unclear. In this study, we investigated the role of the *stcX* gene in ST biosynthesis. As the open reading frame of *stcX* has been undefined, we deleted three putative genes adjacent to *stcW* simultaneously. Deletion of the *region* did not significantly impact growth rate across various growth conditions compared to the control strain. Chemical analyses revealed no significant change in ST production in the deletion strain compared to the control. Furthermore, the three transcripts were largely undetectable during ST biosynthesis, and the expression of other ST cluster genes was unaffected by the deletion. Our results suggest that these genes are not essential for ST biosynthesis in *A. nidulans*, indicating that the cluster should be delineated to exclude *stcX*.

268C

Automated discovery of stress-responsive natural products from anaerobic gut fungi in extreme environments

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Strict anaerobes from extreme environments harbor extensive but largely untapped biosynthetic potential. Yet natural products from these organisms remain poorly characterized because they are difficult to cultivate at scale and many of their biosynthetic pathways are **not organized as contiguous gene clusters**. In anaerobic gut fungi (AGF; Neocallimastigomycota), components of nonribosomal peptide synthetases, polyketide synthases, and associated tailoring enzymes are often **distributed across chromosomes** or embedded within broader stress-response networks, limiting the effectiveness of standard genome-mining approaches.

To systematically uncover stress-responsive biosynthetic activity, we developed an **automated anaerobic workflow** that couples environmental perturbation with microbial co-culture. Leveraging the NSF ExFAB BioFoundry's liquid-handling systems enclosed within an anaerobic chamber, AGF are exposed to gradients of pH, temperature, nutrient availability, and co-cultures with rumen bacteria such as *Fibrobacter succinogenes*. Time-resolved samples generate both nucleic acid and metabolite datasets suitable for transcriptomic and metabolomic profiling.

To sensitively detect activation of distributed biosynthetic pathways, we employ a **multiplex probe-based RT-qPCR library** targeting conserved NRPS, PKS, and tailoring domains, enabling rapid screening of hundreds of conditions. Conditions exhibiting transcriptional activation are prioritized for **untargeted LC–MS/MS**, molecular networking, and functional assays using fungal supernatants against representative rumen microbes. This integration of expression signatures, metabolite families, and microbial interaction effects reveals stress-dependent biosynthetic logic that is not predictable from genome architecture alone.

Together, this platform provides a scalable strategy to discover stress-responsive natural products in anaerobes, decode how environmental and microbial cues regulate fragmented biosynthetic pathways, and build a mechanistic framework for engineering resilient microbial communities.

269C

Investigating the biosynthetic capacity of *Trichoderma koningiopsis* to combat papaya dieback disease caused by *Erwinia mallotivora*

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Globally between 20 and 40 percent of crops are lost annually due to pest species (FAO), and the effects of climate change are only likely to make this worse. These crop losses lead to food insecurity and economic burdens. Ongoing issues with resistance to existing treatment methods mean new forms of pest control are urgently needed. Microbes naturally associated with plants often convey a variety of benefits to their hosts, including plant growth promotion, increased capacity to cope with abiotic stresses and protection against pathogenic or pest species. Fungi from the genus *Trichoderma* are particularly useful as biocontrol agents (BCA), either alone or in synergy with other microbes. We describe three strains of *Trichoderma koningiopsis* isolated from the rhizosphere of papaya trees antagonist to *Erwinia mallotivora*, the causative agent of papaya dieback disease. These strains also induced pathways in the papaya plants associated with improved tolerance of abiotic stress, which could be detected by RNASeq analysis.

LC-MS analysis of fungal metabolites from our strains showed the presence of polyketides with anti-fungal properties, including trichodermatides and koningins. Genome mining and RNASeq revealed the presence of over forty biosynthetic gene clusters (BGC), including a novel cluster of twelve genes, upregulated in association with the production of koningins. This cluster, which was only partially identified by antiSMASH, included a type I highly reducing, iterative polyketide synthase (PKS) showing similarity to the brefeldin A PKS and to the lovastatin PKS *lovF*. Heterologous expression of several genes from this cluster in *Aspergillus oryzae* resulted in the production of multiple compounds not detected from wild type *A. oryzae*. Working is ongoing to characterise these metabolites and confirm any therapeutic properties.

Trichoderma sp. have also been found to show synergistic effects when combined with bacterial species, such as *Bacillus*sp. We identified a strain of *B. cereus* which showed promise as a biocontrol agent and using genome mining have identified multiple BGCs, including a high proportion of RiPPs and siderophores, which may also have antimicrobial properties.

These results demonstrate that *T. koningiopsis* holds promise as a BCA for papaya dieback disease, however further work is required to identify the best methods of inoculation and determine if any synergies result from co-inoculation with *B. cereus*.

270C

Structural insights into mechanisms of zinc scavenging by the *Candida albicans* zincophore

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Candida albicans causes over 400,000 life-threatening, and an additional half a billion of mucosal infections annually. In response to infection, the host limits essential micronutrient availability, including zinc, to restrict growth of the invading pathogen. As assimilation of zinc is essential for *C. albicans* pathogenicity, limitation induces secretion of the zincophore protein Pra1 to scavenge zinc from the host. Pra1 also plays a number of important roles in host-pathogen interactions and is conserved in most fungi. However, the structure of fungal zincophores is unknown. Here, we present cryo-EM structures of *C. albicans* Pra1 in apo- and zinc-bound states, at 2.8 and 2.5 Å resolution respectively. Our work reveals a hexameric ring with multiple zinc binding sites. Through genetic studies, we show that these sites are essential for *C. albicans* growth under zinc restriction but do not affect the inflammatory properties of Pra1. These data create a foundation for future work to explore the structural basis of Pra1-mediated host-pathogen interactions, *C. albicans* zinc uptake, as well as therapeutics development.

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Genetic characterization of N-acetylglucosamine utilization in *Aspergillus nidulans*

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The *Aspergillus nidulans* transcription factor XprG plays important roles in regulation of autolysis and nutrient acquisition, including catabolism of N-acetylglucosamine (GlcNAc). We identified the putative genes of the GlcNAc catabolic pathway in *A. nidulans*, most of which are clustered adjacent to the *xprG* gene on chromosome VII. Extracellular GlcNAc is predicted to be imported into the cell by the GlcNAc transporter NgtA, phosphorylated by the hexokinase HxkC to GlcNAc-6-phosphate, then deacetylated by DacA to glucosamine-6-phosphate, and subsequently deaminated by DamA to ammonium and fructose-6-phosphate, which can enter nitrogen and carbon metabolism, respectively. Deletion of the *hxkC* gene was previously shown to confer reduced GlcNAc utilization. We deleted the *ngtA*, *dacA* and *damA* genes by gene replacement. Each deletion mutant showed impaired growth on media containing GlcNAc as a sole nitrogen, carbon, or nitrogen and carbon source. We also identified a putative GlcNAc sensor and histone deacetylase, NgsA, expected to interact with XprG and regulate GlcNAc utilization, based on the phenotypes and interactions of their orthologs in *Candida albicans* and *Trichoderma reesei*. Deletion of the *ngsA* gene confers decreased ability to utilize GlcNAc. Furthermore, we show by construction of double mutants that *xprG* and *ngsA* exhibit genetic interaction. We identified a second potential GlcNAc transporter in *A. nidulans*, NgtB. The *ngtB* gene is located on chromosome II. We deleted the *ngtB* gene and the resulting mutant showed slightly reduced growth on GlcNAc as a nitrogen or carbon source. Therefore, NgtB acts in the GlcNAc utilization pathway. The GlcNAc utilization phenotypes of the *ngtA*Δ and *ngtB*Δ single mutants are less severe than those of the catabolic gene mutants. To determine if deletion of both transporter genes prevents growth on GlcNAc, we attempted to construct a *ngtA*Δ *ngtB*Δ double mutant. Putative diploid and heterokaryotic transformants were obtained. A heterokaryon rescue test indicated that simultaneous deletion of both transporter genes is lethal, suggesting that transport of extracellular GlcNAc into the cell is necessary for hyphal growth.

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Elucidating Cell Wall Architecture in Rhizopus and Mucor Species and Melanin Structure in Lichtheimia via Solid-State NMR Spectroscopy

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Zygomycetous fungi are common pathogens in immunocompromised hosts, yet the molecular architecture of their cell walls and melanin remains poorly defined. Leveraging high-resolution solid-state NMR, we recently resolved the structural organization of Mucorales cell walls and found that they are built on a fundamentally distinctive chitin- and chitosan-dominated framework. Multidimensional NMR reveals several polymorphic forms of these polysaccharides that assemble into a dense, semi-crystalline rigid inner core. This core incorporates wall-associated proteins but contains only trace β-glucan, restricted to a single chitin-linked subdomain rather than forming an independent scaffold. In contrast, the outer wall layer is highly mobile and enriched in galactan-, mannan-, and fucose-containing polysaccharides, forming a chemically and dynamically distinct matrix that overlays the rigid fibrillar network. Using nikkomycin, we further dissect the contributions of specific chitin subtypes to wall integrity. Nikkomycin selectively removes the β-glucan–associated chitin/chitosan complex while leaving other chitin and chitosan allomorphs largely intact. Counterintuitively, loss of this subdomain does not weaken the wall; instead, we observe increased rigidity and thickness of the remaining chitin-chitosan scaffold, consistent with compensatory remodeling of the structural core. These findings demonstrate that Mucorales walls consist of a polymorphic, adaptable chitin-chitosan framework with a pharmacologically sensitive subdomain and a flexible outer glycan layer, underscoring the structural diversity generated by different chitin synthases. In parallel, we present unpublished solid-state NMR analyses of Lichtheimia corymbifera that characterize both its cell-wall structure and the molecular features of its eumelanin.

273C

Trehalose biosynthesis pathway-mediated thermotolerance via tight regulation of glycolytic flux

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Trehalose has been shown to play a key role in the fungal response to high temperature stress, as is found in the transition from ambient temperatures to human physiological conditions. Trehalose is synthesized from glucose-6-phosphate (G6P) and uridine-di-phosphate-glucose (UDPG) in two steps catalyzed by the enzymes trehalose-6-phosphate synthase (Tps1) and trehalose-6-phosphate phosphatase (Tps2) in most fungi. Given the importance of trehalose in the response to high temperature stress, loss of Tps1 or Tps2 leads to loss of thermotolerance. I will present data on the genetic suppression of the *tps1* mutant phenotype under high temperatures in *Cryptococcus deneoformans*, a species in the *Cryptococcus* pathogenic species complex. We found that, as has been found in other fungi, Tps1 is essential for growth at 37°C in *C. deneoformans* and identified spontaneous suppressor mutations that restore the growth of *tps1*Δ mutants under high temperatures. Whole-genome sequencing followed by variant calling analysis led to the identification of several causative loci. I will present the identity and characterization of one of these genes encoding hexokinase 1 (Hxk1), one of two hexokinases in *C. deneoformans*. Several independent mutations were identified in the gene including amino acid changes, protein truncation, and deletions. Biochemical analysis shows that these amino acid changes result in loss of Hxk1 activity. By combining genetics and measurements of metabolic flux in the glycolysis pathway, we will show that overall hexokinase activity increased in the *tps1*Δ mutants and was significantly restored to wild-type levels in the suppressor mutants. By generating gene deletion mutants, we will further present data that deletion of either *HXK1* or *HXK2* can bypass *tps1*Δ in a carbon source-dependent manner. Deletion of *HXK1* or *HXK2* partially restored G6P, ATP, and glycogen levels in the *tps1*Δ mutant to wild-type levels, affecting the glycolytic flux in these mutants. Overall, our study reveals a role for Tps1 in high temperature growth, genetic mutations that bypass this function, and a metabolic basis for genetic suppression in *C. deneoformans*. This presentation will show that the role of trehalose in thermotolerance extends beyond simply stabilizing proteins and membranes.

274C

Powdery Mildew Fungi Manipulate Plant Lipid Metabolism for their Growth & Reproduction

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Powdery mildew fungi are obligate biotrophic pathogens of diverse plant species. Their dominant mode of reproduction is asexual resulting in the production of spores replete with storage lipids. These storage lipids are utilized during fungal germination and early colonization before the fungal feeding structure, the haustorium, develops. We performed integrated molecular genetic, biochemical, and metabolic analyses coupled with confocal microscopy and RNAi silencing of powdery mildew genes to elucidate how powdery mildew manipulate plant lipid metabolism to promote asexual reproduction. We found that powdery mildews induce a localized shift in primary plant metabolism, bypassing the mitochondrial pyruvate dehydrogenase complex pathway, to direct plant resources to lipid accumulation. These lipids accumulate primarily as triacylgycerols (TGs) in cells underlying the haustoria-containing cells and are incorporated into spore TGs. The induced plant TGs that support spore formation are made in the chloroplast via the atypical diacylglyceroltransferase (DGAT) DGAT3 at the expense of thylakoid lipids. Furthermore, DGAT3 is localized to the plastoglobule (PG), a plastidic lipoprotein particle that acts as a hub for lipid metabolism and storage. A powdery mildew effector also localizes to the PG and interacts with the PG core protein fibrillin 2 (FBN2). This interaction may facilitate fungal acquisition of PG or PG composition. Silencing this powdery mildew effector or genes involved in lipid catabolism reduces powdery mildew asexual reproduction. Finally, field trials spraying RNAi biopesticides that target powdery mildew genes in lipid catabolism show the efficacy of this approach for powdery mildew control.

275C

Dissecting the Genetic Control of Antagonistic Fungal Volatile Organic Compounds in *Pinus radiata* Pathogen Suppression

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Dothistroma septosporum is a major fungal pathogen of *Pinus radiata*, causing Dothistroma needle blight (DNB). Typical symptoms of DNB include chlorotic yellow spots or bands on needles that progress to tan lesions and distinctive red-brown discoloration. In New Zealand, *P. radiata* constitutes approximately 90% of all planted forests, making DNB a significant threat to the forestry sector.

In this study, we investigated the bioactivity of fungal volatile organic compounds (FVOCs) produced by *Trichoderma atroviride* against *D. septosporum* using both direct and indirect exposure assays. Our preliminary work identified one FVOC with particularly strong inhibitory effects on *D. septosporum* during direct interaction, which we designated FVOC-T1. To understand the molecular responses triggered by this compound, we analysed transcriptomic data from *D. septosporum* exposed to different FVOC-T1 concentrations across multiple timepoints to identify differential gene expression patterns associated with FVOC-T1 treatment.

Our results showed that even low FVOC-T1 concentrations significantly restricted *D. septosporum* growth under both exposure methods. Transcriptome analysis revealed hundreds of differentially expressed genes (DEGs) in FVOC-T1-treated samples compared with untreated controls. The number of DEGs varied with FVOC-T1 concentration and increased with longer exposure duration. Pathway analysis indicated that FVOC-T1 treatment may influence lipid metabolism, biological oxidation, and fatty acid metabolism, suggesting impacts on energy production and stress responses.

Overall, this study demonstrates that FVOC-T1 produced by *T. atroviride* has measurable inhibitory effects on *D. septosporum* and provides new insights into microbe–microbe interactions with potential applications for biocontrol of forest pathogens.

276C

Large-scale transcriptomics reveal a kexin protease required for the formation of the multicellular host morphology of the fungal pathogen *Coccidioides*

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Coccidioides, a dimorphic fungus endemic to the Southwest United States, Central America, and South America, causes infections in people with or without immunocompromise. The most serious manifestation of infection, meningitis, still causes 30% mortality despite current treatments and survivors often have irreversible neurologic damage. In recent years, the incidence of coccidioidomycosis has continued to rise and its endemic region is spreading. The ability of *Coccidioides* to cause infection depends on an elaborate developmental transition: hyphae found in the soil produce spores (arthroconidia) that are inhaled by mammals and then transform into spherules, a multicellular host morphology unique to *Coccidioides*. Once mature, spherules are filled with hundreds of endospores. The spherules then rupture, releasing endospores to disseminate to other sites within the host, where each can go on to form another spherule in a cycle called spherulation. The molecular determinants of spherulation remain poorly understood, likely explaining our lack of effective treatment options. Candidate effectors involved in spherulation are a family of secreted proteases, subtilases, that have undergone evolutionary expansion in the *Coccidioides* lineage compared to other fungal pathogens that do not form spherules. Using a class-wide serine protease inhibitor targeting the subtilases, we have inhibited both spherulation and endospore release in vitro. Guided by the first large-scale transcriptomic atlas of *Coccidioides* development we recently created, we prioritized subtilases demonstrating dynamic expression over spherulation for deletion. One such subtilase, Sub6, whose transcript abundance peaks during early spherule formation, is required for normal spherule morphology and efficient endospore release. Mutants lacking *SUB6* are more sensitive to cell wall stressors during spherulation. Upon *Coccidioides* co-culture with bone-marrow derived macrophages, *SUB6* influences the size of spherules formed. Using an established intranasal mouse model of infection, we found that *SUB6* is required for virulence. By linking subtilase activity to multiple steps of spherulation, we have demonstrated the importance of further dissecting the role of this protease family in spherule biology and discovered a promising drug target, Sub6, whose inhibition could cure coccidioidomycosis.

277C

Mitochondrial Drug-Binding Strategy Underlies Caspofungin Resistance in *Cryptococcus neoformans*

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Cryptococcus exhibits inherent resistance to echinocandins, which inhibit the synthesis of (1,3)-β-D-glucan, a key component of the polysaccharide cell wall. Although the essential FKS1 gene encodes the catalytic subunit of (1,3)-β-D-glucan synthase and caspofungin effectively inhibits this activity *in vitro* using whole-cell lysates, the drug remains ineffective against Cryptococcus, suggesting mechanisms beyond target insensitivity. Although altered regulation of cell-wall remodeling genes and drug efflux pathways has been suggested, the underlying mechanisms of caspofungin resistance remain unknown. Using boron dipyrromethene (BODIPY-) and fluorescein isothiocyanate (FITC-) labeled caspofungin, we demonstrate, for the first time, that the drug enters the cryptococcal cell wall but then primarily accumulates in the mitochondrial inner membrane rather than the plasma membrane. This unforeseen localization indicates that mitochondrial stress responses play a role in echinocandin resistance and that mitochondrial sequestration diminishes the effective drug concentrations at the intended target. We further establish that this mitochondrial accumulation is driven by caspofungin's strong affinity for cardiolipin. Notably, the interaction between caspofungin and cardiolipin also takes place in human cells, establishing a mechanistic connection to caspofungin-related cardiotoxicity. Our findings reveal a previously unrecognized mechanism of echinocandin resistance in Cryptococcus and emphasize cardiolipin as an important factor in caspofungin distribution and toxicity.

278A

Semi-automated high-throughput screening accelerates strain development in *Trichoderma reesei*

Norman Adlung, Tino Koponen, Nina Aro Technical Research Centre of Finland

Trichoderma reesei is a filamentous fungus widely used in biotechnology for industrial protein production. Despite being studied and modified for decades, traditional *T. reesei* strain engineering and screening approaches are often labor-intensive and time-consuming, which limits throughput and slows down development cycles. To address these challenges, we developed a semi-automated high-throughput screening workflow by downscaling cultivation to a microtiter-plate format and utilizing laboratory automation tools such as a colony picker and a liquid handling robot.

T. reesei strains secreting a recombinant laccase were generated to optimize cultivation parameters such as plate type, cultivation time and media composition. Improved microtiter plate cultivation led to similar laccase yields as 24-deep-well cultivation, while standard variation between technical replicates remained slightly higher. The use of automated colony picking and the establishment of transformant sporulation on 96-well agar plates with automated spore harvesting further increased transformant screening throughput. Correlation across microtiter-plate cultivation, 24-deep-well plate cultivation, fed-batch processes, and bioreactor cultivation was studied to evaluate scalability. The developed workflow was used to screen transformants from an overexpression library containing genes involved in protein production. A top-performing transformant from this screening exhibited a more than 2-fold increase in laccase production during bioreactor cultivation, demonstrating the platform's effectiveness for rapid strain optimization.

279A

Synthetic nuclease gene drives as a novel mode of plant pathogen biocontrol

Ben Auxier Wageningen University

Rising antifungal resistance in plant pathogens is a growing concern. Our reliance on chemical fungicides, with negative effects on both environmental and human health, leaves society exposed to ongoing risk. Current agricultural practices and resistance breeding are insufficient to control these diseases, motivating novel control methods. In insects, synthetic Cas-based gene drives are being actively developed to control populations, but in fungi this approach is underdeveloped. Progress appears to be blocked due to our lack of understanding of 1) when to time nuclease activity and 2) genetic targets that can lead to supression. To understand the timing issue, major deficiencies exist in our understanding of gene expression during the sexual cycle. Promoter

that lead to expression exclusively during early sexual stages are expected to be highly effective in Cas-based gene drives due to increased homology-directed repair that occur during meiosis. A more major concen is the gene drive target. In insects a common strategy is that the drive has no effect when heterozygous, allowing it to spread in a population, but lethal when homozygous. At first, this seems difficult to apply in haploid fungi. I predict the sexual stage is critical, as during sex, the brief diploids creates a developmental window that could be heterozygous. The obligate sexual cycles in pathogens like *Zymoseptoria tritici* and *Leptosphaeria maculans* make them key targets for such gene drives. Currently, no genes are known for this stage, although recessive sexual mutations have been described for several decades in *Neurspora*. Addressing safety concerns, the large population size and reproductive isolation of fungal plant pathogens may bypass many safety concerns about uncontrolled spread. Understanding laboratory-scale model populations can inform resistance allele formation and gene flow patterns. Clearly, several critical insights are still needed into the sexual stage of fungi, as well as the dynamics of Cas nucleases in hyphal cells, before expansion is possible towards functional gene drives in diverse systems. Future laboratory results can provide the starting point for the necessary societal discussions prior to eventual targeted applications.

280A

From lab to table: Harnessing fungi to convert globally abundant food and agricultural byproducts into sustainable food

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Food waste or loss accounts for one-third of global annual food production and results in half of total GHG emissions from the global food system. New technologies are therefore needed to reduce or repurpose food waste, sustainably feed an ever-growing population, and mitigate the negative planetary impacts of the current food system. To help address the food waste problem, we have created a novel and sustainable food stream by upcycling human-generated food and agricultural byproducts (e.g. plant-based milk by-products, fruit and vegetable pomace) using *Neurospora intermedia* – a filamentous fungus capable of converting diverse byproducts into sustainable, nutritious, and protein-rich food. Traditionally used in Indonesia to produce oncom – a fermented food derived from soymilk waste – *N. intermedia* is safe to eat and has attractive nutritional and organoleptic profiles, making it ideally suited for upcycling waste into edible biomass. In collaboration with Michelin star chefs, we recently developed several novel food prototypes including meat and dairy alternatives derived from solid state fermentation of *N. intermedia* on diverse plant-based food-ag byproducts. Our initial prototypes demonstrate the remarkable biochemical capabilities of *N. intermedia* for converting food waste into delicious and nutrient-dense foods – a key challenge for circularity of modern food systems.

To enhance the utility of *N. intermedia* for targeted nutrient biosynthesis and waste degradation, we next developed an effective CRISPR-based method for targeted gene editing of *N. intermedia* and demonstrated for the first time its tractability for genetic engineering in the lab. We also generated expansive metabolomic and transcriptomic datasets by fermenting *N. intermedia* with diverse food-ag byproducts, and identified key genes and metabolic circuits involved in the production of nutrients and bioactive molecules relevant to human health. Using our genetic tools and substrate-specific omics profiles in tandem, we are generating new cultivars of *N. intermedia* with enriched nutrient profiles including strains overproducing vitamin A and ergothioneine – a potent antioxidant with potential roles in disease prevention.

Overall, our work demonstrates the biotechnological potential of *N. intermedia* to produce nutrient-dense foods, and provides a strong foundation for building modular genetic tools to characterize and manipulate gene expression in edible non-model fungi.

281A

CrispR/Cas9 mediated co-editing strategy in genetically recalcitrant fungi

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Fungal pathogens threaten global-health, food-security, and ecosystems by infecting animals, plants, and insects. In humans, they cause deadly diseases such as aspergillosis, cryptococcal meningitis, and multidrug-resistant infections. In agriculture, better control of fungal plant pathogens could yield enough food to feed over 600 million people. Conversely, these organisms hold immense potential across biotechnology, medicine, and agriculture (*e.g.* mycoremediation, mycoprotein and metabolites production). However, unlike model organisms such as *S. cerevisiae*, genetic manipulation in most fungi remains a significant challenge due to limited selectable markers, the difficulty to transform them and their particularly low efficiency in homologous recombination. Even though genetic tools were successfully developed in few, specific fungal systems, these technics are difficultly translatable to other species. This lack of efficient and broadly applicable genomic engineering methods has delayed both academic research and industrial exploitation of fungal resources.

To overcome this limitation, we provide robust and standardized protocols that can be applied across multiple fungal species. By leveraging RNP-mediated CrispR technologies, we achieved efficient genetic manipulations in various fungal species, including gene deletions, point-mutations and C-terminal tagging. Furthermore, we managed to develop a marker-free co-editing strategy enabling efficient point-mutations and N-terminal tagging. We anticipate that our approach will unlock opportunities these organisms hold and enable their fuller utilization in science/industry.

282A

Empowering Fungal Research: Cultivarium tools to accelerate study and engineering of non-model fungi

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Cultivarium, a not-for-profit research organization, is aiming to accelerate discovery in diverse non-model fungi by developing high throughput open access tools for the research community. Our goal is to provide inexpensive and accessible protocols, software, and devices to overcome challenges in fungal cultivation, genetic manipulation, and data accessibility. For cultivation, we are developing an affordable incubator-imager designed to capture time-lapse images of fungal morphologies under standard conditions. Such visual reference databases will make it possible to rapidly compare and validate samples across labs and growth conditions. For fungal genetics, Cultivarium is developing high throughput screens to identify protocols for DNA delivery. In our

preliminary results, we demonstrate successful electroporation into several established model fungal species. This success paves the way for applying the approach to novel non-model organisms. These organisms either pose potential threats to public health or possess valuable intrinsic properties, such as unique metabolic pathways or bioactive compounds.We offer free access to our organism portal and data search software tools. Finally, we offer access to our AI-guided protocol video-capture system called Protocol Recording for Improved Scientific Methods (PRISM) that helps reproduce, distribute and train on fungal lab techniques. By making our tools accessible to the fungal community, we hope to lower the barrier for genetic tractability in new fungal species, and empower researchers with accelerated research to discover new fungal biology, prepare for emerging fungal pathogens, and enable new fungal biotechnology applications.

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A fungal community driven development of the oCelloScope™ image analysis platform to quantify growth using deep learning in Medical-, Crop Sci-, Food research and Strain Development

Soren Busch BioSense Solutions

Through years of strategic collaboration with mycology professionals in both academia and industry, the oCelloScope live-cell imaging platform has emerged as a comprehensive tool for analysing fungi, yeasts, and oomycetes across diverse applications from plant pathology to industrial strain development. Through this community-driven development, the technology combines a patented non-invasive time-lapse imaging system with advanced deep learning modules that recognize, count, and track spores, conidia, and mycelial networks in standard microtiter plates and on agar surfaces.

In this presentation, recent assay developments will be highlighted, including a no-stain live/dead assay for yeast and deep-learning-based workflows that quantify susceptibility of oomycete plant pathogens such as Plasmopara viticola (downy mildew) and Phytophthora infestans (late blight) in 96-well formats. Additional assays capture key stages of fungal development, from spore germination kinetics and early hyphal outgrowth to branching patterns and mycelial expansion, enabling high-throughput screening of antifungals, biological control agents, and engineered production strains in both “clean” and “dirty” samples.

Compared to conventional microscopy and optical density measurements, the oCelloScope platform provides faster readouts, automated image analysis, and robust handling of non-uniform fungal morphologies, while preserving cells under native growth conditions. These capabilities have already been applied in multiple peer-reviewed fungal studies and BioAg development projects, supporting rapid assay transfer from research to applied settings. The aim of this presentation is to engage the scientific community in the ongoing development of essential assays for quantification of fungal processes, leveraging our technological capabilities alongside the extensive expertise available at Asilomar.

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OMICS analyses to disclose tolerance mechanisms to the antimicrobial SO_2 in the wine spoilage yeast *Saccharomycodes ludwigii*

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In winemaking, sulphur dioxide (SO_2) stands as the most commonly used antimicrobial to prevent wine spoilage, given its capability to inhibit growth of contaminating microorganisms. However, some species can still tolerate this compound and thrive in sulphited wines. This is the case of the spoilage yeast *Saccharomycodes ludwigii*. *S. ludwigii* is a non-conventional yeast belonging to the poorly characterized *Saccharomyceadea* family, sister to the much more studied *Saccharomycetacea* to which the paradigmatic *Saccharomyces cerevisiae* belongs to. Our laboratory has been involved in genomic and phenotypic characterization of *Saccharomycodeacea* yeasts, having provided the first genome sequence of a *S. ludwigii* wine strain, UTAD17. The genome of UTAD17 disclosed, among other aspects, what seem to be two homologues of the *S. cerevisiae SSU1* gene, predicted to also serve as sulphite efflux pumps given their similarity to the *S. cerevisiae* gene. In this work, we show results concerning the functional analysis of these presumed *S. ludwigii* sulphite transporters, using as a comparative platform the Ssu1 from *S. cerevisiae*. Additionally, we will also discuss the results of comparative transcriptomic and genomic analyses undertaken with strains of *S. ludwigii* exhibiting different degrees of susceptibility to SO_2. Overall, we expect that these combined OMICS studies may shed light into the molecular mechanisms mediating the SO_2 tolerant phenotype of *S. ludwigii* strains, contributing to the design of more efficient wine preservation strategies.

285A

Breaking Barriers in Fungal Engineering: Development of the Serine Recombinase-Assisted Genome Engineering (SAGE) Toolkit

Kyle A Davis[1], Carrie Eckert[1], Kyle Pomraning[2], Adam Guss[1], Joanna Tannous[1] [1]Synthetic Biology, Oak Ridge National Laboratory, [2]Biological Conversion, Pacific Northwest National Laboratory

Filamentous fungi and yeasts are indispensable workhorses of modern biotechnology, powering the production of enzymes, fuels, pharmaceuticals, and other high-value compounds. Yet, their full potential remains underexploited because current genome engineering methods are limited, particularly in non-model filamentous species. Traditional approaches are hindered by low homologous recombination (HR) rates, the dominance of non-homologous end joining (NHEJ), and the lack of naturally occurring, autonomously replicating plasmids in most species. Here, we introduce the Serine recombinase-Assisted Genome Engineering (SAGE) toolkit, a transformative platform designed to overcome these barriers. SAGE enables precise, site-specific, and iterative integration of up to 12 DNA constructs. Unlike traditional approaches, it obviates the need for replicating plasmids and intricate, long donor DNA sequences, significantly reducing cost and complexity. Moreover, its ability to recycle selectable markers via a secondary serine recombinase further enhances flexibility and scalability. This powerful and versatile tool unlocks new opportunities for industrial biotechnology, including the construction of

promoter libraries, functional gene analysis, and metabolic pathway engineering. In this study, we demonstrate the ability to implement this technology across four industrially relevant fungal hosts, and show its successful use in several downstream applications, highlighting its robustness and broad applicability. With its efficiency, modularity, and adaptability, SAGE redefines the genetic engineering landscape for fungi and yeasts. We anticipate that this system will not only accelerate discovery but also catalyze next-generation innovations in ecology, biotechnology, and industrial microbiology.

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Utilizing the FIND-IT technology to accelerate strain development across microbial species

Hanna Muller-Esparza, Myrsini Natsopoulou, Sarah Klarberg, Stephen M Fuchs, Toni Wendt Traitomic

Genetic modification of microbial strains has the potential to improve the performance across the product pipeline. Unlocking new functionalities, deleting unwanted subproducts and optimizing processes can all be achieved by the identification and modification of a genetic target, leading to a phenotype with added value. Novel genetic engineering tools have certainly accelerated strain development, nevertheless they still have technical limitations when it comes to strain transformability and efficient heterologous protein expression, in addition to facing regulatory and consumer constrains in many markets. This is especially true for fungal species.

Here we present a conventional strain improvement approach, which harnesses the broad applicability of mutagenesis together with the high-throughput screening power of digital PCR (dPCR). The technology, termed FIND-IT (Fast Identification of Nucleotide variants by droplet DigITal PCR) [1], offers a plug-and-play option for hard-to-modify organisms, in addition to a non-GM strain improvement alternative with global regulatory and consumer acceptance. Through FIND-IT, we can achieve the screening and isolation of desired microbial variants in less than 8 weeks.

Altogether, FIND-IT is a powerful tool for studying genotype-phenotype relations without species constraints, and accelerates the development of biotechnologically relevant strains in commercial settings.

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2. Poulsen C., Harholt J. (2023) *Modulation of protein levels* (WO 2023/170272 A1). World Intellectual Property Organization.

287A

Asymmetric mitonuclear interactions trigger transgressive inheritance and mitochondria-dependent heterosis in hybrids of the model system *Pleurotus ostreatus*

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Mitonuclear interactions are crucial in governing mitochondrial function, development and responses to environmental stress in eukaryotic systems. In this study, we investigated how variation in mitochondrial haplotypes modulates phenotypic traits and oxidative stress responses using hybrids of the basidiomycete *Pleurotus ostreatus* (*P. ostreatus*) as a model system. By generating reciprocal crosses between monokaryotic strains carrying distinct nuclear backgrounds and divergent mitochondrial genomes, we uncovered marked differences in growth performance, intracellular accumulation of reactive oxygen species (ROS), and gene expression patterns. Hybrids carrying incompatible mitonuclear combinations exhibited reduced mycelial growth and elevated expression of genes—some displaying transgressive inheritance—linked to the electron transport chain and antioxidant defense mechanisms, indicating a compensatory response to oxidative imbalance. Hybrids sharing identical nuclear genomes but differing in their mitochondrial haplotypes revealed pronounced mitochondria-dependent heterosis, highlighting the strong contribution of mitochondrial variation to quantitative traits relevant for fungal vigour. Our findings provide experimental evidence that mitonuclear incompatibilities can compromise cellular homeostasis and, ultimately, fungal performance, underscoring the importance of mitonuclear co-adaptation in this edible mushroom. This experimental approach establishes *P. ostreatus* as a powerful model for exploring the functional consequences of mitonuclear interactions and offers valuable insights for optimizing mushroom breeding programmes. Incorporating mitochondrial variation and compatibility into hybrid design strategies may enhance yield-related traits and improve the efficiency of commercial strain development.

288A

Genetic Engineering of *Trichoderma atroviride* for Functional Studies in the Rhizosphere

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Filamentous fungi are critical members of the rhizosphere, where they shape soil microbial communities and influence plant health. For example, members of the *Trichoderma* genus form direct association with plant roots and directly modulates growth of rhizosphere bacteria and inhibits fungal pathogens. Despite the widespread use of *Trichoderma* species as biostimulants to promote the growth and resilience of diverse agricultural crops, the molecular basis of these interactions between *Trichoderma* and other organisms remains poorly understood at the molecular and genetic level. To address this gap, we developed a CRISPR-Cas9 toolkit that enables precise genetic modification of the biocontrol fungus *Trichoderma atroviride* to study and manipulate its interactions in the rhizosphere. This method involves two key steps: first, integrating a *pyrG* gene cassette into a defined genomic location, and second, recycling the *pyrG* marker in a locus-specific manner, allowing additional rounds of editing. To validate the system, we deleted a biosynthetic gene cluster (BGC) responsible for spore pigmentation and achieved successful gene targeting in 12/12 tested transformants. In a second validation step, growth and phenotype assays confirmed the correct insertion and recycling of the *pyrG* marker at the target locus, enabling further genome edits. Through bioinformatics analysis, we identified twenty-seven clusters predicted to encode for secondary metabolites with potentially important roles in rhizosphere interactions and deleted them in *T. atroviride*. We are currently investigating how these mutations affect the growth of a library of rhizosphere-associated

bacteria, as well as plant and fungal pathogens. In the long term, these tools will enable functional studies and engineering of *Trichoderma* and other rhizosphere fungi supporting the development of new bioproducts and bioactive compounds for sustainable agriculture.

289A

A Bitter Tug-of-War: Exploiting fungal sexual reproduction for better blue cheese

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The fungus Penicilium roqueforti is used in the production of blue cheeses such as Stilton, Gorgonzola and Roquefort. All such cheeses go through a “bitter phase” during ripening, during which they are unpalatable. However, the timing, duration and final results of taste changes during this phase are highly variable, especially when using different strains of the fungus. Previous research has identified that small, hydrophobic peptides generated during cheese ripening are likely responsible for the bitter taste, but the exact chemical identities of these molecules in blue cheese is currently unknown. Linked to this, the genetic basis of variation in bitterness between different strains of the fungus P. roqueforti is poorly understood, which makes strain improvement challenging. There is also evidence that umami and salty tasting molecules can interfere with bitterness.

My work is investigating why P. roqueforti strains show different bitterness profiles, aiming to identify genes whose expression contributes to varying levels of bitterness in cheese products. An electronic tongue has been applied to investigate changes in taste over time in a cheese simulation medium, using two strains known to generate more or less bitter cheese. This demonstrated how bitterness and umami levels vary between the two. Following this, 18 potentially-relevant candidate genes that might contribute to formation of bitter peptides have been identified and investigations into their expression in a cheese-like environment have been conducted. These include an aspartic protease AspA, which has already been identified as particularly important enzyme in the cheese maturation process, so investigations into its activity have been prioritised. Additionally, a Stilton cheese's taste and peptidome have been tracked over time using e-tongue and LC-MS-MS approaches to observe how maturation affects these qualities. Significant trends in taste were observed, and 938 unique casein peptide sequences were recorded and rated for likely bitterness using machine learning models. My current hypothesis is that the balance in expression levels of proteases and peptidases is responsible for determining bitterness levels in the final product. This is being investigated further using CRISPR Cas-9, qPCR, proteomic and e-tongue experimental approaches on both cheese simulation medium and actual cheeses.

290A

The impact of developmental structures on pure mycelial materials made from *Aspergillus nidulans*

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The vast majority of the world’s manufactured materials are nonrenewable and energy-intensive to produce. Viable alternatives must be developed, and “mycelial materials” made from filamentous fungi are an exciting option. Companies manufacturing various mycelial materials (e.g., textiles, food products, packing foams, building supplies) have attracted hundreds of millions in commercial investment, and the global market is predicted to soon exceed $5 billion. Yet, the current approach to modifying material properties is typically through trial-and-error, using different fungal species, altering nutrients used for growth, or changing the growth environment. Because it’s unclear how these inputs lead to *specific* changes in material properties, advances have been limited. To address this limitation, we are exploring the use of genetic approaches to modify fungi to be used in materials. Our central hypothesis is that genetic means can be used to tune fungal morphological phenotypes, and that these phenotypes can be combined to enable the design of mycelial materials with specific sets of material properties. Initial studies have involved the model fungus, *Aspergillus nidulans*. We initially hypothesized that disabling the cell wall integrity (CWI) signaling pathway, which leads to weaker-walled hyphae, would result in weaker material. Surprisingly, material from an *mpkA* deletion strain (D*mpkA*) was significantly stronger than material generated from an isogenic control. Comprehensive phenotype testing implied increased material strength was due to lack of fungal developmental structures. Subsequent experiments, with several developmentally-disabled strains (D*brlA*, D*flubA*, D*fluG*) confirmed this.

291B

Laboratory evolution of the all-rounder yeast *Metschnikowia pulcherrima* shows different mechanisms of adaptation depending on genotype and ploidy

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Strains of *Metschnikowia pulcherrima* are useful industrially across a wide range of applications in oenology, biocontrol, probiotics, and sustainable oils for cosmetics and food. Part of the allure of these, and similarly less well-studied yeasts, may be their poorly circumscribed nature that can suggest few defined limits on their potential uses while offering enough similarity to models to allow clear routes for exploitation. Adding to the suggestions of potential, the *M. pulcherrima* group of yeasts is thought to include natural populations with high genetic variation, few barriers to gene flow across what has been at times been considered many different species, and variation in ploidy. In addition to this natural variation, a major route for improved industrial outcomes has been through various laboratory evolution approaches. Here we use several different strains of *M. pulcherrima* representing different genetic backgrounds and ploidy in simple clonal laboratory evolution experiments. To uncover patterns of genetic change that are genotype specific, we evolved the strains in different selective conditions, for example, temperature stress, acid stress, microbial competition, or salt. Evolution that involves adaptation via large duplications is less common in haploid strains than those with diploid or more complex ploidy, but all strains show patterns of abundant copy number variation. We also describe the spatial distribution of genetic variation over *M. pulcherrima* genomes and observe patterns of change in intergenic space that suggests possible novel molecular mechanisms of genetic regulation.

292B

Improved recombinant protein production and culture rheology in pure oxygen aeration fermentation using a hyphal dispersion strain of *Aspergillus oryzae*

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Aspergillus oryzae is a filamentous fungus widely used as an industrial production host in submerged cultivation. Following inoculation, wild-type strains rapidly form spore aggregates that develop into pelleted mycelia, which can impose substantial diffusion constraints on oxygen and nutrients. In contrast, dispersed mycelia enhance mass transfer but markedly increases culture viscosity and non-Newtonian properties, thereby compromising mixing efficiency. We previously reported that deficiency in the cell-surface polysaccharides α-1,3-glucan (AG) and galactosaminogalactan (GAG) generates a hyphal dispersion strain (AGΔ-GAGΔ) exhibiting reduced culture viscosity and enhanced recombinant protein production. Such rheological control is particularly crucial in large-scale fermentations, where excessive viscosity markedly increases mixing energy requirements and limits bioprocess scalability. Although AGΔ-GAGΔ strain represents a promising host for industrial enzyme manufacturing, the reduction in culture viscosity remains insufficient, indicating a need for further rheological optimization.

In this study, we sought to further enhance recombinant enzyme production in stirred-tank fermentation by cultivating either the wild-type or AGΔ-GAGΔ strain under air or pure-oxygen aeration. Comparative analyses were conducted on recombinant enzyme productivity, culture viscosity, and pellet size distribution. Pure oxygen aeration increased enzyme productivity compared with air aeration and induced a morphological shift in the AGΔ-GAGΔ strain toward finer pellets. Quantitative pellet analysis revealed that pelleted mycelia accounted for 0 % of total biomass up to 36 h of cultivation but increased significantly thereafter. Most wild-type strain pellets were larger than 700 μm in diameter, whereas those of AGΔ-GAGΔ strain ranged from 200 to 700 μm. Rheological measurements revealed that decreases in viscosity and non-Newtonian behavior of culture correlated with increased abundance of finer pellets. The formation of smaller pellets in AGΔ-GAGΔ strain likely alleviates intrapellet diffusion limitations for oxygen and nutrients. Collectively, these findings demonstrate that pure-oxygen aeration in AGΔ-GAGΔ strain improves both culture rheology and recombinant enzyme productivity, underscoring its potential to enhance mixing efficiency and reduced agitation power in filamentous fungal bioprocesses.

293B

Transcriptomic Insights into Isomaltose-induced amylolytic enzyme production in *Aspergillus nidulans*

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Amylolytic enzymes, which degrade starch, are of great importance in both ndustrial applications and fundamental research. In *Aspergillus* species, the expression of amylolytic enzyme genes is regulated by the fungal-specific transcription factor AmyR. Among known inducers, isomaltose is the most effective and direct activator of AmyR compared to glucose or maltose. We previously showed that isomaltose uptake is mediated by proton-coupled symporter[1)] and identified a novel isomaltose transporter, ImtA, in *Aspergillus nidulans* through spontaneous mutagenesis[2)]. However, isomaltose uptake pattern and further isomaltose-mediated AmyR activation mechanism remain poorly understood. In this study, HPLC analysis demonstrated that isomaltose is slightly taken up inside the cell in *Aspergillus oryzae* and *A. nidulans*, and this uptake is dependent on the novel isomaltose transporter ImtA. Altogether, a tiny amount of isomaltose is transported into the cell and subsequently triggers AmyR nuclear localization to activate amylolytic gene expression in *Aspergillus* species. RNA-seq analysis further revealed differentially expressed genes in the isomaltose transporter-deficient or AmyR-deficient strain in the presence of isomaltose to make clear observation of isomaltose-mediated AmyR activation pathway. Interestingly, the expression of amylolytic genes including *amyR* is dependent on ImtA in the presence of isomaltose.

1) Shoki Fujita et al., Biosci Biotechnol Biochem, 89, 1391−1399 (2025)
2) Jeong et al., The 33rd Conference on Fungal Genetics and Molecular Biology (P-42) (2024).

294B

Quantification of Viable Fungal Spores Using a PMAxx Dye-Based Viability-qPCR Assay

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Accurate quantification of viable fungal spores is essential for evaluating fungal responses to environmental stresses, assessing virulence, and supporting disease diagnostics. In this study, we evaluated the performance of a PMAxx dye–based viability qPCR (vPCR) assay for distinguishing viable from non-viable spores of several plant pathogenic fungi, including members of the *Fusarium*, *Botrytis*, *Colletotrichum*, and *Magnaporthe* genera. PMAxx selectively binds to the DNA of compromised spores, thereby preventing their amplification during qPCR. To systematically assess assay performance, defined mixtures of viable and heat-killed spores were prepared. The resulting Ct values exhibited a strong inverse correlation with the proportion of viable spores, with each one-cycle increase corresponding to an approximate 50% reduction in viable spore content. Compared with traditional plating methods, the vPCR assay provided faster turnaround time, reduced labor requirements, and greater scalability for high-throughput analysis. These findings highlight PMAxx-vPCR as a reliable, culture-independent molecular tool for quantifying fungal viability and support its potential application in fungal physiology research.

295B

Building the Future of Roads: Biotechnological Optimization of Mycelium-Based Binder

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Fungal biotechnologies are advancing and finding applications across many sectors. One rapidly growing area is the development of mycelium-based materials. While these materials have been explored in controlled, non-load bearing applications, their use as load-bearing materials in outdoor conditions with continuous mechanical and environmental stress remains challenging.

The motivation for this work comes from the growing need in the road industry to find alternatives to petroleum-based binder, bitumen, and to reduce the high CO_2 emissions linked to concrete production. We developed a mycelium-based binder, visiBINDER, which leverages the natural ability of fungal mycelium to cohesively bind the material it grows through. This biogenic binder offers significant advantages, lower energy consumption in pavement production, independence from fossil-based raw materials, and improved safety for paving workers.

In this work, we present the development of the mycelium-based binder for pavement construction, including the process of screening and selection of suitable fungus–substrate combinations, advances in fermentation optimization, binder integration into mineral aggregates, and the first industrial implementation of visiBINDER in an asphalt production plant with the results from real use-case testing.

296B

Functional analysis of Mbp1 in cell wall synthesis and mycelium material properties in *Pleurotus ostreatus*

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White-rot fungi, which can be efficiently cultivated on wood and agricultural waste, are considered sustainable bioresources for producing eco-friendly materials, such as mycelium leather, packaging materials, and bricks. Molecular breeding is gaining more attentions as an effective means to strengthen the mechanical properties of these mycelium materials. Previous studies suggest that cell wall of white-rot fungi contributes to the mechanical strength of mycelium materials, making it a promising target of molecular breeding. However, the regulatory mechanisms underlying cell wall synthesis remain unclear in white-rot fungi. To elucidate the cell wall synthesis regulatory system in white-rot fungi and the utility of molecular breeding for improving mycelium materials, this study investigated the function of a transcription factor Mbp1, predicted to be involved in regulating cell wall synthesis in the white-rot fungus *Pleurotus ostreatus*, and the effects of *mbp1* disruption on mycelium mats and composites.

mbp1 disruption strains exhibited lower mycelial growth rate and ability to develop aerial hyphae, suggesting that Mbp1 is required for normal vegetative mycelial growth. *mbp1* disruption decreased cell wall thickness, relative percentage of β-glucan and relative expression levels of β-glucan synthase genes. Furthermore, abnormal transcriptional profile of chitin synthase genes was observed in *mbp1* disruption strains. Therefore, Mbp1 is suggested to play a crucial role in the regulation of β-glucan and chitin synthesis.

To prepare mycelium mats and composites using *P. ostreatus*, dikaryotic wild-type (WT) and *mbp1* disruption strains were generated by mating. Mycelium mats of dikaryotic *mbp1* disruption strains exhibited higher Young's modulus. Because dikaryotic *mbp1* disruption strains exhibited a significantly higher mycelial density, this mechanical modification may be primarily attributed to the increased density. In addition, dikaryotic *mbp1* disruption strains showed a reduced ratio of proteins to polysaccharides. Since proteins are generally regarded as plasticizers, the decreased protein ratio may also contribute to the stiffer mats. Interestingly, *mbp1* disruption enhanced the hardness of mycelium composites. To the best of our knowledge, this study is the first to demonstrate that molecular breeding can improve mycelium composites, thereby providing a foundation for further enhancement and border applications of mycelium materials.

297B

Episomal maintenance of introduced plasmid with or without AMA1 sequence in the basidiomycete *Pleurotus ostreatus*

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White-rot basidiomycete fungi have attracted considerable attention for industrial utilization, especially due to their unique wood-degrading enzymes, and, in some case, as edible and medicinal mushrooms. Nevertheless, establishing highly efficient transformation systems for these organisms remains a substantial challenge. In filamentous ascomycetes, plasmids carrying the AMA1, which is known as autonomous replicating sequence, have been widely used to enhance transformation efficiency. However, their functionality in basidiomycetes has not been fully assessed. In this study, pAMAHyg plasmid was constructed that harbored AMA1, in addition to a hygromycin B resistance marker gene derived from the pPHT1 plasmid. pAMAHyg was introduced into the white-rot basidiomycete *Pleurotus ostreatus* and yielded more hygromycin-resistant colonies than those with pPHT1, despite its larger size, suggesting a positive impact of AMA1 on transformation efficiency. However, unlike previous reports in ascomycetes, frequent loss of drug resistance was not observed upon subculturing. And plasmid rescue assays and long-read sequencing demonstrated the capacity for episomal maintenance, regardless of AMA1, of introduced plasmids in the original or rearranged structures. Methylation-sensitive restriction enzyme analysis of the extracted plasmids indicated the loss of bacterial Dam methylation and the acquisition of CpG methylation, suggesting the replication of these plasmids in *P. ostreatus*. Southern blot analysis revealed genomic integration of the introduced plasmid as well. Together, these results suggest that the integrated and episomal plasmid sequences may coexist in these *P. ostreatus* transformants. This study provides experimental evidence supporting the extrachromosomal existence of circular plasmids in the fungus, even in the absence of AMA1.

298B

Azole-resistant development in *Aspergillus fumigatus* and its related antifungal strategy with novel nano-salt particles used as the aerosolized disinfectant

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The incidence of invasive fungal infections has increased steadily, driven by the growing population of immunocompromised patients. *Aspergillus fumigatus* is one of leading fungal pathogens and allergen of humans worldwide while invasive pulmonary aspergillosis is one of deadliest fungal infections with high mortality rates. The management of invasive fungal infections is further complicated by difficulties in accessing appropriate antifungal agents and the rising prevalence of antifungal resistance in clinical settings. This study focuses on how development and evolution in these *A. fumigatus* isolates embedding antifungal drug resistance/tolerance and persistent and then to enable the development of new therapeutic strategies for life-threatening fungal infections. Currently, it has been recognized that fungi primarily enter the human body through the respiratory tract via airborne transmission resulted in invasive fungal infections. However, related safety aerosolized disinfectants and related technologies are limited. Sodium chloride, a vital inorganic salt, plays a key role in regulating biological fluid balance. At high concentrations, it also exhibits potent disinfection properties. This study focuses on the design and synthesis of novel nano-salt particles. Using anti-solvent precipitation coupled with high-energy nanonization, we produced ultrafine sodium chloride powder at the nanoscale. These nano-salt particles are intended for use as aerosolized disinfectants against predominantly existed almost all test human fungal pathogens including antifungal azole susceptible and resistant *Aspergillus* spp, *Candida albicans* and *Cryptococcus neoformans*. Notably, the fungicidal mechanism of action against fungal conidia of *A. fumigatus* has been investigated. Data demonstrated that these novel nano-salt particles can disrupt the cell wall and membrane's integrity by creating pores and then leads to leakage of essential cellular contents and ultimately resulted in cell lysis and death. Findings in this study assess the disinfectant efficacy of this innovative nano-formulation and explore its potential practical applications.

299B

Genetic Modulation of Glycosylation Pathways Enhances Protein Stability and Activity in Filamentous Fungi

Yun Luo, Lori A Maggio-Hall IFF

Filamentous fungi such as *Trichoderma reesei* are important hosts for industrial enzyme production, but proteolytic cleavage and suboptimal glycosylation can reduce protein stability and function. In this study, we engineered *T. reesei* strains comprising a deletion of the TreSec152 gene (Trire2_105808), and either restoration or over-expression of the *mpg1* gene, which encodes GTP: α-D-mannose-1-phosphate guanylyltransferase, a key enzyme involved in N- and O-linked glycosylation. The parent strain lacks the native *mpg1* gene, which was either restored or over-expressed in the engineered strains. These modifications increased the proportion of intact endoglucase II from approximately 50% to about 95%, effectively protecting it from endogenous proteolytic cleavage. Additionally, restoration of *mpg1* in glucoamylase-producing strains improved glycan occupancy and thermostability, resulting in higher catalytic performance in starch saccharification assays. Together, these results demonstrate that targeted engineering of glycosylation pathways via *mpg1* restoration or over-expression can improve the stability and activity of multiple industrially relevant enzymes in filamentous fungi. This approach offers a robust strategy for enhancing protein yields and functional properties in bioprocessing, pharmaceuticals, and biofuel production.

300B

Role of hydrophobins in gas entrainment/foaming during fungal fermentations

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Foaming and gas entrainment are commonly encountered phenomena during fermentations with filamentous fungi. Overexpression of a native *Trichoderma reesei* hydrophobin (*hfb2*) was previously reported to significantly increase foaming, suggesting that native levels of these proteins could contribute to problematic fermentations as well. Hydrophobins are amphipathic secreted proteins that associate with cells and thus alter their interactions with aqueous fermentation media. Of the 6 Class II hydrophobin homologues in *T. reesei*, only *hfb1* and *hfb2* appeared to be relevant under submerged fermentation conditions. Using transcriptomics, we have observed lineage-specific differences in expression of *hfb1* and *hfb2*. Deletion of *hfb1*, the dominant hydrophobin in one of our high-performance *T. reesei* protein manufacturing strains, was found to mitigate gas entrainment without negative impacts on growth or the production of several different enzymes.

301B

Harnessing the edible fungus *Neurospora intermedia* to convert waste into food

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The modern global food system is inefficient and presents a striking paradox: on one hand, the amount of food produced globally well exceeds the amount needed per person, yet on the other, billions of people around the world are starving or malnourished. One emerging strategy to address this challenge is converting byproducts—waste streams such as soymilk waste and brewers grain that are generated as part of regular food processing—into food suitable for human consumption. Despite being edible, many byproducts end up in landfills or are used as animal feed because they are indigestible or have undesirable sensory qualities. This represents a missed opportunity for human nutrition. We recently uncovered a low-cost solution to convert byproducts into delicious and nutritious human food. The strategy involved fermentation with *N. intermedia,* an edible fungus domesticated in Indonesia, to turn soymilk waste into a meat substitute called oncom. In addition to its ability to create oncom from soymilk waste, *N. intermedia* can grow on diverse substrates such as bran, fruit pomace, and hulls, breaking down insoluble carbohydrates and increasing bioavailable amino acids, vitamins, protein, and antioxidants.

Here, we build a platform for using *N. intermedia* to convert diverse byproducts into nutritious and delicious foods, including novel meat and dairy substitutes. Our approaches leverage a Design–Build–Test–Learn framework, bridging insights from the lab with approaches from gastronomy to develop and evaluate food prototypes. We first optimized fermentation growth of *N. intermedia* on selected byproducts to maximize growth rate and its resistance

to various stressors. We also identified key nutrients and metabolites produced during growth on waste. Based on these insights, we designed three food prototypes targeting dairy and meat applications: a cheese alternative based on 66% waste, a patty containing 40% waste, and a salami-type product incorporating 34% waste. Finally, we evaluated nutritional value of the product and used life cycle analysis to understand the environmental impact of these novel foods. These results indicate that *N. intermedia* can turn diverse byproducts into novel foods and sets the stage for further engineering efforts using CRISPR-Cas9 to tune flavor and nutrition for desired outcomes.

302B

A biosynthetic survey and a heterologous production platform for molecules from biocontrol fungi

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Pests are responsible for the loss of up to 40% of global crops. Widespread resistance to pesticides has emerged due to the overuse of a limited set of compounds and targets. Many fungal species form symbiotic relationships with plants while antagonizing insects, nematodes, and bacteria, making them valuable sources of sustainable biocontrol agents and biopesticides. However, despite their potential, only a few species are currently used in biocontrol, and their biosynthetic capabilities remain poorly understood.

Here, we used metabologenomic approaches to catalog the biosynthetic repertoire of 82 plant- and insect-associated species within the Hypocreales order. We annotated over 5,000 biosynthetic gene clusters (BGCs), assigned putative products to more than 1,000 of them, and experimentally linked over 100 BGCs to molecules. In parallel, we established black yeasts as novel heterologous hosts for fungal natural products. These hosts combine the genetic tractability of conventional yeasts with the metabolic robustness of filamentous fungi. We developed genetic engineering tools and automation protocols enabling cheap, efficient, and precise gene integration and genome editing, allowing us to heterologously produce several natural products from biocontrol species.

Our work highlights the vast and underexplored chemical repertoire of biocontrol fungi and demonstrates the potential of black yeasts for systematic discovery, characterization, and production of natural products with pest-controlling activities. We believe this provides a strong foundation for developing sustainable pest control strategies in agriculture.

303B

Fungal Biocomposites from Regolith Simulant and Whole or Lipid-Extracted Microalgae for Circular Resource Use in Space Habitats

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Space-based life support and propulsion systems are likely to rely on microalgae, generating substantial quantities of both whole biomass and lipid-extracted residues that cannot simply be discarded. At the same time, launching structural materials for construction remains prohibitively expensive, despite the presence of mineral-rich regolith at destination sites. This study explores whether fungal growth on mixtures of lunar regolith simulant with either whole algal powders or lipid-extracted algae can produce biocomposites suitable for early construction concepts, including molded and eventually 3D-printed forms. *Trametes versicolor* is inoculated onto a series of blends that vary in regolith content and algae type, and basic growth characteristics—such as time to visible colonization, extent of mycelial coverage, and qualitative robustness of the formed pieces—are recorded. Simple mechanical assays are used to compare the relative strength and cohesion of materials grown on whole versus spent algae across different blend ratios, providing an initial map of where fungal binding is sufficient to produce blocks that can be handled, cut, or reshaped. Blends that show both reasonable growth and promising strength are further evaluated for their ability to be mixed into pastes for extrusion, as a first step toward layered fabrication. Together, these experiments outline a circular use of resources in which algae support life support and fuel production, while both whole biomass and post-extraction residues are combined with locally available regolith and fungal growth to form structural materials, reducing launched mass and integrating waste management with habitat construction. This same strategy is also relevant on Earth, where industrial algal wastes and mineral fillers could be converted into mycelium-based materials instead of entering waste streams. More broadly, the work illustrates a circular bioeconomy approach in which biological growth links resource capture, energy production, and structural material generation within a single, integrated system.

304C

Genetic library screening in filamentous fungal cells using a genome integration system restrictive for single variant expression

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Efficient genetic library screening in filamentous fungi is hindered by frequent tandem or ectopic integration during pooled transformations, complicating downstream analysis of expression clones. Here, we describe a single-copy “landing pad” genome integration system in *Trichoderma reesei* that restricts expression to a single construct per cell. The engineered “landing pad” is comprised of a full-length promoter (cbh1), a unique CRISPR Cas9 cut site, followed by a split selectable marker. A compatible library is comprised of a truncated, non-functional promoter (cbh1), a gene-of-interest, followed by the other half of the split selectable marker. Alternatively, it may contain the entire selectable marker followed by a sequence homologous to genomic region downstream of the integration site. To test the "landing pad” system we built two sets of libraries (total of four): one set with a full-length promoter and the other with the truncated promoter each harboring different expression targets (alpha-amylase and phytase). Transformation of an equal mixture of the alpha-amylase and phytase full-length promoter libraries into the “landing pad” strain resulted in 32% of the clones expressing both alpha-amylase and phytase, demonstrating frequent multi-copy integration. However, transformation of an equal mixture of the truncated promoter libraries into the “landing pad” strain resulted in only clones expressing either alpha-amylase activity or phytase activity, not both. Moreover, transformation of the truncated promoter libraries into a host strain without the “landing pad” resulted in clones that lacked any detectable alpha-amylase or phytase activity, demonstrating that the truncated promoter was insufficient to drive expression. In conclusion, this strategy has enabled single variant library engineering and downstream high-throughput screening in *T. reesei* and highlighted the impact of engineering design in streamlining industrial protein expression workflows.

305C

Toward a platform for space mycotecture

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Long-duration spaceflight and off-world exploration will require materials that can be produced in situ while tolerating extreme thermal, vacuum, and radiation environments. Mycotecture—architectural composites grown from fungal mycelium and lignocellulosic or regolith-based feedstocks—offers a biologically regenerative route to such materials, with potential to reduce upmass and integrate with bioregenerative life support and ISRU architectures. We are developing a modular “mycotecture for space” platform to systematically link fungal strain, substrate formulation, growth parameters, and post-processing to mechanical and environmental performance metrics relevant to space exploration. Using three basidiomycete strains (Ganoderma lucidum, Ganoderma spp., Pleurotus ostreatus) on a custom NASA Woody Substrate with wheat or flax supplements, plus a commercial Ecovative Inc. reference material (ERM), the system generated low-density foams whose elastic modulus and compressive strength approach those of expanded polystyrene. To probe flight-relevant robustness, baked mycomaterial coupons were treated in a planetary simulator under flight-relevant conditions. Ongoing work extends this platform toward flight certification, e.g., flammability and fire-response testing under terrestrial and spaceflight-relevant protocols. Together, these results position mycotecture as a tractable fungal biomaterials and biomaterials–systems biology topic for space exploration, and outline a generalizable platform for evolving genetically tractable fungal strains into space-rated materials.

306C

Employing Machine Learning Methods to Explore Thermotolerance Genetics in Saccharomycotina Yeasts

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Over the course of hundreds of millions of years, budding yeasts from the Saccharomycotina subphylum have evolved and speciated into over 1,100 known unique species, each with their own ecological niches and specific set of optimal growth conditions. Of particular interest are yeasts that grow at human body temperature, as these are most likely to function as human pathogens. The primary objective of this project was to elucidate which genes are responsible for yeast growth at 37°C, a characteristic also referred to as yeast thermotolerance.

To explore the genetic underpinnings of thermotolerance, we employed random forest models for their versatility and interpretability. We trained our random forest models to predict growth at 37°C using codon optimization data and growth data derived from the experimental findings of the Y1000+ project (https://y1000plus.wei.wisc.edu/). We split the data using an 85/15 train/test ratio and used Monte Carlo cross validation and K-Fold cross validation for model training. To balance the number of positive and negative growth classes, we used random under sampling. In total, we used codon optimization values from 4,439 genes across 835 species of yeasts to train the models. After model training, we employed SHAP feature importance analysis to find which genes were most significant for the emergence of thermotolerance phenotypes.

From the trained models, we were able to achieve an ROC AUC value of 0.825 and an overall confusion matrix accuracy score of 0.7708. These results signify that our predictions were better than random chance. Conducting a SHAP feature importance analysis on these models showed that FPR3, BUD23, and TIF6 were the top 3 most important genes for predicting thermotolerance in yeasts. More specifically, FPR3 expression increases, BUD23 expression decreases, and TIF6 expression decreases in thermotolerant yeasts. These genes are involved in nucleosome assembly and histone methylation, methyltransferase production and nuclear export of ribosomal subunits, and the binding of large ribosomal subunits, respectively.

The results of this study indicate that yeast thermotolerance is primarily a stress response, indicating that most yeasts do not prefer to live and grow at 37°C. Findings suggest that most yeasts decrease their gene expression rates and metabolic rates when exposed to these specific temperature conditions. As a future study, we’ll be looking at growth rates at various temperatures for yeasts that fall outside of this rule.

307C

Development of genetic transformation methods in anaerobic gut fungi

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Microorganisms hold great promise for applications in biotechnology, including the renewable production of fuels and chemicals, the biosynthesis of therapeutics, and even the creation of novel materials. However, it is well known that only a relatively small percentage of microbial species are isolated and characterized sufficiently to be amenable for engineering purposes, thus limiting our repertoire of available biological tools. This issue is particularly true for hard-to-culture organisms. One such group of organisms, the anaerobic gut fungi (phylum Neocallimastigomycota), hold significant, untapped biotechnological potential. These fungi, which are found predominantly in the digestive tracts of herbivores, possess an expansive array of uncharacterized carbohydrate-active enzymes, indicating a high-degree of potential for applications involving the processing and conversion of lignocellulosic material. Furthermore, as these fungi natively exist in a competitive microbial environment, they are believed to possess diverse secondary metabolites with potential for therapeutic applications.

However, studies of anaerobic gut fungi are severely limited by an almost complete lack of genetic tools. The genetic intractability of these organisms also hinders efforts at strain engineering for biotechnological applications. To overcome these restrictions, we are in the process of developing tools and techniques for the genetic manipulation of these fungi. A critical first step in this regard is to develop methods for introducing exogenous DNA into the fungal cells for stable or transient expression. We are investigating multiple mechanisms to carry out this genetic transformation, including natural competency-based transformation, PEG-mediated transformation, electroporation, and bacterially-mediated transformation. Yet, establishing these transformation techniques is made difficult by the extreme nature of the fungi, both in terms of their obligately anaerobic growth as well as the severe AT-bias of their genomes. As such, we are adapting existing transformation protocols as well as developing novel approaches to accommodate the unique features of these organisms. The development of the aforementioned systems into functional transformation tools in anaerobic gut fungi will significantly

expand the scope of studies that can be performed on these organisms and will usher in the development of engineered fungal strains to address pressing biotechnological applications.

308C

Implementation of robotics and high-throughput imaging to capture growth rate data for *Neurospora crassa* cultured under different conditions.

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Growth rate is an important metric for determining the virulence of plant pathogens, the suitability of an organism for biotechnological applications, species roles in ecological niches and many other applications. Traditional methods of determining growth rates on solid media typically capture basal hyphae extension in a single plane over time. These established methods are often laborious and time intensive, and do not typically yield the density or height of the colonies. Working with the NSF-funded Biofoundry for Extreme and Exceptional Fungi, Archaea, and Bacteria (ExFAB) at the University of California, Riverside, we have developed a high-throughput imaging pipeline to determine the growth rate of *Neurospora crassa* strains, that should be broadly applicable to other filamentous fungi. To establish this method, ~500 knockout mutants lacking genes encoding transcription factors or those implicated in cellular signaling were selected from the *N. crassa* knockout collection and screened under various conditions. These conditions include several alternative carbon and nitrogen sources and conditions known to induce stress. Utilizing the resources available in the ExFAB facility, we have automated normalization of spore concentration, spore inoculation, and time-lapse imaging of hyphal growth. Imaging analysis was performed utilizing Phenotypic, a software program developed by ExFAB that determines the integrated intensity or area of fungal colonies, which can be extrapolated to growth rate. This pipeline presents a high-throughput method that accounts for multiple colony attributes that may contribute to growth in filamentous fungi.

309C

Construction of the *Aspergillus oryzae* strain producing high levels of kojic acid by CRISPR/Cas9-mediated multicopy gene integration

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Background and Objective: *Aspergillus oryzae* is widely used as a host for productions of recombinant proteins as well as secondary metabolites. Recently, we have successfully integrated multiple xylanase genes into transposon-like sequences on chromosomes of *A. oryzae* by a CRISPR/Cas9-mediated gene editing to achieve high production (3 g/L) of xylanase in flask culture. However, it remains undetermined whether this multicopy gene integration system is applicable to genes other than xylanase. Therefore, we aimed to construct an *A. oryzae* strain inserted with multiple copies of *kojA,* the gene encoding a key enzyme in biosynthesis of kojic acid, a secondary metabolite of *A. oryzae*, and to evaluate kojic acid production.

Methods: The *kojA* gene, driven by the strong promoter P*glaA142* was cloned into a transposon-targeting plasmid, which was subsequently used for transformation of *A. oryzae*. Gene insertion was confirmed by PCR after an induction of Cas9 expression.

Results: We successfully obtained a strain containing 13 copies of the *kojA* gene (hereafter referred to as the multicopy strain). We compared the kojic acid production levels, as well as the transcription and translation products of *kojA*, between the multicopy strain and a single-copy (1-copy) strain. The multicopy strain produced 5.5-fold more kojic acid than the 1-copy strain. The qRT-PCR analysis demonstrated that the *kojA* expression levels were significantly increased in the multicopy strain compared to the 1-copy strain. SDS-PAGE analysis of the intracellular proteins revealed that a protein with the estimated molecular weight of KojA was detected as the most abundant protein in the multicopy strain, suggesting that the technique is also effective for high-level production of intracellular proteins. In conclusion, we have successfully developed the *A. oryzae* strain capable of high-level kojic acid production, demonstrating that our multicopy gene integration method is applicable to various genes, including those involved in the biosynthesis of secondary metabolites.

310C

Less environmental bitterness: filamentous fungi as a valorisation route for coffee bean wet-processing effluents

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Fungal-based biotechnologies can be versatile platforms for converting complex organic waste streams into commercially and environmentally sustainable outputs, offering new solutions for resource recovery in industries with substantial by-product burdens. Coffee bean production is one such sector, generating solid residues (husk, pulp, spoiled beans) but also large volumes of wastewater, particularly from the predominant practice of wet processing. For every ton of produced bean, approximately two billion litres of wastewater will be discharged. There is a high financial cost to mitigate the environmental burden of this effluent due to high biochemical and chemical oxygen demand (BOD, COD) and eutrophication risks, but also an opportunity due to its high content of fermentable sugars from the coffee pulp. In collaboration between researchers in Ireland and Vietnam, the second biggest coffee producer worldwide, the enzymatic diversity, metabolic plasticity, and substrate tolerance of filamentous fungi is investigated with the aim of valorising these waste fractions into nutrients (with a focus on mycoproteins), enzymes and bioactive metabolites. Fifty well-characterized filamentous fungi were assessed for biomass accumulation when cultivated using coffee production effluent, from which eight high-performing candidates, including *Pleurotus eryngii*, *Pleurotus ostreatus*, *Cordyceps militaris*, and *Lentinula edodes*, were selected for optimisation of growth parameters (pH, dilution factor). In parallel, fifteen endophytic yeasts were isolated from coffee cherries, and one hundred yeasts were isolated from the effluent. Two strains were found in both environments and identified as *Pichia kluyveri* and *Pichia kudriavzevii*. They exhibited promising fermentative capabilities, producing up to 7% ethanol and generating tropical fruit aroma profiles when cultivated on wastewater substrates. The following steps will include quantifying the mycoprotein content of the resulting fungal biomass and characterising the nutraceutical compounds using metabolomic analyses. The genetic and enzymatic toolkit of selected strains will also be examined through transcriptomic and proteomic approaches. These early findings highlight the metabolic potential of fungal communities to upcycle coffee production liquid residues, and establish a foundation for developing sustainable and scalable fungal bioprocesses that can achieve waste reduction while also generating of value-added products.

311C

Elucidation of basidiomycete-specific chitin synthase roles in the white-rot fungus *Pleurotus ostreatus*

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Chitin is a key structural component of fungal cell walls, produced by chitin synthases (CHSs). CHSs have a broad range of reported roles in ascomycetes; however, remain poorly understood in basidiomycetes, despite large differences in their cell wall structures. White-rot species like *Pleurotus ostreatus* also have sustainable biomaterial applications as they can grow on agricultural waste. We previously identified nine *P. ostreatus chs* genes including two myosin motor domain (MMD) *chs*s in conserved clades V and VII, one in conserved clade IV, two *chs*s in the filamentous clade III, and four novel *chsb* genes in clades BI-III. This research aims to investigate the roles of basidiomycete-specific CHS (CHSB) functions.

Through homologous recombination-based gene disruption, we isolated disruption strains for all *chsb* genes in *P. ostreatus* (*chsb1-4*) and simultaneously disrupted *chsb*s with a shared clade. Morphology and cell wall analysis revealed that clade BI (*chsb1*) disruption caused severe mycelial growth defects, reduced cell wall thickness and significantly altered chitin/β-glucan content; clade BII (*chsb2* and *chsb3*) single disruption had mild effects, whilst double disruption caused severe growth defects, cell wall remodelling, almost no aerial hyphae production and abnormal septation; and clade BIII (*chsb4*) disruption had minimal effects aside from slightly thinner cell walls and slight aerial hyphae defects, suggesting a more specific role in aerial hyphae development. These results highlight the importance of *chsb*s in growth and suggest clade-specific functional differentiation between *chsb*s. Preliminary assessments of mycelium mats derived from *chsb* mutants revealed differences in flexibility and texture, suggesting that *chsb*s have the potential to alter the physical properties of mushroom materials. Additional work to understand the impacts of *chsb* disruption on fruiting body formation as well as *chsb* overexpression are ongoing.

Preliminary analysis of the remaining *chs* genes (*chs5-9*) demonstrates reduced growth and cell wall integrity, though less severely than *chsb1* and *chsb2chsb3* disruption. Disruption strains of *chs5*, the second MMD *chs* have not yet been obtained despite numerous attempts, suggesting some essential function. These findings highlight the importance of understanding basidiomycete *chs*s, clear functional differentiation among *chsb* clades, and the potential for altering mycelium biomaterials through *chs* gene targeting

312C

Bridging Continuum Mechanics and Fungal Biology to Enable Mycelium-Based Biosensing Technologies

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Filamentous fungi form spatially distributed networks capable of detecting and responding to diverse chemical cues, yet the physical mechanisms governing how these signals move through the mycelium remain incompletely characterized. We seek to develop predictive models of fungal chemical sensing—of both nutrients and hazards—using tools from continuum mechanics traditionally applied to synthetic polymeric materials.

Our early-stage work explores two complementary modeling approaches. The first is a hyphal-resolved description that treats individual hyphae as directed one-dimensional conduits for transport and reaction, allowing explicit representation of geometry-dependent bio-chemical propagation of chemical information. The second is an effective three-dimensional continuum model that homogenizes the network architecture into mesoscale parameters, enabling analysis of signal dynamics at the scale of the colony without requiring detailed hyphal reconstruction. Together, these models aim to characterize how local chemical inputs evolve across space and time within a growing, branching network and to identify the structural and physical features that dominate information flow.

In parallel with these modeling efforts, collaborators are generating experimental data to refine model assumptions and parameter ranges. By integrating mechanistic modeling with biological insight, we aim to establish a framework that can ultimately inform the design and control of fungal systems for biotechnology and living material applications.

313C

The increase in cell volume and nuclear number of the koji-fungus *Aspergillus oryzae* contributes to its high enzyme productivity.

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While the ratio of nuclei to cell volume is well regulated, it remains largely unexplored in multinucleate organisms. The koji-fungus *Aspergillus oryzae*, traditionally used in Japanese brewing and fermentation for over a thousand years, is now widely utilized in modern biotechnology as a host for enzyme production. We discovered that, over time in culture, hyphae become thicker, resulting in a tenfold increase in cell volume, and the number of nuclei in hyphal cells also increases tenfold, exceeding 200. The increase in cell volume and nuclear number is unique among the investigated *Aspergillus* species and correlates with its high enzyme production capabilities. Since nuclear number and cell volume are correlated, both must increase simultaneously for either to expand. Our analyses identified genetic factors and nutritional environmental signals involved in each of these increases. Increases in nuclear number and cell volume were also observed in other fungi bred for industrial use. This study not only deepens our understanding of the evolutionary processes that promote high enzyme productivity through fungal breeding, but also provides insights into the molecular mechanisms regulating cell volume and nuclear number in multinucleate organisms.

314C

Discovery and Characterization of Novel Endogenous Protease Inhibitors from *Trichoderma reesei* for Enhanced Heterologous Protein Production

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Trichoderma reesei is one of the most important industrial workhorses for recombinant protein production owing to its exceptional secretory capacity and well-established genetic tools. However, heterologous protein yields often fall short of theoretical expectations with extracellular proteolytic degradation representing a major bottleneck. While strain engineering strategies have focused on deleting known protease genes, the endogenous regulatory mechanisms controlling proteolytic activity remain incompletely understood.

In this study, we report the identification and functional characterization of novel protease inhibitor encoded within the *T. reesei* genome. Through systematic genomic analysis, we identified a candidate gene with predicted inhibitory activity against subtilisin-like serine proteases, a protease family known to contribute significantly to heterologous protein degradation in filamentous fungi.

Biochemical characterization confirmed that the identified protein exhibits potent inhibitory activity against subtilisin-like proteases native to *T. reesei*. To assess the physiological relevance of this finding, we evaluated the impact of this inhibitor on heterologous protein stability during bioreactor cultivation. Our results demonstrate a measurable protective effect against proteolytic degradation under industrially relevant fermentation conditions, establishing a direct connection between inhibitor activity and improved product integrity.

These findings expand our understanding of the endogenous proteolytic regulatory network in *T. reesei* and highlight a previously unrecognized mechanism that may be exploited to enhance recombinant protein production. The discovery of this inhibitor opens new avenues for strain improvement strategies, potentially complementing existing protease-deficient host strains or providing alternative approaches for protecting sensitive protein products during large-scale manufacturing processes.

315C

Mycotechnology to the rescue: Biosustainable Production of secondary metabolites by filamentous fungi using carbon sourced from Polystyrene and Polyethylene Plastic Waste

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Plastic waste is an acute threat to the environment and human health. The complete elimination of plastics, however, is an inconceivable solution due to their revolutionary impact on daily life and immense economical contribution. Consequently, long-term plastic sustainability is an urgent matter that requires new approaches to achieve, such as the utilization of microorganisms. We previously have demonstrated the conversion of polystyrene (PS) and polyethylene (PE), two common plastic polymers with poor recycling rates, into metabolically relevant fungal substrates. Further, the sustainable impact of fungi can be broadened by their ability to create complex secondary metabolites that have the potential to replace our current environmentally hazardous synthetic processes like those used in dye manufacturing. In this talk I will present our groups efforts to produce various useful secondary metabolites using our hybrid chemical biological approach. The talk will demonstrate the power of mycotechnology to address issues in plastic waste and sustainability.

316C

CRISPR/Cas9-Mediated Multicopy Integration into 13 Chromosomal Loci Enables Hyperproduction of Xylanase in *Aspergillus oryzae*

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Background and Purpose : *Aspergillus oryzae* is widely used as a host for recombinant protein production. Increasing gene copy number is an effective strategy to improve protein yields. However, *in A. oryzae*, multicopy integration typically occurs as tandem repeats at a single chromosomal locus, which may in turn lead to the loss of repeat sequences through homologous recombination. In this study, we aimed to establish a genome engineering method that enables the simultaneous insertion of exogenous DNA fragments into multiple chromosomal loci in a single transformation procedure.

Methods and Results: *A. oryzae* RIB40 strain contains 13 highly conserved DDE1 transposon-like sequences on its chromosomes. We examined whether CRISPR/Cas9-mediated cleavage of these sites could facilitate multicopy gene integration. A guide RNA was designed to target a conserved region near the center of the transposon-like sequence, allowing Cas9 to introduce a double-strand break at all homologous loci. The donor DNA consisted of an *xynF1* (xylanase F1) expression cassette flanked by ~300-bp homology arms corresponding to the regions upstream and downstream of the Cas9 target sites. All elements required for multicopy integration, including xylose-inducible Cas9, the gRNA cassette, the donor DNA, the AMA1 replicon, and a selectable marker, were assembled into a single plasmid and then introduced into the *A. oryzae* Δ*ligD* strain. Transformants were initially maintained under Cas9-repressive conditions (glucose) and then transferred to xylose plates to induce genome editing. Conidial PCR verified successful integration of the *xynF1* cassette at all 13 targeted loci. Furthermore, evaluation of the enzyme productivity of the constructed multicopy strains revealed that the edited strain exhibited an approximately 10-fold increase in xylanase production compared with the single-copy strain, reaching a high titer of over 3 g/L.

317C

Production of hen egg ovalbumin by *Trichoderma reesei* using precision fermentation technology and testing of its techno-functional properties

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Egg white protein ovalbumin is a highly functional food ingredient, yet replacing it with plant proteins is challenging due to its unique structural and functional properties. To provide a non-animal source of ovalbumin, we utilized the fungal host *Trichoderma reesei* for the biotechnical production of recombinant hen ovalbumin (TrOVA). The protein was expressed using both the strong synthetic SES promoter and the native *cbh1* promoter, reaching up to 2 g/L in lab scale bioreactors. The SES promoter strain produced fewer background proteins, simplifying downstream purification and providing higher product purity.

Biochemical studies revealed that TrOVA degradation was caused by a specific endogenous protease, which was identified and removed from the *T. reesei* genome, enabling stable accumulation of intact protein. Following purification, the TrOVA amino acid sequence and molecular weight were verified using mass spectrometry. Glycan analysis showed that TrOVA carried N-glycans typical of *T. reesei* proteins, consisting of mannose chains Man_{5-11} linked by GlcNAc residues. Although minor differences in glycosylation were observed compared to native ovalbumin, overall similarity remained high, with $(Man)_5(GlcNAc)_2$ identified as the predominant glycoform shared by both.

Purified TrOVA displayed native secondary structure, excellent foaming characteristics, and heat-induced gelation. These results demonstrate that *T. reesei* is a promising host for precision-fermentation-based production of functional egg proteins.

318A

Novel Cysteine bZIP Transcription Factors and Their Role in the Oomycete *Phytophthora infestans*

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Basic leucine zipper transcription factors (bZIPs) are important regulators of many cellular functions and have been studied in detail in many organisms. In the oomycete *Phytophthora infestans*, however, there is a novel feature that has not been studied to the same extent, the cysteine bZIP. In most organisms, bZIPs bind DNA with a so-called “invariant asparagine” in their binding domain, analogous to the asparagine at position 235 in the yeast protein GCN4. In *Phytophthora infestans*, this asparagine is present in only 17 of the 37 bZIPs, replaced by cysteine in 15, valine in four, and tyrosine in one. Preliminary experiments show that many of these bZIPs are necessary to respond to oxidative stress. Based on this and the fact that cysteine is sensitive to oxidation, it is hypothesized that the cysteine residue functions as a redox switch. In order to understand the function of these bZIPs, dimerization patterns of the bZIPs were tested with yeast two-hybrid. This revealed that homodimers are more likely to form regardless of the type of amino acid in the 235 position, and that cysteine bZIPs are more likely to form heterodimers with other cysteine bZIPs. FRET is then being used to quantitatively study some of these interactions in more detail, using different oxidative conditions and in the presence of the DNA binding site. Oxidation states of different life stages of *P. infestans* are being investigated for oxidative stress levels by the use of a strain of *Phytophthora infestans* transformed to express roGFP (redox-sensitive green fluorescent protein), which displays altered fluorescence depending on its redox state. Mycelia, sporangia, sporulating mycelia, zoospores, germinating cysts, and plant infection stages are all being studied. This system is also being used to investigate the effects of factors like starvation, heat, osmotic stress, heavy metals, and oomyceticides on oxidative stress.

319A

The role of atypical Thioredoxins in *Candida albicans*

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Candida albicans is a commensal organism of humans that poses a significant health concern due to its potential progression into life-threatening systemic infections, particularly in individuals with a compromised immune system. This problem is exacerbated by the limited therapeutic options for fungal infections and the emergence of antifungal drug-resistant strains. Innate immune cells play a key role in protecting against infection by *C. albicans* by creating a respiratory burst, characterized by rapid release of reactive oxygen species (ROS). Therefore, being able to withstand ROS is crucial for fungal survival in the host. *C. albicans* employs a range of antioxidant mechanisms, but among these thioredoxin plays a key role. *C. albicans* encodes three thioredoxin-related genes: one typical thioredoxin (*TRX1*) and two atypical thioredoxin genes that have not been well studied (*TRX2*, *TXL1*). Deletion mutant analysis was used to demonstrate that the atypical thioredoxins play distinct roles compared to *TRX1*. *TRX2* deletion mutants (*trx2Δ*) show no phenotypes related to oxidative stress sensitivity but *TRX2* was important for yeast-to-hypha transition. Deletion of *TXL1* (*txl1Δ*) resulted in increased sensitivity to organic peroxides and a minor virulence defect in the systemic candidiasis mouse model. We also identified additional roles for *TRX1,* showing that Trx1 contributes to the development and maturation of biofilms in *C. albicans,* possibly through downstream peroxiredoxin-dependent pathways. We next examined how redox imbalance affects other cellular structures as oxidative stress is known to disrupt a wide range of cellular processes. Actin, for example, was shown to form intra- and intermolecular disulfide bonds in these conditions. In *S. cerevisiae,* this results in Oxidation-Induced Bodies (OABs), structures proposed to form as a type of protection against oxidative stress. To test this, we developed strains harboring different constructs of the live cell actin marker LifeAct and demonstrated the formation of OABs in *C. albicans.* These strains are being used to better define how OABs assemble and the role of thioredoxins in promoting their disassembly during the recovery phase. Overall, our work shows that the three thioredoxin genes have a diverse range of functions that contribute to the ability of *C. albicans* pathogen to invade tissues and establish infection.

320A

Metabolic crosstalk between the opportunistic pathogen *Candida albicans* and the gastrointestinal tumor microenvironment

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C. albicans infections are highly prevalent in cancer patients, largely due to gastrointestinal (GI) dysbiosis triggered either by broad-spectrum antibiotics or chemotherapeutic agents. Current epidemiological and experimental evidence supports *C. albicans* involvement in GI cancer, including an association of these pathogens with poor prognosis. While the ability of *C. albicans* to colonize tumors is well established, it remains unclear whether these fungi act as oncomicrobes promoting cancer progression or instead exploit the tumor microenvironment to thrive and evade immune surveillance. To address this question, we are employing *in vivo* models of GI cancer to profile the metabolic responses of multiple *C. albicans* clinical isolates to the tumor environment. Using genetic approaches, we have identified specific *C. albicans* nutrient transporters and receptors that confer physiological advantage under this niche. Our findings suggest that tumor secreted metabolites support fungal proliferation, pointing to a potential metabolic crosstalk between tumour cells and *C. albicans*. By dissecting the molecular mechanisms that enable some environmental fungi to thrive in the tumor niche, our goal is to clarify whether these organisms act as passive bystanders or active participants in GI carcinogenesis.

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321A

The RNA-binding protein Psc1 sequesters transcripts in biomolecular condensates to regulate cryptococcal tolerance to CO_2

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The fungal pathogen *Cryptococcus neoformans* causes lethal cryptococcal meningoencephalitis in those with compromised immune systems, leading to nearly 110,000 deaths annually. Its virulence has mostly been attributed to three virulence traits: thermotolerance, melanin pigment production, and polysaccharide capsule formation. Nonetheless, these factors alone do not fully explain its pathogenicity. *C. neoformans* can adapt to high host CO_2 levels (≥5%), which differs drastically from its ecological niche of ~0.04% CO_2. The molecular mechanisms enabling adaptation to host conditions remain poorly characterized. Our previous research into CO_2 tolerance placed the *Regulator of Ace2p Morphogenesis* (RAM) pathway at the center of the signaling network, allowing this fungus to adapt to host conditions. Loss of Cbk1, the terminal kinase of the RAM pathway, impairs growth at high CO_2. A *cbk1*Δ natural suppressor screen identified Psc1, which contains an RNA-binding domain and is highly disordered, whose deletion partially restores *cbk1*Δ's growth defects. We found Psc1 is enriched in daughter cells and increasingly forms biomolecular condensates in response to high CO_2. We hypothesized sequestration of mRNAs by Psc1 in the absence of Cbk1 may prevent translation of bound mRNAs, causing growth defects in high CO_2. To test our hypothesis, we identified the mRNA transcripts associated with Psc1 through HyperTRIBE. We further visualized one of the transcripts, *ZDS3*, in live cells with the MS2-MCP tagging system. We discovered Psc1 interacts with *ZDS3* mRNA molecules required for CO_2 adaptation in the cytosol, regulating their subcellular localization and translation in response to CO_2 and Cbk1 activity. Our investigation has begun to reveal mechanisms of post-transcriptional control that enables *C. neoformans* to adapt to high CO_2. These insights may offer critical targets to combat the devastating burden of disease.

322A

Actin-based cell crawling in chytrid zoospores illuminates potential mechanisms of ancestral fungal locomotion

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Understanding the nature of the last universal fungal ancestor (LUFA) provides essential insight for how fungi evolved and diversified into the millions of species we see today. To explore what the biology of this ancestor may have looked like, we study chytrids, a fungal lineage that diverged before the radiation of Dikarya–the group that includes commonly studied yeasts and multicellular fungi–and that share many features with animals that have been lost in other fungal lineages. Chytrids are characterized by a biphasic life cycle, alternating between sessile walled sporangia and motile zoospores capable of swimming through the water and crawling across surfaces like an amoeba–behaviors lost in Dikarya but inferred to have been present in the LUFA. Although the flagellar-based motility of zoospores appears well conserved with that of diverse lineages, the mechanisms that drive crawling motility vary greatly across eukaryotes, leaving it unclear what mechanisms chytrids and their fungal ancestors may have used to migrate across solid surfaces. By studying the motility of chytrid zoospores, we may infer how the fungal ancestor locomoted.

Using the "frog-killing" chytrid Batrachochytrium dendrobatidis, we show the crawling motility of zoospores involves highly dynamic pseudopod fronts, relying on pushing forces generated by branched actin networks and actomyosin contractility. These mechanisms are similar to that of amoeboid migration seen in other parts of the Eukaryotic tree of life, such as animal cells, the Amoebozoan Dictyostelium and and the Heterolobosean Naegleria. We also show that the flagellum is immobile during crawling, indicating this migration is not dependent on forces arising from flagellar beating. Remarkably, retention of the flagellum and its associated basal body are important for stable polarity of migrating cells; the flagellum is located at the rear of the cell during migration and loss of the flagellum results in erratic and multiple competing pseudopod fronts. This behavior is consistent with the ability of the fungal ancestor to utilize machinery for both flagellar swimming and cell crawling, as well as the presence of cross talk between these two networks.

323A

Exploring the interaction between the septin cytoskeleton and the cell wall integrity pathway MAPKs MpkA and MkkA in *Aspergillus fumigatus*

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Aspergillus fumigatus is the primary etiological agent behind invasive aspergillosis (IA), one of the most deadly and costly invasive fungal diseases. IA is notoriously difficult to treat due to increasing resistance to antifungal drugs and limited therapy options. The echinocandins, including caspofungin, target the cell wall, but are only fungistatic against *A. fumigatus*. This fungistatic response requires signaling through the cell wall integrity (CWI) pathway. Similarly, the septin AspB is also required for caspofungin fungistatic effect. Septins are a family of highly conserved eukaryotic GTP-binding proteins. Despite their functions varying across species, they notably act as scaffolds to recruit proteins for specific cellular functions. Previous epistasis analyses in *A. nidulans* also showed a connection between MpkA and the septins. Furthermore, our preliminary proteomics pulldown data showed that the CWI pathway kinases, MkkA and MpkA, co-immunoprecipitated with AspB. Based on this, we hypothesize that the *A. fumigatus* septin cytoskeleton contributes to CWI signaling. To test this hypothesis, we performed epistasis analyses between the *aspB, mpkA*, and *mkkA* genes and found that the Δ*aspB*Δ*mpkA* and Δ*aspB*Δ*mkkA* double deletion strains had reduced susceptibility at sub-lethal concentrations compared to the Δ*mpkA* and Δ*mkkA* single deletion strains. Western blot analyses showed that the Δ*aspB* strain had less total MpkA, but more phosphorylated MpkA under basal conditions. We will determine if loss of septins during caspofungin exposure prevents CWI signal to the nucleus using a strain that expresses MpkA tagged with GFP in the wild-type and Δ*aspB* strains. This work will help uncover the connection between the CWI pathway and septins, as well as provide insight into unlocking the fungicidal potential of caspofungin for use against IA.

324A

Short-term experimental adaptation of *Fusarium oxysporum* f. sp. *mori* to respiratory inhibitors fungicides

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Fusarium oxysporum is a plant pathogen responsible for vascular wilt diseases in a wide range of hosts. Traditional management of these diseases relies heavily on preventive measures and repeated fungicide applications. However, the increasing emergence of *F. oxysporum* populations exhibiting reduced sensitivity or resistance to these chemicals is undermining the efficacy of such crop protection strategies. Despite the critical nature of this issue, there remains a scarcity of research examining the phenotypic adaptations of this pathogen in response to repeated fungicide exposure.

Chemical management of *F. oxysporum* predominantly utilizes synthetic fungicides that target essential cellular processes required for fungal survival and proliferation. For example, Quinone Outside Inhibitors (QoIs) block electron transfer at the quinol oxidation site of cytochrome b, while Succinate Dehydrogenase Inhibitors (SDHIs) impair the activity of succinate dehydrogenase in complex II of the mitochondrial respiratory chain. Both fungicide classes disrupt mitochondrial respiration and hinder ATP synthesis, ultimately inhibiting fungal growth.

In this study, we investigated the adaptive responses of *F. oxysporum* f. sp. *mori* to fungicides using a short-term experimental adaptation framework that simulates repeated chemical exposure. The adapted *F. oxysporum* f. sp. *mori* strain not only acquired resistance to respiratory inhibitor fungicides but also exhibited notable phenotypic changes, including altered growth rates, altered conidial production and germination, and distinct hyphal morphologies. By adopting this comprehensive approach, our research aims to deepen understanding of fungal adaptation mechanisms and development of innovative strategies for sustainable disease management and prevention.

325A

Multilayered regulation of TORC1 signaling by Ait1, Gcn2, and SEAC/GATOR during nitrogen limitation and starvation

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The Target of Rapamycin kinase Complex I (TORC1) is a central hub in the cell growth and metabolic control network of eukaryotes. How its upstream regulators cooperate to tune signaling across environmental conditions remains unclear. Here, we combine quantitative phosphoproteomics, global proteomics, TORC1 activity assays, and targeted genetic perturbations to dissect TORC1 regulation in *Saccharomyces cerevisiae* during transitions from a high-quality nitrogen source (glutamine) to a low-quality nitrogen source (proline), and on to complete nitrogen starvation. TORC1 activity decreases rapidly after nutrient downshift but then diverges depending on nitrogen availability. In proline medium, Ait1 and Gcn2 attenuate TORC1 activity, establishing a partially inhibited “Low Nitrogen Adaptive” (LoNA) state marked by sustained reprogramming of nutrient transport and metabolism without growth arrest. In contrast, during nitrogen starvation, SEAC, Ait1, and Gcn2 act together to fully inactivate TORC1, driving broad dephosphorylation of TORC1–Sch9 targets and entry into quiescence. Comparative phosphoproteomic profiling revealed that nitrogen limitation and starvation elicit similar early signaling, but diverge over time as SEAC enforces complete repression. Together, these findings reveal a multilayered regulatory circuit in which Ait1 and Gcn2 establish partial TORC1 inhibition under limitation, while SEAC reinforces repression during starvation. This architecture enables yeast to balance growth and metabolic remodeling across nutrient conditions—a logic likely conserved across eukaryotes.

326A

Quantitative single-molecule FISH reveals differential subcellular localization of *flb* family mRNA in the filamentous fungus *Aspergillus niger*

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Aspergillus niger is a filamentous fungus important in carbon cycling due to its ability to degrade diverse organic materials. During growth, it forms colonies with distinct regions, where peripheral zones show enhanced growth and protein secretion. While colony heterogeneity is well described, it is unclear whether spatial organization arises from localized gene expression at the hyphal level. To investigate this, we analyzed the spatial distribution of the Flb

proteins and their mRNAs in *A. niger* using reporter proteins and single-molecule FISH. The *flb* gene family (*flbA*–*flbE*) regulates growth, sporulation, and secretion. FlbA and its mRNA were evenly distributed, but FlbB–E showed distinct spatial patterns. Notably, *flbD* mRNA localized at hyphal tips, while its protein was nuclear throughout the hyphae. This mRNA localization was disrupted in Δ*flbE* and Δ*rrm4*, reducing nuclear FlbD levels. These findings suggest that spatial mRNA localization affects protein distribution, possibly contributing to *A. niger*'s growth and colony organization.

327A

Echinocandin Resistance: Mapping CWI-SIN Crosstalk in *Aspergillus nidulans*

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Echinocandin resistance poses a major challenge in clinical mycology. Alongside azoles, amphotericin B, and flucytosine, echinocandins are one of only four therapeutic options available to treat systemic mycoses – diseases that claim an estimated 3.75 million human lives annually. Resistant fungi include *Aspergillus spp.*, who's filamentous morphologies enable resistance through septation of hyphae. This study investigates the complex interaction between the Cell Wall Integrity (CWI) and Septation Initiation Network (SIN) signaling pathways in *Aspergillus nidulans* that drive echinocandin resistance through the dynamic regulation of septum density. We used a microscopy-based assay to measure the septation response to echinocandin-induced (micafungin) wall stress. We observed a strong correlation between micafungin treatment and septum density in wild-type strains, showing that septation is actively regulated in response to wall stress. Through targeted knockouts of CWI and SIN signaling proteins, we then identified two protein kinases: MpkA in CWI as the crosstalk initiator and SepH in SIN as the crosstalk target. Following this discovery, we broadened our study to determine the molecular mechanisms driving crosstalk between MpkA and SepH. Namely, we conducted septation response screens for knockouts of each non-essential *A. nidulans* kinase. In these screens, we have identified hypo-, hyper-, and non-responding- septators. In particular, the identification of non-responding septators will significantly narrow our mechanistic search, allowing us to efficiently use proteomic, enzyme-substrate, and interactomic experiments to determine underlying molecular mechanisms.

328A

Crosstalk between nonsense-mediated decay (NMD) and Tor alters fluconazole susceptibility of *Cryptococcus neoformans*

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Cryptococcus neoformans is an opportunistic fungal pathogen that primarily causes disease in immunocompromised individuals. While the current frontline antifungals for cryptococcal infection remain efficacious, these drugs require hospitalization to administer and are not readily available in regions predominantly impacted by *C. neoformans*. Thus, a need for identifying novel antifungal targets is present in treating *C. neoformans* in these under-resourced regions. Nonsense-mediated decay (NMD) is a mechanism of RNA decay that targets mRNAs with premature termination codons (PTCs) for degradation. This pathway is regulated by a conserved set of Upf (1-3) proteins whose role is identifying PTC-containing mRNAs. In *C. neoformans*, deletions of any one of the Upf proteins causes sensitivity to fluconazole, a drug that typically only exerts a fungistatic effect on *C. neoformans*. This suggests that targeting NMD may be a viable means of boosting the efficacy of fluconazole in treating *C. neoformans* infection. However, the mechanism by which NMD regulates fluconazole tolerance and other aspects of *C. neoformans* biology is unknown. In this work, we determine that the magnitude of fluconazole sensitivity as well as resistance to the Tor inhibitor rapamycin observed in the *upf1*Δ mutant is dependent on the presence of glucose as a carbon source. Additionally, the rapamycin resistance of the *upf1*Δ mutant can be further modulated by growth at increased temperatures. We further show that the presence of sub-inhibitory concentrations of rapamycin also reduce the *upf1*Δ mutant's sensitivity to fluconazole. Investigation into the Tor-dependent signaling of *C. neoformans* revealed that the *upf1*Δ mutant possesses altered signaling through the cell wall integrity pathway (CWI) as well as activation of the eIF2α kinase Gcn2 indicative of hyperactivation of Tor. In addition, mutants lacking upstream regulators of Tor display defects in growth and signaling similar to the *upf1*Δ mutant. Growth in the absence of glucose, at elevated temperatures, and with the addition of rapamycin were able to partially restore the defects in signaling observed in the *upf1*Δ mutant demonstrating complex crosstalk between NMD and Tor signaling in *C. neoformans* that will identify targets to improve the efficacy of fluconazole against cryptococcal infection.

329A

Characterization of *Histoplasma* micro- and macroconidia reveals key distinguishing features

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Histoplasma is a thermally dimorphic fungal pathogen of humans that causes disease even in immunocompetent individuals and is thought to be the number one cause of fungal respiratory infections. At ambient temperatures in the environment, *Histoplasma* hyphae produce conidia, which may become aerosolized and inhaled by a mammalian host, triggering the morphological transition to the pathogenic yeast form. Whereas many other fungi produce multiple types of spores, *Histoplasma* is the only thermal dimorph that produces two types of asexual spores: micro- and macroconidia. The physiological or functional differences between these spores is a long-standing mystery in the field, especially since the need for biosafety level 3 containment has constrained prior studies. Additionally, it is notoriously difficult to reproducibly generate micro- and macroconidia in the laboratory. Here, we describe optimization of conidiation conditions to allow for simultaneous production of both spore types. While these spores have similar cell wall composition, ultrastructural imaging reveals that macroconidia have thicker cell walls than microconidia. Macroconidia also have higher lipid droplet content and may be multinucleate, suggesting these spores may be able to withstand environmental stresses more readily than microconidia. Since *Histoplasma* is an intracellular pathogen that replicates within and lyses host macrophages, we compared the fate of murine bone marrow-derived macrophages infected with each spore type. These macrophages phagocytose both spore types equally efficiently, but macrophages infected with macroconidia show higher cell death than those infected with microconidia, despite the higher germination rate of microconidia *in vitro*. Future studies will measure the stress resistance of both spore types as well as their relative ability to cause disease in a murine model of histoplasmosis.

330A

How a shapeshifting fungus builds hyphal networks: hyphal initiation and growth in polymorphic fungus *Aureobasidium pullulans*

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Aureobasidium pullulans is a polymorphic fungus that, in a given cell cycle, can grow by budding, multi-budding, non-budding mitosis, meristematic, or hyphal growth (Goshima 2022; Ramos and García Acha 1975; Slepecky and Starmer 2009). It has been suggested that this morphological plasticity is what allows *A. pullulans* to thrive in a wide range of environments (Gostinčar et al. 2019). This polyextremotolerant fungus has been found in soil, plants, oceans, ice, house dust, and food products (Gostinčar et al. 2019; Prasongsuk et al. 2018). While new tools have enabled cell biology studies in this emerging model organism, research has mostly focused on the budding form of the fungus (Colarusso et al. 2025; Petrucco et al. 2024; Wirshing, Petrucco, and Lew 2024). Here, to further our understanding of morphological plasticity, we ask how *A. pullulans* grows as a hypha and how it transitions from yeast cells to hyphae. We describe the polarity requirements for hyphal growth, general characteristics of *A. pullulans* hyphae, and the process of hyphal initiation from yeast cells. We determine that the Rho-GTPase Rac1 is essential for hyphal growth, while Cdc42 is dispensable. We also find that hyphal initiation is probabilistic, rather than deterministic, with cells being more likely to initiate a hypha as they increase in nuclear number. Moreover, we observe that hyphal initiation is characterized by short initial polar growth events that often pause and resume either from the same hyphal tip or another site.

331A

Characterization of the galactosaminogalactan pathway genes in *Neurospora crassa*

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In *Aspergillus fumigatus* galactosaminogalactan (a bioactive polysaccharide) has been found to be capable of confusing the human immune system. It is made as a fully acetylated polysaccharide and subsequently deacetylated by Agd-3. Once galactosaminogalactan is deacetylated, it can surpass the immune cells of human.The genes for galactosaminogalactan synthesis and deacetylation are found throughout the fungal kingdom and the function of galactosaminogalactan in other fungi has not yet been fully elucidated. While studying this in *Neurospora crassa*, we observed that galactosaminogalactan biosynthetic pathway is encoded in a gene cluster and that Ncw-1 (NCU05137) is a homolog of Agd-3. Through compositional analysis of the polysaccharides from Δ5137 and wild type (WT), we observed that in Δ5137 we have N-acetylated galactosamine peak which was not present in WT, demonstrating that NCW-1 is a galactosaminogalactan deacetylase. We characterized mutants lacking each of the galactosaminogalactan biosynthetic pathway genes and found them to be morphologically indistinguishable from the wild type. The genes encoding NCU05137 (ncw-1, GAG deacetylase) and NCU05136 (GAG hydrolase) were cloned and expressed in wild type *Neurospora* cells with a HIS6 tag (both proteins). The proteins were purified on nickel columns and characterized. We found the NCW-1 mutant generates a hyphal matrix with less tensile strength than the wildtype and the mutant hyphae are defective in their ability to adhere to a glass surface. Furthermore, we found that the binding characteristics of galactosaminogalactan was dependent upon their state of acetylation. The fully acetylated galactosaminogalactan bound to wheat grass, chitin, activated charcoal, alumina, and celite.

We present a model of the galactosaminogalactan as a malleable adhesin which binds to different materials and facilitates adhesion to different materials in a location-specific manner within a growing fungal colony.

332A

Peroxisomal targeting via organellar transit

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Peroxisomes are vital eukaryotic organelles that are required for diverse oxidative metabolic pathways including the breakdown of fatty acids. The majority of peroxisomal matrix and membrane proteins can reach peroxisomes following their translation in the cytosol. Here, we discuss alternative pathways that involve an intermediate destination in mitochondria or in the endoplasmatic reticulum (ER) in the two fungal model systems *Saccharomyces cerevisiae* and in *Ustilago maydis*. The protein phosphatase Ptc5 is first imported into mitochondria; a fraction is rerouted into peroxisomes at organellar contact sites thereby regulating peroxisomal redox homeostasis. The tail anchor protein Pex15 can enter peroxisomes directly and via ER. We have used the latter pathway to identify novel factors for ER to peroxisome protein sorting. In summery, we provide insight into alternative protein targeting pathways, which might be relevant also outside the fungal kingdom. We believe that direct targeting and indirect targeting are coexisting pathways that regulate peroxisome formation and function.

333A

How fungal antigen subcellular localization affects translation and antigen processing in macrophages

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The basidiomycete fungus Cryptococcus neoformans poses a deadly risk for the immunocompromised population and is the most common cause of fungal meningitis in people living with HIV/AIDS. Despite ongoing effort in vaccine development, there is no clinically available vaccine for any fungal disease. To develop needed therapeutics against cryptococcal species, the field has focused on identifying potential drug targets and antigenic proteins for vaccine development. Based on mRNA vaccine design against parasites, the subcellular localization of antigens following in-host translation can greatly influence vaccine protection and supportive immune responses. However, it is unclear if cellular trafficking of fungal antigenic proteins is the same in fungal cells as in mammalian cells, and if the same antigens targeting different subcellular localizations would elicit different host responses. To test this, I have generated four isoforms of mCherry: cytosolic, GPI-anchored, transmembrane bound, and secreted. Expression of each isoform of mCherry using fungal targeting sequences should traffic as expected in cryptococcal cells, and the corresponding mCherry isoform mRNA constructs are expected to traffic in a similar manner after uptake by mammalian cells. By identifying the antigenic constructs allowing for the correct localizations in both cryptococcal and mammalian cells, mRNA constructs for fungal antigens can be adjusted for ideal vaccine responses. Elucidating the impact of protein subcellular localization in such

eukaryotic cells will be of use to guide vaccine development for known and new antigenic proteins. I hope this work contributes to antigen design for vaccines against C. neoformans and other pathogenic fungi.

334A

Trk1 potassium transport is crucial for effective *Candida* (*Candidozyma*) *auris* pathogenesis

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Candida (*Candidozyma*) *auris* is an emerging public health threat that colonizes human skin resulting in increased risk for subsequent invasive infection. The mechanisms underlying *C. auris* survival in the skin niche, however, remain poorly understood. We investigated the growth requirements of *C. auris* in a synthetic sweat media and identified potassium (K^+) as a crucial nutrient for efficient growth in skin niche condition. We aimed to understand the potential role of K^+ in *C. auris* growth on skin. To do this, we generated a strain with genetic disruption of the putative high-affinity K^+ transporter *TRK1*. The *trk1Δ* mutant demonstrated a severe growth defect in low K^+ conditions and on ex vivo human skin. Further studies also revealed that the loss of *TRK1* resulted in significantly impaired K^+ uptake, decreased intracellular K^+, plasma membrane hyperpolarization, decreased intracellular pH, and increased sensitivity to cationic stress. These findings demonstrate that *TRK1* is necessary for maintaining K^+ homeostasis and is critical for the effective growth and colonization of *C. auris* on human skin. Given that *TRK1* has no known human homologs, it represents a promising therapeutic target that can be used in developing novel strategies to prevent, decolonize, and treat *C. auris* colonization and potentially invasive infection.

335A

Mutants escaping from growth inhibition between colonies of the same strain in filamentous fungi

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In filamentous fungi, growth inhibition occurs between colonies of different species or strains, known as “antagonistic effect”, which has been studied for a hundred years in terms of a biological control. In contrast, we found that colony-colony growth inhibition occurs between the same strain in *Aspergillus oryzae*. This phenomenon would mean the presence of self/non-self recognition even between colonies of the same strain. Previously, we demonstrated that the inhibition involves FlbA/FadA-mediated G-protein signaling. In this study, to further elucidate mechanisms regulating colony-colony growth inhibition, we performed mutant screening using UV-induced mutagenesis. Under the multi-spot inoculation, colony growth normally stopped without contacting to each other. In the screening, 73 mutants that extended into the growth-inhibited zones were isolated, and thorough a subsequent analysis by a paired-culture 40 mutants escaping from colony-colony growth inhibition were obtained. As some of the mutants exhibited conidiation-deficient phenotype typically seen in Δ*flbA*, gene sequencing revealed that mutations in the *flbA* were commonly found, which supports the importance of FlbA/FadA-mediated G-protein signaling. Furthermore, whole-genome sequencing identified mutations in several uncharacterized genes. Reintroduction of the wild-type genes restored the inhibition, suggesting their involvement in the regulation of colony-colony growth inhibition. Collectively, mutant screening revealed candidate genes involved in the inhibition, which would help clarify the overall regulatory mechanism underlying colony-colony growth inhibition between the same strain.

336B

Death or disomy: perturbing spindle checkpoint kinases in the fungal pathogen *Cryptococcus neoformans*

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Fungi change shape and reconfigure their growth & division processes in response to environmental stresses. Under severe stress, such as during human infection, individual fungal cells adapt rapidly. Adaptation is often accompanied by large-scale genomic alterations including polyploidy and aneuploidy (an abnormal number of chromosomes). Aneuploidy produces genetic diversity within the fungal population and selection will favour those cells best suited to growth in the stressful conditions. Aneuploidy can also generate drug-resistance, which is a serious problem in clinics world-wide.

During an infection, Cryptococcus cells become large and polyploid and this protects them from immune cells. These polyploid cells then produce haploid daughters, through a fascinating reductive division. We study cell division, chromosome segregation and aneuploidy in *Cryptococcus neoformans,* including mechanisms of error correction and spindle checkpoint signalling. This checkpoint acts as a surveillance system: only when all chromosomes are attached appropriately is the checkpoint satisfied, allowing high fidelity chromosome segregation to take place.

We will report our analysis of *Cryptococcus mad*, *bub1* and *mps1* mutants, the use of SAC-specific protein kinase inhibitors and perturbation of PP1 and PP2A mitotic phosphatases. Certain perturbations lead to increased rates of chromosome mis-segregation and aneuploidy. Our recent analysis of *mps1* and *bub1* deletion mutants demonstrates that both are very defective in the formation of viable, polyploid titan cells. In addition, both null mutants appear to be kept alive by extra copies of chromosome 6. On the hunt for specific suppressors, we are evolving these strains in the lab. We are also studying, through quantitative whole cell proteomics, how these mutants cope with the proteotoxic stress caused by their aneuploidy.

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337B

Expanding experimental tractability across the *Schizosaccharomyces* clade of fission yeast

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The *Schizosaccharomyces* clade of fission yeast has grown to include over seven described species, providing valuable models for studying evolutionary events. Among these, *Schizosaccharomyces pombe* remains the most extensively characterized growing as single rod-shaped cells in YEA medium and forming smooth colonies on plates. Upon glucose deprivation this species enters a state of dormancy characterized by cytosolic freezing, reversible upon return to nutrient-rich conditions; and upon nitrogen starvation undergoes meiosis to form four spored-asci. In contrast, little is known about its relatives, limiting comparative functional studies.

Schizosaccharomyces octosporus is studied by a handful of labs and exhibits many striking differences to *S. pombe*. This yeast tends to grow in clumps in YEA and forms rough colonies on plates, though growth in YPD reduces aggregation. During meiosis, it generates 4–8 spores due to an additional mitotic division after meiosis. Glucose deprivation induces the formation of large internal structures distinct from vacuoles. We show using electron microscopy that this may represent condensed cytoplasm due to thickening of the cell wall. *Schizosaccharomyces cryophilus* shares many traits with *S. octosporus* but grows optimally at lower temperatures, resulting in slow propagation. It also rarely undergoes meiosis, limiting its experimental utility.

I will present our work to define the lifecycle and optimal growth requirements for these species and efforts to enhance their tractability, enabling deeper evolutionary insights and expanding opportunities for comparative research within the *Schizosaccharomyces* clade.

338B

A novel beta-propeller protein in *Aspergillus nidulans* required for microtubule stability

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We discovered a fungal-specific protein required for microtubule (MT) stability, *wmcA* (*W*D-repeat *m*itotic *c*atastrophe) in *Aspergillus nidulans*. Cells lacking *wmcA* exhibit striking abnormalities: the cells are extremely cold sensitive and undergo mitotic catastrophe characterized by the absence of microtubules and consequent lethal fragmentation and dispersion of chromosomes. Several lines of evidence point to a role in MT homeostasis, including (1) at the non-permissive temperature, absence of GFP-tagged MTs and mislocalization of the MT (+)-end binding protein ebA::GFP, and (2) genetic interactions with a null mutation in Tubulin-binding Cofactor A (TBCA) and with a MT-hyperstabilizing mutation in beta-tubulin. Generation of suppressors of the cold-sensitivity of delta-*wmcA* revealed additional genetic interactions involving translation and ubiquitin-mediated processes. Localization studies demonstrate that *wmcA* is both cytoplasmic and nuclear. Functional domain studies show that the *wmcA* N-terminus prior to the first WD repeat is essential, whereas the highly conserved C-terminus following the last WD repeat is fully dispensable. Given that WD-repeat proteins typically serve as scaffolds in multi-protein complexes, and that cold-sensitive mutations, such as occur in the delta-*wmcA* mutant, often reflect the destabilization of protein complexes at low temperature, it is likely that *wmcA* functions to stabilize a multi-protein complex. Colabfold modeling suggested beta-tubulin and/or TBCA as candidate physical interactors with *wmcA::GFP*. Current efforts are aimed at testing these candidates by co-IP to identify *bona fide* binding partners and thereby gain insight into the mechanism by which this previously unstudied protein influences MT stability and dynamics.

339B

Lipid homeostasis in antifungal resistance of *Cryptococcus neoformans*

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The fungal pathogen *Cryptococcus neoformans* exhibits innate resistance to the echinocandin class of antifungal drugs, through mechanisms that are poorly understood. Previous work has identified Cdc50, the regulatory subunit of lipid flippase, as a key contributor of resistance to the echinocandin drug caspofungin. The *cdc50*D mutant has impaired flippase function, resulting in increased phosphatidylserine (PS) levels at the outer leaflet of the plasma membrane. We hypothesize that phospholipid distribution in at the plasma membrane alters the interaction between caspofungin and its target, b-1,3-D-glucan synthase (Fks1). To test this hypothesis, we are using a cell-free system, in which Fks1 is expressed in liposomes with varying concentrations of PS, to examine interactions between caspofungin and Fks1. We are also examining how PS levels affect the Fks1-caspofungin interaction by altering levels of the PS synthase enzyme Cho1. As *CHO1* is an essential gene, we have generated a CTR4 promoter-based system to conditionally reduce Cho1 expression, in order to test if altering PS synthesis affects drug sensitivity. These studies provide mechanistic insight that can be used towards drug discovery, which will focus developing inhibitors of lipid flippase and PS synthase.

340B

The role of eisosomal proteins in pathogenesis of *Cryptococcus neoformans*

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Cryptococcus neoformans is a human fungal pathogen responsible for the death of ~20% of AIDS related patients.The cell wall and plasma membrane (PM) are critical for survival of a pathogen in the host and pathogenicity. Several protein complexes associated with the PM sense the unfavorable host environment and initiate compensatory mechanisms to cope with stress. Eisosome is one such protein complex important for nutrient acquisition, stress response and pathogenesis. Eisosome complex is well-studied in the model yeasts of the ascomycetes phylum; however, no study has been conducted yet to define eisosome's role in *C. neoformans* which belongs to basidiomycetes. In *Saccharomyces cerevisiae*, Pil1 and Lsp1 proteins are the core

components of eisosomes that shape the curvature of the PM based on their conserved AH-BAR domains. We identified respective homologues of Pil1 and Lsp1 in *C. neoformans*. Our results revealed two localization patterns for Pil1 tagged with the GFP: the canonical punctate localization to the PM and a previously uncharacterized localization to the mother-bud neck. The *pil1*Δ and *lsp1*Δ mutants exhibited inverse sensitivity when grown under various stress conditions suggesting a novel mechanism of how Pil1 and Lsp1 cooperate in stress response. Cells lacking Lsp1 exhibited fewer punctate PM-associated signals of Pil1-GFP that were also notably brighter as compared to those found in the wild-type, suggesting a role of Lsp1 in eisosome formation and dynamics. Interestingly, elimination of Lsp1 had similar consequences on the localization of the septin Cdc12-GFP, which together with other septins, forms filamentous punctate assemblies at the PM specifically when cells are stressed with 37°C. As the well-described function of septins is at the mother-bud neck during cytokinesis, our results point to a novel connection between, septins and eisosome components that involves PM homeostasis and cytokinesis. Strikingly, the double mutant (*pil1*Δ*lsp1*Δ*)* revealed defects in two major virulence factors, polysaccharide capsule and secretion of urease, pointing to an important contribution of eisosomes to pathogenicity of *C. neoformans*. As eisosome components have no resemblance to human proteome, they constitute a promising therapeutic target.

341B

DIA-PASEF Proteomic Profiling Reveals MpkA-Dependent Iron Stress Responses and Metabolic Reprogramming that Influence Siderophore Biosynthesis in *Aspergillus nidulans*

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Filamentous fungi play important roles in the biotechnology industry, human health, and as plant pathogens, yet comprehensive proteomic resources remain somewhat limited. To address this limitation, we developed a new, data-independent acquisition–parallel accumulation serial fragmentation (DIA-PASEF) approach for high-depth proteome profiling of *Aspergillus nidulans*. By leveraging ion mobility information from DDA-PASEF experiments, our DIA-PASEF workflow identified 3,904 proteins across biological triplicates grown in rich medium (YGV), representing a 140% increase in proteome coverage with over five-fold shorter analysis time compared to previous studies. We have applied this workflow to investigate the impact of iron limitation and *mpkA* kinase deletion (Δ*mpkA*) through integrated proteomic and phosphoproteomic analyses. In minimal media, with and without iron, the Δ*mpkA* strain expressed approximately 500 additional proteins and showed increased phosphorylation at over 1,800 unique phosphosites. This was accompanied by markedly increased differential expression and phosphorylation under both iron-replete and iron-deplete conditions. Gene Ontology (GO) analysis revealed broad metabolic and stress-responsive adaptations in the Δ*mpkA* strain under iron limitation. Notably, under iron-limited conditions, the Δ*mpkA* strain displayed upregulation of central metabolic pathways that generate precursors for siderophore biosynthesis compared to the control strain. Based on these results, we hypothesized that MpkA deficiency enhances siderophore production under iron limitation. Consistent with this, Chrome Azurol S (CAS) assays confirmed that Δ*mpkA* mutants produced significantly more siderophores under iron-deficient conditions. Together, these data establish a comprehensive proteomic reference for *A. nidulans* and uncover MpkA-dependent regulatory networks that link iron stress responses to siderophore biosynthesis.

342B

Dynamic localization of the septin Cdc3 in the predator yeast Saccharomycopsis schoenii

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Saccharomycopsis schoenii is a predatory yeast which preys on other yeasts or filamentous fungi when starved e.g. for methionine. Prey cells, therefore, include *Candida auris and Saccharomyces cerevisiae*, but predator yeasts are occasionally also cannibalistic on other *Saccharomycopsis* cells. For predation, *S. schoenii* recognizes its prey cell and forms a penetration peg to infect the prey cell. Predation leads to the death of the prey cell, allowing *S. schoenii* to take up nutrients required for its growth. Septin proteins, filament-forming GTPases, are found in all eukaryotes except land plants. Septins are involved in cell division and have been implicated in fungal pathogenesis but have not been previously studied in *S. schoenii*. To investigate the involvement of septins in predation by *S. schoenii*, we followed the localization of the fluorescently tagged septin homologue Cdc3 in *S. schoenii* (*SsCDC3-GFP*). We also tagged the *S. cerevisiae CDC3* with mCherry (*ScCDC3*-mCherry) to observe any septin patterns in the prey cell during predation. During normal growth, *S. schoenii Ss*Cdc3-GFP localized to the bud neck between mother and daughter cell either in the form of a ring/collar or as bars parallel to the mother-daughter axis. In large-budded cells the *Ss*Cdc3-GFP bars transitioned to form rings which subsequently contracted in cells that appeared to undergo cytokinesis, a septin dynamic which has not yet been described in literature. Additionally, during active growth, *Ss*Cdc3-GFP accumulated lightly at the growing tip of *S. schoenii* cells. During predation, *Ss*Cdc3-GFP localized to the incipient site of penetration peg formation and remained at the base of the penetration peg. *Sc*Cdc3-mCherry in *S. cerevisiae* did not exhibit unusual septin organization or localization while being preyed upon, except upon cell death, when septins occasionally localized as puncta at the cell perimeter. These data show previously uncharacterized dynamics of fungal septins and suggest that septins play an important role in predation by *S. schoenii*.

343B

Septins and cytokinesis in the polymorphic fungus *Aureobasidium pullulans*

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During cytokinesis of animals and fungi, a contractile actomyosin ring (CAR) assembles at target locations and constricts to drive cell separation. In animal cells, the position of the CAR is determined by the mitotic spindle, so that the cleavage plane is perpendicular to the mitotic axis. However, in budding yeasts, the location of CAR assembly is specified by a cortical septin cytoskeleton that recruits CAR components to the neck. In the polymorphic fungus *Aureobasidium pullulans*, we show that septins assemble at mother-bud necks and predict the site of CAR assembly. Cells lacking septins stochastically failed to assemble CARs at a subset of bud necks. However, even cells lacking all four core septins were able to assemble CARs at 75% of bud necks, indicating that there is a mysterious alternative mechanism to assemble CARs at mother-bud necks.

344B

Cryptococcal mRNA vaccine development based on GPI-anchored mannoproteins

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Systemic infections caused by *Cryptococcus* claim over 112,000 lives annually, with global mortality rate close to 70% despite antifungal therapies. Currently, no vaccine is available. Due to major scientific and technological breakthroughs and the establishment of mRNA-vaccine infrastructures, stabilized mRNAs with modified uridine encoding immunogens packaged in and delivered through LNPs have become a clinically proven and effective vaccine technology. To develop an effective multivalent vaccine against this free-living opportunistic eukaryotic pathogen, it is critical to identify protective antigens. We previously discovered *ZNF2*^{oe} strains elicit protective host immune responses and increase the abundance of antigens present in the capsule, which is required for its immunoprotection. Capsule is a defining feature of *Cryptococcus* species and composed of polysaccharides and mannoproteins. Here, we found increased levels of exposed mannoproteins in *ZNF2*^{oe} cells. As mannoproteins are the primary components recognized by anticryptococcal cell-mediated immune responses and few have been characterized, we systemically screened all 49 predicted GPI-mannoproteins in *Cryptococcus neoformans* for enhanced host recognition. We identified those highly present in *ZNF2*^{oe} cells and found Cig1 to be a protective antigen against cryptococcosis either as a recombinant protein vaccine or an mRNA vaccine. Cig1 is induced by iron limitation and is highly expressed by this fungus in infected mice and in patients with cryptococcal meningitis. Remarkably, iron restriction by the host induces cryptococcal cells to express iron-uptake proteins including Cig1, which act as cryptococcal antigens and in turn enhance host detection. Our results highlight an arms race between the pathogen and the host centered on iron competition, and the trade-off between cryptococcal iron acquisition and antigen exposure. These findings demonstrate the potential of leveraging this host–pathogen interaction for vaccine development.

345B

Circadian clock is critical for regulating pathogenicity in *Fusarium oxysporum*

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Circadian clocks have evolved to enhance the fitness of organisms by improving their ability to adapt to daily environmental changes. Circadian clocks are known to modulate host immune responses to pathogen infections, yet their role in influencing pathogen pathogenesis remains unclear. To investigate the specific role of the circadian clock in pathogenic fungi, we examined the presence of a functional circadian clock in *Fusarium oxysporum,* which has multiple circadian clock genes *frqs* due to gene duplication events, with *Fofrq1* being the primary circadian clock gene. The pathogenesis of *F. oxysporum* in plants is controlled by its circadian clock, with infections causing severe disease symptoms at dawn. Notably, disruption of clock genes dramatically reduces fungal pathogenicity. Mechanically, circadian clocks regulate fungal-plant interactions by controlling the expression of pathogenic factors, including FoZafA, which enables the pathogen to adapt to zinc starvation within the plant, and FoCzf1, which governs the production of the toxin fusaric acid. In summary, we elucidated the important function of the circadian clock in modulating the pathogenicity of *F. oxysporum* by regulating zinc starvation response and secondary metabolite production.

346B

Quantification of *Aspergillus nidulans* Actin Dynamics during Early Growth and Septum Formation

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Filamentous fungi present unique challenges for live-cell imaging due to their three-dimensional growth patterns and non-adherent nature, limiting quantitative analysis of dynamic cellular processes. We modified a live cell imaging system to constrain *Aspergillus nidulans* growth to a single focal plane, enabling high-resolution time-lapse imaging of actin dynamics throughout development using a Lifeact actin marker. We performed kymographic analysis to quantify actin velocity and hyphal extension rates during early development. Our results revealed two distinct growth phases: germ tube extension (0.58 µm/min) and hyphal extension (1.52 µm/min). Actin exhibited bi-directional transport along hyphae with biased movement toward the spore body. Actin was also observed re-localizing from hyphal tips to sites of septal formation indicating active redistribution of cytoskeletal resources based on cellular demands. This new system overcomes limitations in fungal live-cell imaging and provides a powerful platform for quantitative systems-level analysis of mycelial development, offering new insights into the spatiotemporal coordination of cytoskeletal dynamics during filamentous growth.

347B

FluG confers conidial heterogeneity in *Aspergillus fumigatus*

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Although conidia (asexual spores) are genetically homogeneous, their germination timing varies. In the human pathogenic fungus *Aspergillus fumigatus*, we found that D-galactosamine (D-GalN) more effectively promoted dormancy breaking than D-glucose (D-Glc). To identify genes involved in D-GalN utilization, we constructed several gene disruptants based on D-GalN-induced transcripts identified by RNA-seq. Contrary to our expectation of finding a mutant unresponsive to D-GalN, the *fluG* disruptant (Δ*fluG*) displayed notable phenotypes: its growth began earlier than that of the parental strain in minimal medium with D-Glc, resembling the D-GalN condition. Thus, FluG appears to negatively regulate dormancy breaking, mirroring the effect of D-GalN. While FluG is essential for conidiation in *A. nidulans*, it is dispensable in *A. fumigatus*. We therefore investigated the role of FluG in conidial dormancy breaking in *A. fumigatus*. Although Δ*fluG* conidia were morphologically similar to the parental strain, they germinated earlier in all tested carbon sources. Dormancy breaking was quantified by β-1,3-glucan exposure. At 6 h in minimal medium, ~90% of Δ*fluG* conidia showed dormancy breaking compared to

~40% of conidia of the parental strain. These results indicate that conidial germination is heterogeneously regulated under nutrient-limited conditions, and that FluG plays a key role in establishing this heterogeneity in *A. fumigatus*.

348B

The *Aspergillus fumigatus* growth determinant Afu6g07200 is the first granulin described in fungi

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Granulin is a secreted growth factor conserved among eukaryotic organisms. In humans, it is related to neuronal, autoimmune, and cancer diseases because it has a role in survival, growth modulation, migration, inflammation, and wound repair. It was thought that fungi had lost this type of domain since there is no sequence homolog, but in this work, we described a conserved protein in filamentous fungi with the same 3D structure. Different approaches using *Aspergillus fumigatus* mutant strains were employed to demonstrate that human and fungal proteins have similar functions and localization. Phenotypic characterization of the deletion strain revealed defects in maintaining cell morphology, decreased proliferation and conidiation, increased septa and branch formation, and disorganized polarity. The zigzag phenotype of the hyphae was stronger under osmotic pressure and was completely rescued by the deletion of the polarity marker TeaC. At the molecular level, the deletion strain was unable to activate the two major mitogen-activated cascades, cell wall integrity and the high-osmolarity pathway, even after the addition of stressors. In addition, a higher sensitivity of the deletion strain to cell wall stressors, disorganized walls, and increased periplasmic spaces were detected. Finally, functional homology between human granulin A and Afu6g07200 gene was confirmed by the complementation of the deletion strain with human granulin A. The endogenous expression of human granulin rescued the phenotypic defects at the morphological and molecular level.

349B

The general repressor Ssn6 suppresses biofilm formation in *C. neoformans*

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Cryptococcus neoformans is an environmental human fungal pathogen and a model basidiomycete yeast responsible for 19% of AIDS-related deaths. As a model organism, its sexual cycles are well-understood. Mating occurs under nutrient-limited conditions, during which yeast cells fuse, undergo nuclear duplication, exhibit hyphal growth, and ultimately form fruiting bodies. These structures also generate classical biofilms composed of invasive aggregates embedded in an extracellular matrix. These resulting morphotypes have distinct immunogenic and pathogenic properties. For example, overexpression of the regulator of hyphal morphogenesis, *ZNF2*, induces the host's TH1 response, providing protection against lethal cryptococcosis. Due to the tight interconnection between mating and biofilm formation, separating their regulation is challenging. However, biofilms can develop independently of sexual reproduction and meiosis, as observed in patients who develop cryptococcomas. This prompted us to search for regulators of biofilm formation that operate outside the canonical pheromone pathway. We focused on mutants capable of forming biofilms in nutrient-rich conditions, where wild-type cells typically remain yeast-like. We discovered that the general transcription repressor Ssn6 suppresses biofilm formation, as deleting *SSN6* triggers robust biofilm development in rich medium. Using a general repressor mutant provides a less pathway-biased tool for identifying additional biofilm-associated genes. Employing the *ssn6*Δ mutant, we found that biofilm induction is mediated by a Znf2-independent secreted extracellular signal that is subsequently taken up by neighboring cells. Furthermore, we established that the quorum-sensing molecule Qsp1 is crucial for this process. We also uncover that biofilm formation under nutrient-rich conditions depends on mating type and involves Mat2, a transcription factor that regulates the pheromone pathway. Together, our findings reveal a regulator of biofilm formation outside the classical mating pathway and reveal interconnected roles for quorum sensing, biofilm development, and mating pathway activation.

350B

Antifungal resistance: formation of heterochromatin-dependent epimutations in fission yeast

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Global health and food supplies are endangered by antifungal resistance of pathogenic fungi. We employ fission yeast, *Schizosaccharomyces pombe*, as a model system to study mechanisms of resistance. Assembly of histone H3 lysine 9 methylation (H3K9me)-dependent heterochromatin over genes decreases their transcription. In fission yeast and several pathogenic fungi, heterochromatin is normally concentrated at telomeres and around centromeres. However, wild-type *S. pombe* can gain resistance to caffeine and antifungal insults through heterochromatin-dependent epimutations which reduce expression of underlying genes. Epimutations are unstable and resistance is gradually lost in the absence of selection. Thus, heterochromatin-dependent epimutants may allow for bet-hedging within populations and phenotypic plasticity to cope with changing environments.

We have shown that one mechanism by which epimutations can cause resistance is via mitochondrial dysfunction-induced oxidative stress, leading to increased efflux. However, the events upstream of epimutation formation are unknown. We are investigating whether epimutations that allow resistance to different insults pre-exist in the population or are induced. We aim to uncover mechanisms through which epimutations arise at particular loci by investigating genomic and cellular requirements for heterochromatin assembly at common epimutation loci.

Similar unstable epimutations may provide an alternative or precursor to genetic mutations and contribute to antifungal resistance in fungal pathogens.

351B

Spatial coordination of growth and division in *Ashbya gossypii*

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Fungal cells are organized into diverse architectures to grow and divide in changing environments. Extensive work in uninucleate fungi has demonstrated that cell growth is tightly coordinated with nuclear division to maintain a stable nuclear-to-cytoplasmic ratio (N/C ratio). Multinucleated fungi, many of which have also evolved complex branched geometries, face an interesting challenge in coordinating N/C ratio over large cytoplasmic distances. In the multinucleated filamentous-growing fungus *Ashbya gossypii*, N/C ratio is kept remarkably constant, even through drastic increases in cell growth rate. Nuclei are highly mobile, often traveling far from sisters before completing their next division. These nuclear divisions occur asynchronously from neighbors throughout the entire cell and far from growing hyphal tips, suggesting that *A. gossypii* has evolved mechanisms to communicate cell growth information to nuclei at length-scales greater than diffusion can accomplish. The *A. gossypii* genome exhibits high homology with the uninucleate budding yeast *Saccharomyces cerevisiae* but grows exclusively as a multinucleated cell. Interestingly, two distinct cytoskeletal features are conserved: (1) like cytokinetic rings in *S. cerevisiae*, the *A. gossypii* septin cytoskeleton forms cortical rings periodically throughout all hyphae and (2) *A. gossypii* nuclei migrate through conserved interactions between microtubule-motors and cortical anchors, seen in *S. cerevisiae* spindle positioning. I hypothesize that *A. gossypii* has adapted this cytoskeletal machinery to coordinate its N/C ratio over large distances, where septin-based cortical cues communicate long-range growth information to globally couple cell growth with nuclear division, and microtubule-driven nuclear migration events facilitate cell-cycle asynchrony that stabilizes local N/C ratio. With a combination of fixed and live imaging strategies, I report that the distance between cortical septin rings increases with cell growth rate, suggesting that the interval between septin rings may be a record of growth rate that is propagated and maintained far away from the growing hyphal tip. Future work will investigate how these septin rings may locally influence mitotic progression, and how microtubule-dependent nuclear movements facilitate dynamic control of local N/C ratio, important insights in understanding how multinucleated cells may use conserved regulatory logic and machinery to sense and communicate their size.

352B

Small but mighty: Defining the *Cryptococcus neoformans* titanide morphotype as a driver of fungal pathogenesis

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Fungi frequently exhibit morphological changes providing adaption to the host. The pathogen *Cryptococcus neoformans* is predominantly studied as an encapsulated haploid budding yeast (5 µm). However, in host-relevant conditions *C. neoformans* is highly pleomorphic, capable of generating an impressive range of morphologies with substantial differences in total cell body and capsule size/structure that directly influence pathogenesis by facilitating immune evasion, dissemination, and antifungal resistance. To better understand how this heterogeneity emerges, we used microfluidics to study the *C. neoformans* life cycle. We observed that yeast cells are highly plastic and can respond to inducing conditions by propagating as typical "yeast" cells (5-7 µm), transitioning into titan cells (>10 µm), or generating a third alternative, the titanide daughter (2-3 µm).

Titanides are a poorly understood cell type that appears in the lungs of infected mice and can be readily induced *in vitro* under conditions that mimic the host environment. Under these conditions of cellular heterogeneity, titanides appear ~36 hours post-induction and by 72 hours are the dominant morphotype (>70%). Despite titanides being the most prevalent cell type in induced populations and the significance of cryptococcal morphogenesis for virulence, their biology remains unstudied.

To address this, we investigated titanides both *in vivo* and *in vitro*, focusing on key questions regarding their origin and cell fate. We demonstrate that titanides display distinct morphological features to their typical cell mothers; they are small, oval, and possess thin cell walls. Fluorescence microscopy also reveals alterations in cell wall, membrane, and capsule composition following titanide morphogenesis. Additionally, titanides are uniquely non-proliferative under the *in vitro* culture conditions from which they emerge. These features distinguish titanides from previously described *C. neoformans* small cells such as in vivo seed cells (4-6 µm, high cell-wall mannan content) and micro cells (round, <1 µm, with thick cell walls). Interestingly, despite their thin cell walls, we show that titanides display a modest increase in stress resistance and altered antifungal sensitivity profiles relative to yeast.

Overall, this work highlights the morphological plasticity of Cryptococcus cells and raises questions about the role of titanides in environmental and disease contexts.

353B

Anaerobic Gut Fungi Produce Extracellular Vesicle-Like Particles

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Anaerobic gut fungi (AGF; phylum Neocallimastigomycota) are early-branching fungi that wield great biotechnological potential through their exceptional capacity to degrade lignocellulose. Extracellular vesicles (EVs) are lipid nanoparticles secreted by cells that mediate cargo transport and intercellular communication and are genetically regulated secretion products in many model fungi; however, their existence and biological roles in AGF remain unexplored. Here, we report the isolation and characterization of EV-like particles from three phylogenetically distinct AGF species: *Anaeromyces robustus*, *Caecomyces churrovis*, and *Neocallimastix* sp. GF-Ma3-1. To enable EV detection, rumen-derived growth media were depleted of background particulates using differential centrifugation and filtration prior to fungal inoculation. After four days of growth, conditioned media were clarified and EV-like particles were isolated by either ultracentrifugation or size exclusion chromatography (SEC). Particle populations were characterized using nanoparticle tracking analysis (NTA) and transmission electron microscopy (TEM). All three AGF species produced highly concentrated particle populations with size distributions consistent with fungal EVs and absent from non-cultured media controls. TEM imaging confirmed the presence of membrane-bound vesicle-like structures. SEC fractions exhibited low protein-to-particle ratios, as measured by microBCA and NTA, supporting enrichment of vesicles rather than

protein aggregates. Together, these results provide the first experimental evidence that anaerobic gut fungi produce EV-like particles. Ongoing molecular profiling will define their protein and nucleic acid cargo and will guide future studies of the genetic pathways governing vesicle biogenesis and cargo loading as genetic manipulation tools in AGF continue to advance. This work expands EV biology into an evolutionarily distinct fungal lineage and establishes AGF as a new experimental system enabling future genetic studies of fungal secretion and intercellular communication.

354B

Characterization of two *Neurospora crassa* GH72 family enzymes and their roles in cell wall biogenesis

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The fungal cell wall is cross-linked matrix of polysaccharides and glycoproteins. The types of polysaccharides and glycoproteins present in the cell wall is highly regulated and can vary from one cell type to another. In *Neurospora crassa* vegetative hyphae, chitin, β-1,3-glucan (laminarin), and a mixed β-1,3-/β-1,4-glucan (lichenin) are the major cell wall polysaccharides. Glucan transferases from the glucosyl hydrolases 72 family (GH72) play a critical role in cross-linking cell wall polysaccharides together and generating a cell wall matrix. GH72 enzymes have been shown to function as b-1,3- glucanases and glucanosyltransferases and can function in crosslinking b-1,3-glucans together. To characterize the enzymatic activities of GEL-1, we expressed the encoded protein with a HIS6 tag in *N. crassa.* A chimeric MBP::GEL-2 was produced in *E. coli*. Purified GEL-1 and GEL-2 were used to characterize their enzymatic activities. We employed thin-layer chromatography (TLC) and polyacrylamide carbohydrate gel electrophoresis (PACE) assays to visualize GEL-1 and GEL-2 hydrolase and transferase activities on lichenin and laminarin substrates. We determined that GEL-1 functions as a laminarinase (b-1,3-glucanase) and as a laminarin transferase. We found that GEL-2 can function as a laminarinase and as a licheninase (b-1,3-/β-1,4-mixed-glucanase) and can crosslink b-1,3-glucans together. We demonstrated that GEL-2 can form laminarin:enzyme intermediates and lichenin:enzyme intermediates, proving evidence that GEL-2 can function as a lichenin transferase as well as a β-1,3-glucan transferase.

355C

Dissecting turgor-sensing mechanisms required for appressorium-mediated plant infection by the rice blast fungus *Magnaporthe oryzae*

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Appressorium-mediated infection by *Magnaporthe oryzae* relies on the ability of the fungus to generate, sense, and respond to extreme turgor pressure in its infection cell. The histidine kinase Sln1 functions as a central turgor sensor that triggers appressorium repolarisation and host penetration, yet the molecular mechanisms underlying its function remain poorly understood. To define the signalling networks by which Sln1 coordinates turgor-dependent developmental transitions, we mapped the Sln1 interactome using an ultra-high-throughput yeast two-hybrid (Y2H) screen of an appressorium-stage cDNA library. Screens using full-length Sln1 (minus the transmembrane domain) and its isolated response-regulator domain identified 11 and 66 candidate interactors, respectively. These include β-oxidation enzymes, glycerol-generating enzymes, cytoskeletal motors, cell wall–modifying proteins, and COP9 signalosome components, highlighting a broad regulatory network linking metabolism, cytoskeletal dynamics, and cell wall remodelling to turgor sensing.

Additionally, complementary immunoprecipitation–mass spectrometry (IP/MS) with in-locus Sln1-3xFLAG and Ypd1-3xFLAG *M. oryzae* strains in both wild-type and turgor-deficient (*rsy1*–) backgrounds revealed 47 significant interactors. Sln1 associates with distinct protein cohorts depending on turgor status. In Guy11, interactors are enriched for wall-modifying enzymes and signalling factors, whereas in Δ*rsy1 mutants*, Sln1 engages proteins involved in wall biosynthesis and vesicle trafficking, including kinesin II and chitin synthase 1. Sln1 also associates with core melanin biosynthesis enzymes, and Δ*sln1* mutants fail to repress melanin deposition, linking phosphorelay signalling to the regulation of melanin biosynthesis and turgor maturation. These findings support a model in which Sln1 dynamically rewires its interactome in response to mechanical cues.

Finally, in yeast, Sln1 controls Hog1 MAPK activity through a conserved phosphorelay, and *SLN1* deletion is lethal due to constitutive Hog1 phosphorylation. Leveraging this conserved mechanism, we are testing whether Osm1 phosphorylation can serve as a quantitative proxy for Sln1 dephosphorylation and activation during turgor generation. Establishing this relationship will enable time-course assays to pinpoint when Sln1 becomes active as appressoria mature and mechanical pressure accumulates, as pre-requisites to plant infection.

356C

New tools to tweak the appressorial *Mo*TOR

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The rice blast pathogen *Magnaporthe oryzae* is a major global threat to food security, having expanded from rice to infect wheat, barley, millet and other cereals, causing substantial agricultural and economic losses. Although fungicides can limit disease, their environmental toxicity and impact on soil and human health highlight the urgent need for new, mechanism-based control strategies.

Disease initiates with the arrival, and immediate germination, of three-celled conidia on a plant surface. If the germling senses a hard, hydrophobic surface, free of nutrients, a specialized infection structure, the appressorium, will form, which, driven by autophagy of the initial three cells accumulates high glycerol concentrations, and becomes melanized enabling the generation of hydrostatic pressures of up to ~8 MPa. This pressure is then used to force a penetration peg through the leaf cuticle, a prerequisite for plant colonization.

The Target of Rapamycin (TOR) kinase has emerged as a central regulator of this developmental program. In *Saccharomyces cerevisiae*, TOR functions through two complexes, TORC1 and TORC2, which control growth, autophagy, membrane stress responses, and cell-cycle progression. In *M. oryzae*, chemical inhibition of *Mo*TOR using Torin completely abolishes appressorium formation (Loewith lab, unpublished), underscoring its essential role during infection.

Our work focuses on *Mo*TORC1 regulation during early infection. Using an optimized CRISPR-Cas9 system, we generated mutants with impaired or hyperactivated *Mo*TORC1 signalling. Phenotypic analyses revealed that *Mo*TORC1 transduces environmental cues (nutrients, surface characteristics, etc) to appropriately regulate infection-related development. To facilitate these studies, we are developing an AI-based tool for automatic classification of conidial and appressorial morphotypes. This platform quantifies spore size, morphology, and developmental transitions with high accuracy, enabling rapid analysis while reducing human error.

Together, these genetic and computational tools provide new insights into *Mo*TOR regulation and open avenues for identifying novel antifungal targets.

357C

Characterization of the Structure and Function of the Septal Pore Apparatus in *Coprinopsis cinerea*

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Vegetative mycelia of agaricomycetes can be exceptionally large and long-lived. The hyphae of these fungi are compartmentalized by cross-walls (septa) containing pores with thickened cell walls (dolipores) and sophisticated cap structures, referred hereafter as septal pore apparatus. This apparatus ensures that nutrients and signals are distributed between the hyphal compartments via cytoplasmic bulk flow. Like in ascomycetes, the apparatus can also block cytoplasmic bulk flow between compartments by septal pore plugging e.g. as a response to hyphal damage and maybe also internal cues. Since the septal pore apparatus ultimately controls the cytoplasmic bulk flow in a fungal mycelium, it is an essential organelle for multicellular fungi. In contrast to multicellular ascomycetes, however, very little is known about the molecular composition of the septal pore apparatus and the mechanism of septal pore plugging in multicellular basidiomycetes. We set out to determine the function, the structure and the protein composition of the septal pore apparatus in the model agaricomycete *Coprinopsis cinerea* using a combination of microscopic, genetic and biochemical approaches. These findings will contribute to our understanding of cytoplasmic bulk flow dynamics within coenocytic systems.

358C

A novel functional cell biology platform for the fungal symbiont domesticated by leafcutter ants

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Leafcutter ants farm the basidiomycete fungus *Leucoagaricus gongylophorus* whose ecological success depends on the differentiation of specialized hyphal cells, called gongylidia, that the ants consume. These cells exhibit striking subcellular adaptations, including large vacuoles that store plant-degrading enzymes the ants later vector back to the fungus via fecal droplets. We recently used live-cell fluorescence microscopy to show that these gongylidium vacuoles are further specialized to exclude reactive oxygen species (ROS) that ostensibly promotes this vectoring of stable enzymes and improves their value as a domesticated food crop. To further probe the underlying genetic mechanisms, we developed a robust protoplast isolation protocol for *L. gongylophorus*, providing direct access to viable wall-free cells for live-cell imaging, viability testing, and automated quantification. Current work focuses on protoplast regeneration and the establishment of transformation methods to identify and manipulate candidate genes regulating gongylidium differentiation and nutrient exchange. Together, these advances provide the first experimental framework to integrate fungal cell biology, genetics, and symbiotic coevolution in this obligately farmed and domesticated fungal crop.

Keywords:
Leucoagaricus gongylophorus, protoplasts, symbiosis, cell differentiation, ROS compartmentalization, regeneration, fungal biotechnology

359C

Unisexual reproduction in the global human fungal pathogen *Cryptococcus neoformans*

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The human fungal pathogen *Cryptococcus* species complex (encompassing *C. neoformans*, *C. deneoformans*, and the *C. gattii* species complexes) exhibit diversity in sexual reproduction, including a-**a** mating, pseudosexual reproduction, as well as unisexual reproduction initiated from a single isolate or between isolates of the same mating type. A central conundrum is that while most *Cryptococcus* natural populations exhibit significant a mating-type bias, genetic and genomic analyses show recombination occurs in nature. The discovery of unisexual reproduction in *C. deneoformans* provided insight; however, thus far unisexual reproduction has never been directly observed in the predominant global pathogenic species *C. neoformans*. Here, we provide evidence that mutating the *RIC8* gene, which encodes a conserved guanine nucleotide exchange factor (GEF) involved in both chaperoning and activating Ga proteins, enables unisexual reproduction in *C. neoformans*. Additionally, we show that genetic variation in the natural population promotes unisexual reproduction, and unisexual reproduction in *C. neoformans* involves canonical meiotic recombination. Finally, we found that deletion of both *GPA2* and *GPA3* in the *MAT*a background leads to self-filamentation without sporulation, suggesting that differential modulation of the Ga proteins, likely involving Ric8, could underlie the switch between different modes of sexual reproduction in *Cryptococcus*. Our study further highlights that the highly conserved Ric8 GEF can act as an important regulator of cellular development in response to environmental stimuli and could modulate sexual reproduction in nature. We hypothesize that unisexual reproduction occurs much more frequently in nature than currently appreciated, and possibly in other fungi and microbial eukaryotes as well.

360C

Investigating the role of the Nuclear Dbf2-related (NDR) kinase Cot1 in polarized growth and infection-related development in the blast fungus *Magnaporthe oryzae*

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The rice blast fungus *Magnaporthe oryzae* is a major threat to global cereal production, infecting rice, wheat, and other grass species. Plant infection depends on the formation of the appressorium, a specialized pressure-generating cell that breaches the host cuticle and initiates biotrophic infection. Despite its central role in pathogenicity, the molecular mechanisms regulating appressorium morphogenesis remain incompletely understood. To uncover key regulators, we performed a fluorescence microscopy–based genetic screen for mutants defective in cytoskeleton-mediated appressorium repolarization. Here, we have identified a mutant, M41, exhibiting severely compromised polarized growth and malformed appressoria with abnormal subcellular architecture. Whole-genome sequencing revealed a G402A missense point mutation in *COT1*, encoding a conserved Nuclear Dbf2-related (NDR) kinase within the AGC kinase family which are known to broadly regulate cell growth and proliferation. Complementation of M41 with the wild-type *COT1* allele fully rescued all mutant phenotypes. Live-cell imaging demonstrated that Cot1 undergoes bi-directional trafficking along microtubules in a Hook1-dependent manner, consistent with localization to motile early endosomes, which likely act as spatial hubs for morphogenetic signaling. Additionally, we have found that Cot1 is critical for the colonization of host tissue likely by its role in the polarized growth of invasive hyphae. Taken together, our data highlights a role for Cot1-mediated regulation of appressorium morphogenesis and plant infection.

361C

Identification of *Aspergillus fumigatus* Septation Effectors through Proximity-labeling with TurboID

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Aspergillus fumigatus is a leading cause of invasive fungal infections, and novel therapeutic strategies are urgently needed. Previous work in our laboratory suggests that septation inhibition may be one such strategy, as loss of septation generates avirulence characterized by the loss of tissue-invasive hyphal growth. We have shown that the terminal Septation Initiation Network (SIN) signaling complex, composed of the kinase SidB and its activator MobA, is essential for proper septation, virulence, and echinocandin resistance. However, the downstream effectors of this complex remain largely unknown. As proteins that interact with the SIN are likely important effectors for septation and virulence, we employed proximity-based labeling of the SidB/MobA kinase module using TurboID for the first time in *A. fumigatus*. TurboID can identify transient protein-protein interactions exhibited by kinases such as SidB, making it a putatively useful tool to study this pathway. LC-MS/MS analyses of streptavidin-precipitated triplicate lysates indicated that SidB-TurboID and MobA-TurboID strains shared significantly overlapping interaction profiles, as expected based on our previous genetic analysis revealing that MobA is essential for SidB's role in septation. Further validating the dataset, both SidB and MobA were among the significantly enriched streptavidin-precipitated proteins commonly identified in both tagged strains. After prioritization of the remaining enriched proteins, 18 candidate proteins were selected for further characterization based on predicted roles in cell wall synthesis or cytoskeletal dynamics. Genes encoding candidate proteins were deleted and initially scored for vegetative growth defects, septation and cell wall stress susceptibility. Most notably, our analysis identified multiple putative components of the STRIPAK complex which has been shown to downregulate SIN signaling in other fungi. Deletion of the gene encoding one component, *pabA*, resulted in hyperseptation and hyperbranching consistent with loss of negative SIN regulation. Among the prioritized genes was also the *A. fumigatus* ortholog of the IQGAP, *sepG*, which functions in scaffolding at the contractile actin ring during cytokinesis in *A. nidulans*. Repression of *sepG* resulted in loss of septation. This work demonstrates successful adaptation of the TurboID technology to *A. fumigatus*, which may be used in the future to delineate other molecular pathways involved in pathogenesis.

362C

Polarized cell wall growth during exit tube assembly in the chytrid *Batrachochytrium dendrobatidis*

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Polarized growth is fundamental for fungal physiology and symbiosis. Unlike animal cells and amoebae that mediate polarized growth using an actin cortex to directly control cell shape, polarized growth in yeast, filamentous fungi, and other Dikarya is defined by spatially restricted cell wall remodeling mediated by directed exocytosis and endocytosis. A key transition in fungal evolution, therefore, was the shift from using the cytoskeleton to control cell shape by directly pushing on the plasma membrane to using the cytoskeleton to control cell shape by guiding vesicle traffic to mediate polarized cell wall growth. Understanding when and where this transition occurred is important for understanding the evolution of fungal morphogenesis. To this end we study chytrid fungi, which diverged before the radiation of dikaryotic fungi. Chytrids develop from a motile cell type that lacks a cell wall (the "zoospore") that controls its cell shape using an actin cortex to a sessile cell type that controls its shape using a cell wall (the "sporangium"). During the sporangial phase of their lifecycle, chytrid fungi build several polarized structures. For example, the frog-killing chytrid *Batrachochytrium dendrobatidis* (*Bd)*, like many chytrids, builds one or more exit tubes that discharge the next generation of zoospores from the sporangium into the environment. To determine how *Bd* regulates polarized cell wall growth during discharge apparatus assembly, we developed an assay to induce synchronous assembly of exit tubes. Using this assay, upwards of 70% percent of cells initiate exit tube assembly within a three-hour window. These exit tubes appear similar in diameter to daughter zoospores, suggesting that sporangia may actively control tube widths. We also stained cells with phalloidin to highlight actin polymers and observed higher density of peripheral actin patches near sites of active exit tube development, consistent with vesicular trafficking of cell wall synthases, similar to polarized cell wall assembly in Dikarya. Currently, we are using small molecule inhibitors to test this hypothesis and probe other mechanisms driving exit tube development in *Bd* to explore the deep evolution of fungal morphogenesis.

363C

The RNA virome of *Saccharomyces cerevisiae*

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Sequencing studies have revealed a remarkable diversity of RNA viruses that infect many fungal species. These viruses, known as mycoviruses, are well known to persist as endemic infections transmitted through cell division or fusion with no known extracellular route. Although generally thought to be asymptomatic, mycoviruses can profoundly affect their fungal hosts, suggesting powerful utility as novel biocontrol agents. By mining RNA-seq datasets for >1000 global isolates of *Saccharomyces cerevisiae*, we have generated a comprehensive RNA virome for the species. We recovered hundreds of viral genomes corresponding to 13 previously-known and 14 novel RNA virus species, with more than half of all yeast isolates being virus-positive. Through this analysis, we identified Osiris, a novel virus belonging to *Narnaviridae*: a family of eukaryotic viruses known to solely encode viral RNA-dependent RNA polymerase on a single +ssRNA genome. Interestingly, Osiris has a second segment encoding two uncharacterized ORFs and is homologous to recently-discovered two-segmented narnaviruses infecting apicomplexan parasites. We also identified ScMV1, a virus belonging to the *Mitoviridae* family known to infect host mitochondria. Additionally, we found several novel M-satellites: satellite viruses of the L-A totivirus which encode “killer” toxin-antitoxin systems. These novel M-satellite toxins may derive from horizontal transfer of chromosomal or M-satellite-derived toxins found in non-*S. cerevisiae* fungal species. We also identified satellite-like miniaturized variants of the L-A totivirus as well as miniaturized M-satellites which retain the preprotoxin N-terminus known to encode the toxin secretion and antitoxin functions. This demonstrates that genome miniaturization is a convergent evolutionary phenomenon of totivirus-killer systems with potential impacts on the yeast killer phenotype. Lastly, by cross-referencing viral infection status with multi-omics datasets available for the global 1000-yeast collection, we identified virus-associated transcriptomic changes and hundreds of virus-associated host phenotypes, including changes to translational and metabolic gene expression and enhancement of chronological lifespan and sporulation capacity linked to *Partitiviridae* infection. Overall, we have doubled the number of known yeast virus species and identified a plethora of novel virus biology which we will dissect through the tractable genetics of *S. cerevisiae.*

364C

The role of beta 1,6-glucans in *Cryptococcus neoformans* cell wall organization

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Cryptococcus neoformans is a basidiomycete fungus that can infect human lungs and cause Cryptococcosis, which occurs both in animals and humans. The primary means of infection is believed to be through inhalation of basidiospores or desiccated yeast cells. A significant virulence factor for Cryptococcus is the cell wall, the organization and structure of which is crucial for infection. Research has shown that the absence of cell wall components such as beta 1,6 glucans have significant impacts on virulence. Here we investigate the cell wall architecture and composition of Cryptococcus of both wild-type and beta-1,6-glucan-deficient cells.

365C

Regulation of cell polarity in a multibudding fungus

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Cell polarity refers to the asymmetric distribution of cellular components. Establishing asymmetry is central to a wide range of processes in fungi, including budding, hyphal growth, and morphogenesis (e.g., shmoo formation, conidiogenesis). Polarity establishment has been most intensively studied in single-budding yeast, in which the master regulator Cdc42 concentrates at a single site, ensuring that each mother cell produces only one bud. However, other fungi are not limited to a single polarity site: filamentous fungi can branch to form multiple hyphae, and pathogens such as *Paracoccidioides brasiliensis* transition to a multibudding phenotype upon host invasion. How fungi integrate physiological and environmental cues to specify the correct number of polarity sites remains an open question. In the multinucleate budding yeast *Aureobasidium pullulans*, a mother cell can produce multiple buds simultaneously. Intriguingly, cells with more nuclei also tend to form more buds.

Here, we ask how *A. pullulans* “counts” its nuclei and uses that information to form different numbers of polarity sites. We find that doubling ploidy increases bud number, so that cells with fewer nuclei make too many buds. This suggests that *A. pullulans* senses DNA quantity to count nuclei. To identify the relevant “nuclear-counting” and polarity factors, we combine top-down (quantitative proteomics, genetic screening) and bottom-up (targeted deletions of polarity components) approaches. Our targeted deletion screen reveals two polarity subcircuits: a Cdc42-centric branch that favours the formation of more polarity sites and a Rac1-centric branch that favours fewer. Moreover, the Rac1 subcircuit inhibits the Cdc42 subcircuit via the p21-activated kinase (PAK) Pak1, yielding a tunable architecture for generating variable numbers of polarity sites. Together, our results provide a preliminary mechanistic framework for the regulation of multipolarity in *A. pullulans* and illustrate how evolution of the conserved polarity circuit can generate striking morphological diversity.

366C

Architecture of Fungal Cell Wall and Remodeling by Antifungals: New Insight from Solid-State NMR Spectroscopy

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Invasive fungal infections have emerged as a major threat to human health, with high mortality rate persisting even after treatment. To combat rising drug resistance, substantial efforts have been directed toward developing novel antifungal agents that specifically target the unique polysaccharides present in fungal cell walls. Despite five decades of molecular and biochemical research, our understanding of many structural aspects of the fungal cell wall remains limited due to the challenges of analyzing the structure and interactions of polysaccharides, such as chitin, glucans, and mannan, within a cellular context. Here, we discuss the recent advances achieved through cellular solid-state Nuclear Magnetic Resonance (NMR) that sheds light on the structural dynamics of cell wall polysaccharides in pathogenic fungal species. This approach allows for the use of living fungal cells while obtaining atomic-level information on polysaccharides within the cell wall. First, the integration of NMR-informed dynamic characteristics of carbohydrate polymers with data on linkage, alkali solubility, and localization from conventional chemical and imaging assays, assisted by functional genomics, have provided an updated perspective on the molecular organization of carbohydrate polymers in the human pathogen *Aspergillus fumigatus* [1]. Second, we identified the structural mechanisms utilized by fungi, including *A. fumigatus* [2], *Mucorales* species [3], as well as *Candida albicans* and *Candida auris* [4] to use polysaccharides for remodeling their cell walls in response to treatment with glucan and chitin inhibitors such as echinocandins and nikkomycin. Third, we have identified five distinct types of α-1,3-glucan structures, each playing diverse roles in *Cryptococcus neoformans* [5]. These structures contribute to both the rigid and mobile domains of the cell wall, facilitate capsule attachment to the cell surface, and support melanin deposition within the inner wall domain. Together, these studies provide an in-depth understanding of fungal cell-wall architecture and the functions of its diverse polysaccharides, as well as the adaptive strategies that fungi employ to cope with stress and endure harsh environmental conditions and antifungal treatments.

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367C

A Bipartite Complex Coordinates Glucan Synthesis and Remodeling to Build the Fungal Cell Wall

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The fungal cell wall is an essential extracellular matrix that underpins growth, morphogenesis, and pathogenesis, and its assembly requires the coordinated action of poorly understood enzyme networks. In *Schizosaccharomyces pombe*, we find that Ghs2, a protein containing a glycoside hydrolase 16 (GH16) domain, functions in strict partnership with the β-1,3-glucan synthase Bgs3. Ghs2 and Bgs3 co-localize to sites of polarized growth and are mutually dependent for localization. They also physically associate in cells and structural modeling predicts a Ghs2-Bgs3 complex in which the Ghs2 catalytic domain is positioned proximal to the Bgs3 glucan extrusion pore. Using solid-state NMR we found that Ghs2 and Bgs3 are required for the β-1,6-glucan content of the cell wall. Thus, we propose a model in which Ghs2 acts directly on the nascent β-1,3-glucan chains produced by Bgs3 to introduce β-1,6-linked branches, identifying Ghs2 as the long-sought factor required for β-1,6-glucan synthesis in *S. pombe*. With the Ghs2-Bgs3 complex representing the first example of a glucan synthase physically coupled to a remodeling enzyme to generate branched glucan networks, these findings establish a new principle of fungal cell wall assembly in which synthase-modifier modules function as inseparable units and highlight an enzymatic activity essential for growth.

368C

Stress-driven emergence of heritable non-genetic drug resistance

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Drug resistance is the chief cause of treatment failure for therapies targeting chronic and infectious diseases. Whether the emergence of resistance is accelerated by environmental exposure to low levels of therapeutics remains controversial. Here, we report a non-genetic mechanism of stress adaptation that promotes heritable resistance to fluconazole and other widely used antifungal drugs. In the human pathogen *Candida albicans,* transient exposure to subtherapeutic fluconazole doses induces a protective response that we term para-resistance. Like conventional resistance mechanisms, para-resistance is heritable. However, it does not arise from genetic mutations and can revert spontaneously. Key regulators of this state and its associated multidrug resistance include the stress-activated MAP kinase Hog1, the histone deacetylase scaffold Snt1, the global chromatin regulator Rap1, the Sko1 transcription factor, and the Yck2 kinase. Molecules that disrupt biomolecular condensation and prion propagation block the induction of para-resistance, and its acquisition is associated with conformational shifts in these regulatory factors. Para-resistance is common in clinical isolates and, remarkably, passage through the mammalian gut triggers its acquisition, compromising fluconazole's therapeutic efficacy. Our work defines a pervasive, prion-like epigenetic mechanism of stress adaptation and highlights potential strategies to mitigate the rapid emergence of drug resistance.

369C

Heterologous expression of *Bipolaris maydis* hydrophobins in *Aspergillus nidulans* and characterization of major hydrophobins in *Aspergillus oryzae*

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For filamentous fungi, the surface hydrophobicity of hyphae and conidia is an important factor contributing to the formation of aerial hyphae and the dispersal of conidia. In plant pathogenic fungi, it is also critical for adhesion to and colonization of host plants. In general, hydrophobins—low-molecular-weight amphiphilic secreted proteins—are known to play major roles in cell-surface hydrophobicity in filamentous fungi. In our previous work, we analyzed single and multiple gene-disruption mutants of the four hydrophobin genes present in the genome of the southern corn leaf blight fungus *Bipolaris maydis*. We reported that disruption of these hydrophobin genes did not cause any observable phenotypic changes, including in vegetative growth, stress sensitivities, or pathogenicity, nor did it affect colony hydrophobicity (16th ECFG). In this study, we examined the functions of *B. maydis* hydrophobins by heterologous expression in *Aspergillus nidulans*. When Hyp1—a Class I hydrophobin generally known to strongly contribute to colony hydrophobicity—was introduced into a *dewA* deletion mutant (Δ*dewA*) of *A. nidulans*, the wettable hyphal phenotype of Δ*dewA* was restored. Surprisingly, heterologous expression of Hyp2–4, which are classified as Class II hydrophobins and are generally considered to contribute only weakly to colony hydrophobicity, also restored the wettable phenotype of Δ*dewA* to varying degrees. These findings suggest that hydrophobins of *B. maydis* retain their intrinsic functions but nevertheless do not contribute to colony hydrophobicity in *B. maydis* itself. Furthermore, functional analysis of hydrophobins in *Aspergillus oryzae* revealed that the major hydrophobins are RolA and HypB: RolA functions mainly on the conidial surface, whereas HypB functions predominantly on the surface of aerial hyphae. Collectively, these results indicate that hydrophobins in filamentous fungi exhibit highly specialized and diversified roles depending on fungal species and cellular structures.

370C

Cell cycle control in the unconventional multi-budding yeast *Aureobasidium pullulans*

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Aureobasidium pullulans (*A. pullulans)* is a ubiquitous and generalist black, yeast-like fungus that thrives in diverse environments. *A. pullulans* is polymorphic, with multi-budding yeast, hyphal, or meristematic growth modes and reversible transitions between them. *A. pullulans* yeast cells display very heterogeneous cell sizes, shapes, and numbers of nuclei. Multinucleate cells can make multiple buds within a single cell cycle, and each bud can inherit one or more nuclei. Studies in the model yeast, *Saccharomyces cerevisiae*, have shown that cell cycle regulators trigger morphogenetic transitions (i.e. budding, cytokinesis), and that cell cycle progression is in turn regulated by cell size and shape (i.e. the presence or absence of a bud). I am interested in whether similar cell cycle-morphogenesis connections are present in *A. pullulans*, and if so, how they operate in an organism whose cells can differ by almost two orders of magnitude in cell size and number of buds. To answer these questions, I monitored cell cycle progression in *A. pullulans* using fluorescent PCNA and histone probes. *A. pullulans* yeast and hyphal cells go through the canonical G1-S-G2-M cell cycle phases, but with quite variable timing. Unlike in *S. cerevisiae*, the G1/S transition occurs very soon after birth, independent of cell size. In addition, bud emergence occurs in the late S or G2 phase, and in a subset of cells, it does not occur at all. Despite the absence of a bud, mitosis proceeds with normal kinetics in these cells. Thus, it appears that there is no size control governing G1/S and no morphogenesis checkpoint governing G2/M in this budding yeast, even though homologs of size control and checkpoint genes (e.g. WHI5, SWE1, MIH1) are conserved. Deletion of conserved cell cycle regulators is underway to understand how the cell cycle machinery has been rewired in this unconventional yeast.

371C

Acyl-coA binding protein AcbdA regulates peroxisome movement via hitchhiking on early endosomes in Aspergillus nidulans

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Motor-driven transport on microtubules is critical for distributing organelles throughout the cell. Most commonly, organelle movement is mediated by cargo adaptors, proteins on the surface of an organelle that directly recruit microtubule-based motors. An alternative mechanism called hitchhiking was recently discovered: some organelles move, not by recruiting the motors directly, but instead by using membrane contact sites (MCS) to attach to motor-driven vesicles and hitchhike along microtubules. Organelle hitchhiking is observed across fungi and animals. In filamentous fungi, nearly all peroxisomes move by hitchhiking on early endosomes (EE). In the fungus Aspergillus nidulans, EE-associated linker proteins PxdA and DipA are critical for establishing EE-peroxisome MCS required for peroxisome movement. Whether peroxisome-membrane proteins exist that regulate peroxisome hitchhiking on EEs is not known. Through a forward mutagenesis screen, we discovered an acyl-CoA binding (ACB) domain-containing protein AcbdA/AN1062 that localizes to peroxisomes via its tail-anchored transmembrane domain (TMD). Deleting the AcbdA gene or only its N-terminal ACB domain perturbs the movement and distribution of peroxisomes. Importantly, AcbdA is not required for the movement of EEs or for the recruitment of PxdA and DipA on EEs. Fatty acid (FA)–induced increases in peroxisome movement require AcbdA, suggesting that peroxisome hitchhiking on EEs is coupled to FA metabolism. Mutating a conserved FFAT motif, predicted to interact with the endoplasmic reticulum (ER), has no effect on peroxisome movement. Taken together, our data indicate that AcbdA is a peroxisome-membrane protein required for peroxisome hitchhiking on EEs. AcbdA's involvement in peroxisome hitchhiking represents a divergence from known functions of Acbd4/5 proteins and adds layers to our understanding of the functionality of the Acbd4/5 family of proteins.

372C

Intra-conidial Development Stages Together with Sir5-Mediated Metabolic Checkpoint Shape UV Response Outcomes

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In many fungal pathogens, asexual spores, known as conidia, are key propagules for dispersal. Traditionally considered as dormant cell cycle-arrested cells that germinate only upon exposure to favorable cues, conidia are now recognized to activate multiple transcriptional programs in response to environmental cues. How developmental heterogeneity among conidia influences stress adaptation remains poorly understood. Here, we characterized two physiologically distinct types of microconidia of the fungal pathogen *Fusarium* oxysporum obtained from cultures in liquid or in solid media. While most cells in both microconidial populations were arrested in G1, conidia obtained from liquid culture were larger and contained a higher proportion of G2 cells than those from solid media suggesting two distinct developmental arrest points what seems like pre-START in the former and G1/S in the latter. Transcriptomic analysis of conidia derived from solid potato dextrose agar (PDA) medium revealed upregulation of ribosome biogenesis genes, whereas conidia obtained from liquid KNO_3 medium exhibited upregulation of cell cycle-related genes, supporting a model of developmental stage-specific arrest. Importantly, liquid-derived conidia exhibited higher survival, reduced mutagenesis, and more efficient DNA repair following UV irradiation than solid-derived conidia. Transcript levels of *FoSir5*, a decrotonylase and ortholog of human SIRT5 known to negatively modulate conidial germination, differed between the two conidial populations. Although *FoSir5* deletion failed to abolish the differences observed between the two types of conidial populations, it resulted in increased UV resistance, suggesting a role of this protein in stress modulation. Together, our results suggest that promoting what appears to be a pre- to post-START like transition, and downregulating FoSir5 activity, increases resilience of fungal conidia against environmental stress.

373A

The conserved C-terminus of the non-receptor GEF RIC8 is important for GDP-GTP exchange on Gα subunits *in vitro* and regulation of growth and development *in vivo*

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RIC8 is a non-receptor Guanine Nucleotide Exchange Factor (GEF) for heterotrimeric Gα proteins that is found in certain fungi, protists and animals. We have previously demonstrated that RIC8 is required for growth; asexual and sexual development; intracellular levels of several heterotrimeric G protein subunits, and *in vitro* GEF activity towards the Gα proteins GNA-1 and GNA-3 in *Neurospora crassa*. We also showed that RIC8 impacts levels of adenylyl cyclase and cAMP signaling in *N. crassa*. Here, we explore the consequences of deleting 54 or 136 amino acids from the highly conserved C-terminus of the RIC8 protein. *in vitro* measurements using RIC8 and Gα proteins isolated after heterologous expression in *E. coli* showed that the two truncations had detectable GEF activity, but that it was greatly reduced relative to wild type RIC8. The largest reduction was observed in reactions containing GNA-1, suggesting a greater dependence on the C-terminus for activity towards GNA-1. Introduction of the two *ric8* truncation alleles into *N. crassa* resulted in some rescue of Δ*ric8* mutant phenotypes, including faster hyphal growth rate and longer aerial hyphae. The two truncation mutants were female-sterile, similar to Δ*ric8* and Δ*gna-1* mutants, but had pigmentation like that of Δ*gna-3* strains during growth on synthetic crossing medium. Taken together, our results support the importance of the conserved C-terminus for RIC8 functions in *N. crassa*.

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Comprehensive Role of the MylA in Fungal Development, Stress Response, and Toxigenicity in *Aspergillus flavus*

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Aspergillus flavus is a ubiquitous saprophytic fungus that contaminates seed crops and produces aflatoxin, a group 1 carcinogen. *A. flavus* primarily reproduces through asexual reproduction, producing conidia, and the process of conidia formation (conidiation) is governed by numerous transcription factors (TFs). Nearly 80 TF families have been identified in the fungal genome, and among them, myeloblastosis (MYB)-like proteins constitute a TF family that is known to play pivotal roles in the biological processes of fungi. In this study, we characterized the function of Myb-like protein A (MylA) in *A. flavus*. Disruption of *mylA* resulted in reduced conidiation and impaired colony growth compared to the control. Moreover, the Δ*mylA* conidia exhibited defects in trehalose biosynthesis, resulting in decreased spore viability and thermal stress tolerance, as trehalose serves as a key protective molecule in fungi. The Δ*mylA* conidia also showed a delayed germination rate and reduced germ tube elongation compared with the control. As *A. flavus* is a plant pathogenic fungus, infected crops generate substantial oxidative stress as a defense mechanism against *A. flavus* infection. To elucidate the role of MylA in oxidative stress tolerance, we exposed each strain to various oxidative stress reagents. The results showed that the Δ*mylA* strain was more sensitive to oxidative stress, suggesting a role for MylA in oxidative stress resistance. To assess its role in pathogenicity and toxigenicity, a kernel bioassay was conducted over two different models, corns and soybeans. The Δ*mylA* strain exhibited reduced conidial colonization, decreased conidial viability, and aflatoxin B1 production when infected in both host crops. Collectively, our findings demonstrate that MylA is a critical regulator of fungal development, stress responses, pathogenicity, and toxigenicity in *A. flavus*.

375A

The GTP exchange factor Ric8 controls alternative morphogen etic fates in pathogenic *Cryptococcus*

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Pathogenic *Cryptococcus* species, encompassing *C. neoformans*, *C. deneoformans*, and the *C. gattii* species complexes, are major human fungal pathogens that cause severe lung infections and life-threatening meningoencephalitis. While **a**-a sexual reproduction has been characterized in species

belonging to all three complexes, unisexual reproduction may differ in how it is regulated. In *C. neoformans* and *deneoformans*, the conserved G-protein regulator Ric8 acts as a repressor that restricts yeast cells from diverse cellular developmental fates, including unisexual reproduction. In this study, we sought to determine how Ric8 might influence unisexual reproduction in species of the *C. gattii* species complex. To this end, we generated *ric8* deletion mutants in all six *C. gattii* species – *C. gattii* (VGI), *C. deuterogattii* (VGII), *C. bacillisporus* (VGIII), *C. tetragattii* (VGIV), *C. hyracis* (VGV), and *C. decagattii* (VGVI) – and in both *MAT*a and *MAT***a** backgrounds. While we did not observe unisexual development in any of the mutant strains, deletion of the *RIC8* gene led to diverse non-yeast-form cellular development in subsets of these *C. gattii* species, including highly branched hyphae that produce blastospore clusters, as well as pseudohyphae lacking true septa. This is consistent with the studies in *C. neoformans* and *C. deneoformans* showing that Ric8 suppresses of non-yeast cell development. Whole-genome sequencing identified additional mutations in these *ric8*D mutants that are likely contributing to the lineage-specific divergence in morphology and promoting genetic diversification and adaptation. To define how these phenotypes relate to unisexual development, we further examined the expression of regulators in the RAM pathway, Ric8-associated signaling, and mating. These analyses indicate that Ric8 interfaces with developmental networks, and that pseudohyphal growth constitutes a developmentally distinct state rather than an intermediate stage of unisexual reproduction. Together, our findings reveal how Ric8 governs unisexual signaling with an alternative pseudohyphal developmental fate in *C. gattii*, establishing how this phenotype fits within the broader developmental network of the species complex. This work uncovers an unexpected developmental strategy that may promote genetic diversification, influence adaptation, and diversify pathogenic potential across the *C. gattii* species complex.

376A
Transcriptional regulation during vegetative reproduction in the Mucoral fungus *Phycomyces blakesleeanus*

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Phycomyces blakesleeanus is a member of the Mucoromycota that grows vegetatively as a mycelium with branched, non-septate hyphae. During asexual reproduction, vegetative hyphae develop fruiting bodies, called sporangiophores, that are long aerial and unbranched hyphae with an apical sporangium filled with spores. Its development is regulated by environmental signals such as light, temperature, or the concentration of nitrogen or CO_2. *P. blakesleeanus* uses light as an environmental signal to activate the biosynthesis of the pigment beta-carotene, for the development of sporangiophores and to guide sporangiophore growth (phototropism). The main photoreceptor is the Mad complex, a light-sensor and transcription factor complex composed of the zinc-finger proteins MadA and MadB, homologs of WC-1 and WC-2 of *N. crassa,* respectively. One of the primary roles of the Mad complex is the regulation of transcription upon light reception. While the transcriptional response to light in vegetative mycelia has been characterized, little is known about how light influences gene expression during sporangiophore development.

We have observed that the transcriptional response to light in vegetative mycelia is different to that of sporangiophores, in particular regarding the number of repressed genes (189 genes repressed in mycelia and 23 genes repressed in sporangiophores, FC>2). However, the transcriptional changes during sporangiophore development are far more extensive than those induced by light (516 regulated by light in mycelia compared to 4,321 regulated during development), and indication of the relevance of this developmental transition in the life cycle of *P. blakesleeanus*. During the development of sporangiophores, *P. blakesleeanus* activates preferentially genes for protein synthesis, while it reduces the expression of metabolic genes. We have identified 214 genes that are induced both in response to light and during sporangiophore development, which reveals an interplay of regulatory networks in this fungus. In addition, we have observed that the Mad complex participates in transcriptional regulation in the dark in mycelia and sporangiophores supporting an additional role for this photoreceptor complex.

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377A
Conservation of the regulatory pathway for conidiophore development across species of the *Aspergillus* genus

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Ascomycete fungi reproduce asexually by conidiation, producing clonal spores. The morphogenetic processes leading to the production of a conidiophore, the structure bearing the asexual spores or conidia, are well defined in the model fungus *Aspergillus nidulans*. The central regulatory pathway (CRP) consists of three proteins: BrlA, which is required for vesicle formation, AbaA, which is required for sterigmata formation, and WetA, which is required for conidial maturation. This CRP includes self- and cross-regulatory loops and is also regulated by additional proteins to ensure conidiation only happens when proper signals are present in the environment.

In this work, we examined the conservation of the CRP proteins as well as associated conidiation regulators in nearly 300 species of the genus *Aspergillus* by first characterizing the corresponding orthologs across the genus. Then, the promoter regions of these orthologs were inspected, finding a variable degree of conservation in the self- and cross-regulatory loops described in *A. nidulans*. These orthologs were further analyzed for variability in their protein sequences, unraveling several group determinant residues across BrlA and AbaA, some of which mapped to or nearby to their DNA binding regions. A complementary evolutionary model based on selected conidiophore characteristics described in the *Aspergillus* genus suggests an evolution through specialization with similarities to what was described originally by Darwin's finches. Together, these results suggest that different versions of BrlA and AbaA might participate in the determination of some of the conidiophore morphologies – such as sterigmata arising in one or two series –, particularly in species belonging to subgenus Fumigati or Nidulantes, as these groups represent highly specialized proteins, as shown by our results.

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378A

Autophagy in the filamentous fungus *Sordaria macrospora*: The roles of SmATG11 and SmATG17 in selective and non-selective degradation

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Selective autophagy is a conserved subcellular process that uses receptors to target specific cargos for degradation in the vacuole. In the filamentous ascomycete *Sordaria macrospora,* neighbor of BRCA1 (SmNBR1) was identified as a receptor for the selective autophagy of peroxisomes (pexophagy). However, little is known about the interactions of SmNBR1 with other proteins. Thus, we aimed to identify SmNBR1-interactors by employing the Biotin Identification (BioID) method. In this method, SmNBR1 is fused to a promiscuous biotin-ligase, which labels neighboring proteins with biotin. Biotin-labelled proteins are then enriched by affinity purification and identified by liquid chromatography coupled to mass spectrometry (LC-MS). In these BioID experiments, we identified several proteins to be putative SmNBR1 interactors, among them SmATG11, a homolog of the yeast autophagy protein Atg11. Since ATG11 homologs in diverse organisms are core scaffold proteins crucial for various selective autophagy processes, SmATG11 is a reasonable SmNBR1 interactor. It was initially believed that yeast Atg11 is solely crucial for initiation of selective autophagy, whereas the equivalent role in non-selective bulk autophagy is played by Atg17. However, emerging studies suggest a less rigid functional separation. Moreover, ATG11 homologs found in *Homo sapiens* and *Arabidopsis thaliana* handle both selective and bulk autophagy, while ATG17 homologs are absent. Interestingly, *Smatg11* deletion leads to a severe impairment in fruiting body development. This behavior strongly resembles the phenotypes of other bulk autophagy mutants. Therefore, it reinforces the theory that SmATG11 – like its homologs in other eukaryotes – also plays an important role in bulk autophagy. Consequently, besides studying SmATG11, we are also interested in the function of SmATG17. We discovered that *S. macrospora* encodes two variants of *Smatg17*, which we termed *Smatg17a* and *Smatg17b*. According to our bioinformatical analysis, the *Smatg17a* locus is highly conserved and encodes for a full-length protein, while the *Smatg17b* locus is less conserved and only produces a non-functional transcript. Therefore, we assume *Smatg17b* to be a pseudo-gene and are currently working on the functional analysis of *Smatg11* and *Smatg17a*.

379A

From dormancy to disease: a life cycle proteome atlas of *Cryptococcus neoformans*

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Cryptococcus neoformans spores are the infectious propagules that initiate disease, yet the proteins that control their germination and transition to host infection are poorly defined. To place spore biology in a full life cycle context, we used data-independent acquisition mass spectrometry to quantify protein expression in *C. neoformans* across the sexual cycle, multiple in vitro stress conditions, and mammalian infection, profiling more than 90 biological samples and about 6,200 proteins, roughly 90% of the genome-inferred proteome. A spore germination time course revealed rapid proteome reprogramming as spores exit dormancy and highlighted numerous secreted proteins and quorum sensing peptides as early responders, with deletion of several candidates compromising efficient germination. We define stage specific protein programs, including spore enriched sets that mark infectious propagules, and identify 43 proteins that are enriched in vivo, 14 of which were detected only during infection. Ongoing work is testing how these factors influence virulence. Together these data deliver the most complete fungal proteome to date and will be accessible through an interactive atlas website.

380A

Quantitative imaging of fungal life cycle transitions through generative deep learning

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The life cycle of biomedical and agriculturally relevant filamentous fungal pathogens involves complex transitions between proliferative and non-proliferative states. However, few experimental setups allow the quantitative study of living filamentous fungi through stages such as germination, mating, sporulation, and filamentous growth. Here, we provide a solution to quantify and track life cycle transitions in fungal organisms using a combination of computer vision algorithms and microfluidics. We used high aspect ratio microfluidic devices to induce transitions from dormant into proliferative states in representative ascomycetes and basidiomycetes. To detect and track specific fungal structures or life cycle stages, we created a deep learning fungal tracking algorithm based on generative image synthesis and life cycle stage-specific segmentation of spores, hyphal tips, branching points, mating events, septa, and fungal biomass on phase contrast images. Using this experimental framework, we quantitatively imaged life cycle transitions in the pathogens Verticillium dahliae, Colletotrichum acutatum, Ustilago maydis, and Neopestalotiopsis spp, and the ectomycorrhizal fungus Hebeloma cylindrosporum, including spore germination, filamentous growth with branching and septation, and alternative life cycle paths, such as microcyclic conidiation. We found that germination follows species-specific kinetics that change in response to environmental conditions, whereas apical tip growth accelerated over time during proliferation, with changes in tip orientation being inversely correlated to the speed of hyphal growth. The quantitative characterization of individual life cycle stages allowed us to track complete asexual life cycles using cyclic nutritional stress. Our experimental framework could be applied to quantitatively investigate other fungal life cycle landmarks such as anastomosis, minichromosome maintenance, microcyclic conidiation, mating, and sporulation. Our code will be available as a free pip-installable user interface “GenFungAI.”

381A

Phosphoproteomic analysis of the Target of Rapamycin (TOR) signalling pathway in the rice blast fungus *Magnaporthe oryzae*

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Eukaryotic growth and development are regulated by the Target of Rapamycin (TOR) signalling pathway, which integrates nutritional cues with anabolic and catabolic processes via the TORC1 and TORC2 complexes. In filamentous plant pathogens, such as the rice blast fungus *Magnaporthe oryzae*, the contribution of TOR signalling to infection-related development remains poorly understood. While treatment of *M. oryzae* conidia with TOR activators inhibits appressorium initiation, TOR inhibitors induce constitutive autophagy and suppresses pathogenicity. Using phosphorylation of the ribosomal protein Rps6 as a biochemical readout, TORC1 activity increased between 1 and 8 hpi during appressorium development. Acute inhibition of TOR signalling with Torin1 abolished Rps6 phosphorylation but led only to a marked delay, rather than complete arrest, of appressorium development. This disconnect between loss of TORC1 activity and continued, albeit slower, development prompted us to map the downstream phosphorylation events that might buffer or relay TOR signals.

We therefore profiled the phosphoproteome of conidia undergoing early appressorium development on a hydrophobic surface in the presence of Torin1 or DMSO. Phosphopeptides enriched through sequential TiO_2 and Fe-NTA purification were analysed by data-dependent LC-MS/MS generating a high-confidence catalogue of 2,973 phosphosites across 1,098 proteins. Among these 698 phosphosites across 439 proteins were significantly regulated by TOR inhibition. Clustering of dynamic phosphosites, coupled with gene ontology overrepresentation analysis, identified discrete modules enriched for lipid-binding factors, regulators of the actin cytoskeleton, and proteins controlling cell-cycle progression. Comparative analyses further identified phosphosites co-regulated with the Pmk1 MAPK pathway as well as sites restricted to appressorium-forming fungi, highlighting putative lineage-specific innovations in TOR signalling.

Together, these data identify candidate signalling nodes through which TOR may coordinate appressorium morphogenesis, including phosphoproteins that were also recovered in TORC1 and TORC2 immunoprecipitates, and help explain why perturbing TOR signalling slows, but does not abolish, infection-related development.

382B

Identification and Characterization of Pathogenicity-Related Transcription Factor in *Aspergillus flavus*

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Aspergillus flavus is a major pathogenic fungus of seed crops such as peanuts and maize. It is the major aflatoxin producer, which is one of the most carcinogenic mycotoxins. Upon contaminating host crops via conidia, *A. flavus* undergoes hyphal growth, invasion, and aflatoxin production. These processes are tightly regulated by numerous transcription factors (TFs) that orchestrate gene expression during fungal development and pathogenicity. To identify pathogenicity-related TFs, we conducted transcriptomic analyses using multiple infection models, including maize and soybean. Based on the results, we selected ten candidate TFs, and further phenotypic analyses were carried out. We found one Zn2Cys6 transcription factor that was hyphae-specific and highly expressed at early stages of infection, which we named *hptA* (**h**yphae-specific **p**athogenicity-related **t**ranscription factor A). The *hptA* deletion mutant was generated, and disruption of *hptA* resulted in impaired fungal development. Also, the Δ*hptA* strain exhibited a significant defect in aflatoxin production compared to the control. Lastly, a kernel bioassay was carried out against maize and soybean. The Δ*hptA* strain showed reduced conidial colonization and aflatoxin production in both infection models. Taken together, these results indicate that *hptA* is a hyphae-specific pathogenicity-related transcription factor that is required for proper fungal development, pathogenicity, and toxigenicity in *A. flavus*.

383B

The sole putative leucine-rich repeat receptor Lrr1 in *Cryptococcus neoformans* facilitates conjugation during mating

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The human pathogen *Cryptococcus neoformans* is a polymorphic fungus whose cell morphology is fundamentally linked to its mode of reproduction. The fungus has a yeast morphology during asexual reproduction. However, during bisexual reproduction between cells of **a** and α mating types, a mating projection protrudes from one cell toward the other in preparation for cell-cell conjugation. After conjugation, the resulting zygote transitions from yeast to hyphal morphology. Following hyphal extension, a fruiting body forms at the hyphal tip bearing meiotic spore chains. The pheromone signaling that affects non-self recognition and leads to zygote formation is conserved and well understood, but the cell machinery that facilitates conjugation is not. We recently found that the sole putative leucine-rich repeat (LRR) receptor with a transmembrane domain, which we've named Lrr1, plays a key role in conjugation in this fungus. During asexual reproduction, *LRR1* is not expressed. However, in the early stages of sexual reproduction, both the mating transcription factor Mat2 and the hyphal morphogenesis regulator Znf2 drive *LRR1* expression, with the resulting Lrr1 protein localized to the tip of the mating projection. Following cell-cell conjugation, Lrr1 is lysosomally degraded and no longer expressed, indicating a role highly specific in conjugation during the cryptococcal life cycle. Lrr1 interacts with the conserved actin modulator Sla2 which facilitates Lrr1 localization through interaction with the Lrr1 cytosolic domain. Lrr1 likely homodimerizes through its extracellular leucine-rich repeat domain based on computational structural analysis, suggesting that Lrr1 proteins embedded in opposing membranes may bind to each other achieve membrane docking and/or fusion. This is a remarkable finding from an evolutionary perspective. While higher eukaryotes like plants and animals maintain large families of LRR-containing proteins, frequently found to function as pattern-recognition receptors with diverse roles in innate immunity and development, the diversification of LRR genes largely happened post evolutionary divergence with Kingdom Fungi, particularly for membrane-spanning LRR-containing receptors. As such, this work may provide insight into the ancestral function of LRR genes in the eukaryotic domain.

384B

GATA-type regulator NsdD-mediated species-specific rewiring of gene regulatory networks in *Aspergillus*

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NsdD is a conserved GATA-type transcription factor that governs development and secondary metabolism in *Aspergillus*, yet its species-specific regulatory mechanisms remain unclear. Using network-based multiomics analyses in *Aspergillus nidulans* and *Aspergillus flavus*, we show that NsdD functions in a cell type– and species-dependent manner. Cross-complementation of *A. flavus* Δ*nsdD* with the *A. nidulans nsdD* gene partially rescued developmental and transcriptional defects, indicating evolutionary divergence of function. Genome-wide ChIP-seq identified 502 direct NsdD targets in *A. nidulans* and 674 in *A. flavus*, including key developmental and metabolic regulators. A conserved binding motif (5′-GATCT-3′) was defined as the NsdD response element. Network reconstruction revealed conserved core modules but extensive regulatory rewiring between species, contributing to differences in asexual/sexual morphology and sterigmatocystin/aflatoxin biosynthesis. These findings provide the first comparative map of NsdD-mediated gene regulatory networks in filamentous fungi and highlight how evolutionary changes enable a conserved TF to acquire species-specific roles.

385B

Analysis of the effects of *wc-2* disruption using CRISPR/Cas9 on fruiting body formation in *Pleurotus ostreatus*

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Light, particularly blue light, is a key environmental factor that induces fruiting in certain agaricomycetes. In this study, we characterised mutant strains of *Pleurotus ostreatus* with disrupted *wc-2,* which encodes one of the white-collar proteins, Wc-2, to investigate the role of light in fruiting in *P. ostreatus*. We introduced two different plasmids containing expression cassettes for Cas9 and two different gRNAs targeting *wc-2* separately into the dikaryotic *P. ostreatus* strain PC9×#64. Among the 11 dikaryotic hygromycin-resistant transformants, 6 strains did not form fruiting bodies. Genomic PCR followed by sequencing analysis suggested that all six fruitless strains were dikaryotic *wc-2* disruptants. Small aggregate structures were not observed in the dikaryotic *wc-2* disruptants grown under light conditions, as in PC9×#64 grown in a red box. These results suggest that Wc-2 is essential for the initiation of blue light-induced fruiting in *P. ostreatus.*

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386B

Homeodomain Transcription Factor is Essential for Toxocyst Formation in the Oyster Mushroom *Pleurotus ostreatus*

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Predation is one of the common ecological interactions across various life forms, with fungi exhibiting diverse strategies to prey on nematodes. The basidiomycete *Pleurotus ostreatus* (oyster mushroom) has evolved toxin-containing toxocyst structure to immobilize and consume nematodes as a nutrient source, particularly under nutrient-limited conditions. However, the molecular mechanisms governing toxocyst formation remain largely unclear. In this study, we conducted a forward genetic screen to identify mutants deficient in nematode paralysis, referred to as *lot* mutants (Lost Of Toxicity), and identified a homeodomain transcription factor *HDT1* (HomeoDomain transcription factor for Toxocyst development) required for toxocyst formation. Deletion of the *hdt1* gene mirrored the *lot* mutant phenotype, with normal growth but a defect in toxocyst development. Comparative RNA-seq analysis between the *hdt1* mutant and the wild-type strain under nutrient-limited conditions revealed significant differential expression, with approximately 700 down-regulated and 280 up-regulated genes. Gene ontology enrichment analysis indicated that most of the down-regulated genes are associated with heme binding, ion binding, and oxidoreductase activity. Notably, two small secreted peptides (SSPs) with intrinsic disordered regions and two lectins were significantly down-regulated in the *hdt1* mutant. Fluorescence protein labeled SSPs (Spc1 and Spc2) and lectins (Lec1 and Lec3) showed that they mostly colocalized together within the hyphae and toxocysts. Deletion of Lec1 impaired toxocyst formation. However, deletion of these SSPs only partially impeded toxocyst formation, suggesting the possibility of functional redundancy of the SSPs.

387B

Mating-type transcription factors from *Aspergillus fumigatus* and *Penicillium chrysogenum*: crystallization of the DNA binding domain and determination of MAT protein DNA binding motifs.

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Mating type (MAT) loci are the most important and significant regulators of sexual reproduction and development in ascomycetous fungi. Usually, they encode two transcription factors (TFs), named MAT1-1-1 or MAT1-2-1. Mating-type strains carry only one of the two TF genes, which control expression of pheromone and pheromone receptor genes, involved in the cell-cell recognition process. Beyond sexual reproduction, these master regulators influence asexual development and secondary metabolism, such as penicillin production in *P. chrysogenum*. In the human pathogen *A. fumigatus*, they are also linked to virulence and environmental stress responses

The present work presents the crystallization for the alpha1 (α1) domain of MAT1-1-1 from the human pathogenic fungus *Aspergillus fumigatus* (AfMAT1-1-1), and the penicillin producer *Penicillium chrysogenum* (PcMAT1-1-1). Crystals were obtained for the complex between a polypeptide containing the α1

domain and DNA carrying the AfMAT1-1-1 recognition sequence. A streak seeding technique was applied to improve native crystal quality, resulting in diffraction data to 3.2 Å resolution. Further, highly redundant data sets were collected from the crystals of selenomethionine-substituted AfMAT1-1-1 with a maximum resolution of 3.2 Å. This is the first report of structural studies on the α1 domain MAT regulator involved in the mating of ascomycetes. We further determined the minimum boundary of the alpha1 box domain of MAT1 and found overlapping binding motifs for MAT1 and MAT2. Finally, we provide experiments to uncover missing partner(s)/new interacting partners of MAT1 and MAT2.

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Unraveling toxocyst development in the oyster mushroom *Pleurotus ostreatus* Yi-Yun Lee, Sheng-Chian Juan, Yen-Ping Hsueh Department of Complex Biological Interactions, Max Planck Institute for Biology Tübingen

Carnivory toward nematodes has independently evolved across multiple fungal lineages. The basidiomycete *Pleurotus ostreatus* (oyster mushroom) paralyzes and kills nematodes via lollipop-shaped structures called “toxocysts,” which form under nutrient-limiting conditions. However, the genetic control of the toxocyst development remains unclear. Using forward genetic screens in *P. ostreatus* strain PC9, we identified multiple *loss-of-toxicity* (*lot*) mutants lacking toxocysts, establishing a strong correlation between toxocyst formation and nematicidal activity. Whole-genome sequencing and genetic mapping pinpointed the causative mutations in three mutants (*lot1*, *lot2*, and *lot3*) to the Pho85-cyclin 11 (*PCL11*), the histidine biosynthesis gene (*HIS1*), and the homeodomain transcription factor 1 gene (*HDT1*), respectively, implicating their roles in toxocyst formation. Yeast two-hybrid assays revealed a physical interaction between Pcl11 and the CDK Pho85, supporting a model where the Pho85-Pcl11 complex regulates Cdc42-dependent polarity during toxocyst initiation or branching. Cdc42 is a central regulator of cellular polarity and morphogenesis by organizing septins and actin. Accordingly, septins and F-actin localize to toxocyst branching and initiation sites, respectively, implying the central role of polarity machinery in toxocyst morphogenesis. To enable large-scale mapping of toxocyst-defective mutants, we generated a congenic strain PC9.15, by inrogressing the *MAT* locus from PC15 into the PC9 genetic background. Using bulked segregant analysis, we mapped causal mutations in 19 *lot* mutants, identifying genes essential for toxocyst development. These include histidine biosynthesis genes (*HIS4* and *HIS5*), a lipid metabolism gene (*DES4*), and a vacuolar trafficking and fusion regulator gene (*MON1*). Together, these findings provide new insights into the genetic and molecular pathways regulating toxocyst formation and nematicidal activity in *P. ostreatus*, with broader implications for understanding fungal predation strategies and cellular differentiation mechanisms.

389B

Decoding hyphal constriction in *Magnaporthe oryzae* using a simple assay Song Hee Lee[1], Eunbyeol Cho[1], Minsoo Jeong[2], Surajit De Mandal[1], Sook-Young Park[3], Seung Won Nam[4], Do Gyeung Byeun[1], Jung Kyu Choi[1], Yong-Hwan Lee[5,6], Jae-Ho Shin[2], Junhyun Jeon[1,7] [1]Department of Biotechnology, College of Life and Applied Sciences, Yeungnam University, [2]Department of Applied Biosciences, Kyungpook National University, [3]Department of Plant Medicine, College of Life Science and Natural Resources, Sunchon National University, [4]Microbial Research Department, Nakdonggang National Institute of Biological Resources, [5]Department of Agricultural Biotechnology, Seoul National University, [6]Plant Immunity Research Center, Seoul National University, [7]Phytobiome Engineering Research Laboratory, Yeungnam University

Filamentous fungi navigate extremely confined environments via hyphal constriction (HC), a mechanism critical for the successful colonization of plant hosts. In *Magnaporthe oryzae*, HC is essential for intercellular movement through pit fields. However, the genetic mechanisms governing HC remain largely unknown due to the challenges of monitoring this process *in planta*. To overcome this limitation, we devised a novel *in vitro* assay based on nitrocellulose membrane (NM) with varying pore sizes, enabling systematic evaluation of HC. Using this assay, we determined that wild-type *M. oryzae* hyphae constricted to 0.22–0.3 μm, whereas the Δ*pmk1* mutant, which was previously shown to be required for hyphal constriction and cell-to-cell movement, failed to pass through even 0.45 μm pores. Electron microscopy revealed extreme constriction of wild-type hyphae during traversing of membrane pores, while Δ*pmk1* hyphae remained trapped in larger matrix pockets without undergoing HC. Screening of mutants in signaling pathways and histone-modifying enzymes identified a previously unrecognized role for the histone deacetylase Sir2 in HC. Combining the NM assay with the Δ*pmk1* mutant, RNA-seq analysis uncovered two regulatory layers controlling HC: a Pmk1-independent activation of membrane-associated genes and a Pmk1-dependent transcriptional program associated with development and host interaction. Comparative analysis across fungal species revealed a strong association between lifestyle and HC capability. This demonstrates that the NM-based assay provides an accessible platform for dissecting the genetic regulation of HC.

390C

A tissue-resolved developmental transcriptome of the mushroom-forming fungus *Psilocybe cubensis* Matthew Meyer, Slot Jason Department of Plant Pathology, The Ohio State University

Mushroom-forming fungi produce some of the most complex multicellular structures in the fungal kingdom, yet the genetic networks that build and maintain their distinct tissues remain poorly understood outside of a small number of model species. Our objective was to determine how gene expression is coordinated across mushroom tissues and whether developmentally regulated genes show non-random organization within the genome. To address this, we generated the first tissue-resolved developmental transcriptome for *Psilocybe cubensis*. Using RNA-seq mapped to a high-quality reference genome, we investigated differential gene expression across vegetative mycelium, primordia, and three differentiated tissues of the mature sporocarp. Multivariate and differential expression analyses revealed pronounced transcriptional differences among tissues and showed that primordia occupy a distinct intermediate state between vegetative growth and differentiated sporocarp tissues. Functional enrichment analyses identified coordinated changes in pathways related to metabolism, cell wall remodeling, transcriptional regulation, and nucleic acid synthesis across tissues, reflecting shifts in gene expression profiles as development proceeds. Several biosynthetic gene clusters, including the psilocybin cluster, exhibited differential expression across tissues. Mapping groups of co-expressed genes onto the genome further allowed us to identify additional gene clusters with developmental-stage and tissue-specific expression. Gene families associated with immune and self/non-self recognition, including loci containing NOD-like receptor domains, also exhibited tissue-specific expression patterns, highlighting them among the broader set of genes regulated during mushroom formation. Together, this work establishes *P. cubensis* as a genome-enabled system for studying tissue-specific gene regulation, genome organization, and the developmental regulation of specialized metabolism in complex multicellular fungi.

391C

A Yak1 ortholog (Dkl) orchestrates dimorphism and global gene expression in *Mucor lusitanicus*

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Mucormycosis is a life-threatening infection caused by fungi of the order *Mucorales*, primarily affecting immunocompromised individuals and associated with high mortality rates. Certain species, such as *Mucor lusitanicus*, exhibit dimorphic development, alternating between yeast-like and mycelial states, the latter being responsible for their pathogenic capacity. Interestingly, in *M. lusitanicus*, hundreds of gene families display dimorphic expression. Using the promoter sequences of two dimorphic gene families in a DNA pull-down assay, we identified **Dkl,** an ortholog of the **Dual-specificity tyrosine-regulated kinase (DYRK)** Yak1. The *dkl* mutant showed growth defects and a complete inability to develop the yeast form, indicating a **key role for this gene in dimorphism**. Consistently, RNA-seq analyses revealed marked alterations in gene expression in this mutant, particularly in genes under dimorphic regulation. Given the strong involvement of *dkl* in transcriptional regulation and its central role in dimorphism in *M. lusitanicus*, we performed phosphoproteomic analyses and TurboID-based labeling of Dkl to define its associated regulatory networks. Additionally, two paralogs of *dkl* (*Mucci31461935* and *Mucci31528747*) were identified, both showing enhanced expression in the yeast form. The corresponding mutants were generated to determine their function in dimorphism and their functional relationship with *dkl*. Moreover, we obtained mutants in the orthologs of *Rhizopus microsporus* to elucidate the relevance of *dkl* in non-dimorphic mucorales. Yak1 and other members of the DYRK kinase family are widely conserved throughout evolution and play important regulatory roles in various dikarya fungi, such as *Candida albicans*, *Cryptococcus neoformans*, and *Aspergillus fumigatus*. Therefore, their characterization in *Mucorales* may shed light on the evolutionary role of this kinase and support the development of new treatments for mucormycosis.

392C

The role of chromatin modifiers and transcription factors during fruiting body development in the filamentous ascomycete *Sordaria macrospora*

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The differentiation of complex, multicellular fruiting bodies in filamentous ascomycetes is accompanied by drastic changes in the transcriptional program during development. In the model organism *Sordaria macrospora*, several transcription factors and chromatin modifiers have been identified that are essential for developmental progression past the stage of young fruiting bodies (protoperithecia). Among these are the histone chaperone ASF1 and the transcription factor PRO44, and an initial hypothesis was that both might act in the same or overlapping developmental pathways. However, transcriptome profiling showed that their influence on gene expression is strikingly different, indicating that they are involved in different aspects of development. Further analyses of ASF1 showed that its histone binding function is required for fruiting body formation, since mutated versions that are no longer able to interact with histones H3 and H4 are also unable to complement the sterile phenotype of the Δasf1 mutant. Part of the role of ASF1 in fruiting body development might be through mediating histone modifications since the histone marks H3K27me3 and H3K56ac are deregulated in the mutant strain. For H3K56ac, we could show that this chromatin mark requires the histone-acetyltransferase RTT109, with a Δrtt109 mutant also being blocked during protoperithecia formation. In contrast to Δasf1 and most other developmental mutants, the Δpro44 mutant is not only blocked in protoperithecia development, but also mislocalizes the developing fruiting bodies so that they are formed within the agar and not at the agar-air interface (their wild type-like localization). PRO44 contains a C-terminal GATA domain, whereas the N-terminus consists mostly of intrinsically disordered regions (IDRs). Preliminary experiments with deletion constructs showed that while several plasmids carrying *pro44* versions with deletions of parts of the IDRs were able to complement the mutant to fertility, correct localization of fruiting bodies was not restored, indicating that the IDRs might be involved in fruiting body localization, but not in the actual maturation of developing fruiting bodies. In summary our results indicate that ASF1 acts as a hub for chromatin-related processes in multicellular development and genome stability, whereas PRO44 is involved in spatial distribution of fruiting bodies as well as their development past the protoperithecia stage.

393C

Capture of the SmSTRIPAK proxiome identifies the Greenbeard proteins SmDOC1/2 as regulators of sexual development in *Sordaria macrospora*

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Hyphal fusion and sexual development in filamentous fungi depend on the coordinated signalling of numerous conserved signaling complexes, such as the Striatin-Interacting Phosphatase and Kinase (STRIPAK) complex and the Pheromone Response (PR) MAPK cascade (MIK2, MEK2, MAK2 and HAM5). The homothallic ascomycete *Sordaria macrospora* (Sm) was used here to screen for putative protein interactors of the SmSTRIPAK complex. We enriched and identified canonical SmSTRIPAK components and a DOC protein using the STRIPAK complex interactor 1 (SCI1) subunit of the complex as bait. DOC proteins have been described in the closely related, heterothallic species *Neurospora crassa*, where they function in allorecognition of germlings and hyphal fusions. We generated single deletion strains ΔSmdoc1 and ΔSmdoc2 and a double deletion mutant ΔSmdoc1ΔSmdoc2 in *S. macrospora*. Deletion phenotypes were paradoxical. Single knockouts (ΔSmdoc1 or ΔSmdoc2) were nearly sterile. Yet the double mutant (ΔSmdoc1ΔSmdoc2) exhibited wild-type fertility and fruiting-body development. This demonstrates non-redundant and mutually antagonistic roles of DOC proteins. Similarly, we demonstrated an impairment of sexual development in the *N. crassa* Δdoc-2 mutant. Using gene tagging at the native locus, we performed TurboID-based proximity mapping with SmDOC1 and SmDOC2 as the bait proteins. This proximity mapping revealed a close association between SmDOC1/2 and components of the PR MAPK pathway, as well as mutual SmDOC1-SmDOC2 proximity. Yeast two-hybrid experiments confirmed a direct interaction between SmDOC1 and the MAP kinases MEK2 and MAK2. Fluorescence microscopy revealed that SmDOC1-TagRFP-T localized to ring-like structures around septal pores. Our results show that the DOC system is not only found in heterothallic *N. crassa* but also plays an important role in the development of fruiting bodies in fungi. These findings suggest that the DOC1/2 proteins form a novel system integrating the STRIPAK and PR pathways. This provides a possible mechanistic explanation for the non-additive deletion strain phenotypes observed.

394C

Investigating the Effects of Temperature and Mycelial Culture Age on *Coccidioides* Arthroconidia Physiology

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Coccidioides is a thermally dimorphic fungal pathogen endemic to the desert and arid regions in the Southwestern United States. It grows as mycelia in the soil and forms segmented hyphae, which differentiate into asexual spores called arthroconidia. When the soil is disturbed, arthroconidia become airborne and can be inhaled by mammals. Upon inhalation, arthroconidia undergo a temperature-induced morphological change into large (up to 100 µm) round structures called spherules. Mature spherules form internal endospores, which are released upon spherule burst. Endospores disseminate within the host, giving rise to spherules, continuing the life cycle that leads to the establishment of coccidioidomycosis (Valley Fever). The environmental cues that govern the developmental phases of *Coccidioides* remain poorly characterized. Here we investigated the effects of temperature—room temperature (RT) and 30°C—and mycelial culture age on arthroconidia yield and viability, and their subsequent spherulation. We predicted that warmer temperatures would facilitate the accumulation of stress-response transcripts in arthroconidia, which may prime *Coccidioides* for survival in the host. First, we assessed how temperature influences conidiation. Our findings show that RT-grown mycelia produce significantly more total and viable arthroconidia compared to 30°C-grown mycelia. While mycelial culture age affects total yield, with maximal conidia yield reached after 6 weeks, viability is not significantly affected by mycelial culture age. Electron microscopy analysis comparing arthroconidia from RT-grown mycelia (AC-RT) and arthroconidia from 30°C (AC-30°C) highlights temperature-induced morphological differences. These differences were also evident in the transcriptome analysis comparing AC-RT and AC-30°C, with an increase in peroxisome biogenesis in AC-RT. Furthermore, we tested the spherulation of AC-RT and AC-30°C under in vitro and ex vivo (co-culturing of arthroconidia with macrophages) conditions. AC-30°C has a delay in transition to spherules under *in vitro* and *ex vivo* conditions. Additionally, in the presence of macrophages, AC-RT produced significantly more and larger spherules, induced macrophage death, and elicited stronger IL-1β release compared to AC-30°C. Together, our results reveal how environmental cues, such as temperature, can affect arthroconidia formation and physiology.

395C

Identification of the early sequestered germline lineage in *Marasmius oreades*

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Mushroom-forming fungi are generally thought to sequester their germline late in development. Their modular nature requires the presence of a totipotent stem cell in every location that is able to develop mushrooms, where sexual reproduction occurs. Contrary to this, we have recently found that in the fairy ring mushroom *Marasmius oreades*, somatic mutations are not transmitted to the offspring. Instead, sterile tissues (stipe, cap) from multiple mushrooms share an identical set of mutations, while fertile tissues (lamellae, spores) within the same mushrooms exhibit distinct, shared mutations, creating a clear genetic divide between sterile and fertile tissues. This means that the germline of *M. oreades* is already sequestered in the mycelium prior to sexual development. We use a combination of molecular techniques to determine the identity and maintenance of this lineage, including tracking the distribution of lineage-specific genetic variants in the mycelium, profiling the DNA methylation and expression landscapes of the lineages in mushrooms, and SNP-sensitive visualization methods to track the lineages *in situ*. Together, these results give a better understanding of the unexpected presence of early germline sequestration in a fungus.

396C

Characterization of the roles of the *Um*AA7 and *Um*AA10 CAZymes in the morphogenesis and pathogenesis of *Ustilago maydis*

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Fungi undergo extensive cell wall remodelling throughout their life cycle, often facilitated by secreted carbohydrate-active enzymes. Specifically, auxiliary activity (AA) enzymes are redox enzymes capable of modifying complex carbohydrates of the fungal cell wall, including the recalcitrant chitin layer. We aimed to characterize the function of three such enzymes, AA10 and two AA7s, in the model fungal pathogen, *Ustilago maydis*. We hypothesized that these enzymes contribute to cell wall remodeling and are essential for virulence. To test this, we employed a suite of biochemical assays, deletion mutant construction, and phenotyping to characterize the role of *Um*AA10 (UMAG_05439), *Um*AA7A (UMAG_10861), and *Um*AA7B (UMAG_11765). Overexpression of *Um*AA10 resulted in increased virulence and a decreased abundance of chitin. Additionally, we found that *Um*AA10 binds to ß-glucans rather than chitin, demonstrating novel binding activity. Conversely, deletion of *Um*AA7A caused sensitivity to osmotic stress and flocculation. The Δ*Um*AA7A mutant also exhibited decreased growth on glycerol and increased growth on galactose. These metabolic and stress response phenotypes suggest a link between cell wall integrity and mitochondrial homeostasis. While no distinct phenotype has yet been observed for *Um*AA7B, investigations into functional redundancy are ongoing. Characterizing these enzymes will provide deeper insights into fungal cell wall dynamics and may inform broader studies across microbial species, as well as support applications in antifungal development or industrial biotechnology.

397C

Environmental drivers and cellular mechanisms of development in the fungal pathogen *Coccidioides*

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The fungal pathogen *Coccidioides* causes the human respiratory disease Valley fever when a patient inhales airborne spores from an environmental reservoir. An estimated 40% of infected individuals develop symptoms, which range from pneumonia to fatal multiorgan failure. Since antifungals against this pathogen are minimally effective, prevention remains the first line of defense. However, without understanding the mechanisms and ecological factors that trigger *Coccidioides* to produce its infectious spores, public health models of Valley fever transmission risk remain limited in accuracy. In one wing of the project, we aim to elucidate the specific environmental conditions that promote sporulation, as well as the mechanistic basis underlying spore development. We present data showing that dry and saline conditions promote sporulation in *Coccidioides*, as well as in closely-related species; this indicates that adaptation to environmental conditions characteristic of their native desert habitat is a conserved trait within the Onygenaceae family. In another wing of the project, we aim to understand the genetic regulators of hyphal development. The transcription factor NRG1 has been implicated in morphological transitions in other fungi; here we show that nrg1 deletion in *Coccidioides* delays spores from developing into hyphae without affecting their development into spherules, the parasitic form of the fungus in mammalian hosts. This supports the hypothesis that in *Coccidioides*, nrg1 expression specifically promotes the spore-to-hyphae transition. Our work in sum sheds light on the environmental drivers of and cellular mechanisms underlying production of *Coccidioides*' infectious spores, as well as the regulators that govern their development, informing future efforts to prevent the spread of Valley fever.

398A

Comparative transcriptomics of Paracoccidiodes and Histoplasma reveals a conserved hyphal regulon

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The family Ajellomycetaceae contains the thermally dimorphic primary pathogens of humans including Histoplasma, Blastomyces, and Paracoccidioides species. Each of these fungi grows as a saphrophytic hyphal form that converts to a pathogenic yeast form at mammalian body temperature. Of these, Histoplasma and Paracoccidioides share a number of properties, including the ability to replicate within and lyse host macrophages. Additionally, virulence factors that are known to be required for macrophage lysis by Histoplasma, such as CBP1 and an expanded family of knottin proteins, are also present in the Paracoccidioides genome. Given the evolutionary relationship between Histoplasma and Paracoccidioides, namely Paracoccidioides is the earliest branching lineage of the dimorphic Ajellomycetaceae, these two genuses are a maximally diverged pair suitable for discovering conserved determinants of thermal dimorphism and virulence. Here, we perform the first comparative transcriptional profiling of Histoplasma and Paracoccidioides under matched in vitro conditions at 37ºC (yeast) or 22ºC (hyphae). While 2,872 orthologous gene pairs showed significant differential expression in at least one genus, this differential expression was poorly correlated between the two genuses. Despite the lack of concordance in the temperature-dependent transcriptomes, there was conserved expression of 136 yeast genes, including the virulence factors CBP1 and SOD3, as well as 445 hyphal genes. Surprisingly, the RYP3 transcription factor, which is required for yeast phase growth in Histoplasma, is hyphal-enriched in Paracoccidioides under our in vitro conditions, which could account for some of the divergence between the phase-specific transcriptomes. Notably, the conserved hyphal regulon is enriched for transcription factors, including the hyphal regulators STU1 and FBC1. Furthermore, this regulon is significantly enriched for hyphal expression in 12 published data sets from 8 genuses of dimorphic ascomycota, implying a deeply conserved hyphal program and highlighting the utility of comparative transcriptomics as a discovery tool.

399A

Opening Pandora's Box: The giant genome of *Pandora neoaphidis*

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Early-diverging fungal lineages provide a powerful system for understanding the evolutionary forces that shape genome expansion and architecture. Mobile genetic elements, such as transposable elements, are major drivers of fungal genome expansion, yet most work has centered on younger fungal phyla such as *Ascomycota*. Recent draft genomes from the order *Entomophthorales*, which is within the first terrestrial phyla of fungi *Zoopagomycota*, have been found to be large and highly-repetitive. However, we still have a limited understanding of the forces driving extreme genome expansion in this clade. To address this gap, we assembled a highly contiguous genome of a prevalent 'zombie' fungal pathogen of aphids, called *Pandora neoaphidis*, and we analyzed a time-series of RNA-seq libraries spanning host infection. We uncovered a remarkably expanded genome, which, at 5Gbp, represents the largest fungal genome yet assembled. We further showed that the genome has a lineage specific transposon landscape and 60% repetitive content along with high heterozygosity. We also found evidence for high levels of repeat-induced point mutation (RIP) pathway signature in this basal fungal lineage, suggesting this mechanism was active somewhere in the early evolution of fungi. Existing research in Dikarya has shown RIP pathway activity occurs during meiosis. This knowledge, in combination with the high level of heterozygosity, in *P.neoaphidis* brings into consideration ancestral fungal ploidy and sexual reproduction within *Entomophthorales*. Together, our analyses provide critical insight on how genome expansion, transposable-element dynamics, and defense pathways change over evolutionary time.

400A

Linking genomic functional traits to lifestyles and hosts in hypocrealean fungi via machine learning

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The fungal order Hypocreales exhibits exceptional ecological diversity, encompassing lifestyles that span the mutualist–pathogen continuum and a wide range of host associations. This ecological breadth underlies both their importance in natural ecosystems and their development as agricultural biocontrol agents against invertebrate pests and phytopathogens. Understanding the genomic basis of these diverse lifestyles is an important step toward improving

pest management strategies by facilitating prediction of host preference and lifestyle from genomic data. In this study, we address this challenge by applying machine learning to genomic trait data derived from more than 900 genomes spanning all clades of Hypocreales. Our analysis focuses on key traits involved in host-interactions, including carbohydrate-active enzymes (CAZymes), proteases, secreted small peptides (SSPs), peptidases, and biosynthetic gene clusters (BGCs), as well as more global signatures such as genome assembly features (genome size, GC content, repetitive elements) and functional profiles (PFAM and other functional domains) to identify patterns that predict potential lifestyle and host associations. Together, these methods provide a framework for linking genomic signatures to fungal lifestyles and functions, supporting both basic research into ecological diversity and applied efforts to identify fungi with biocontrol potential.

401A

Chromosomal translocation of a horizontally-acquired gene cluster and virus-like elements in a cosmopolitan yeast Jassim Al-Oboudi, Chris T Hittinger Genetics, University of Wisconsin-Madison

The instability and dynamic nature of subtelomeric regions in yeasts can facilitate dramatic genomic alterations**.** However, these regions can be difficult to characterize due to lower assembly quality and contiguity from short-read sequencing technologies. Here, we leverage long-read sequencing to resolve cryptic subtelomeric regions in a cosmopolitan yeast, *Torulaspora delbrueckii***.** This approach revealed the organization and varied chromosomal locations of a subtelomeric horizontally-transferred metabolic gene cluster for galactose consumption. Interestingly, we identified genetic signatures of double-strand break (DSB)-mediated repair of fragile subtelomeric regions flanking the cluster, offering insights into the mechanisms that facilitated its integration and chromosomal translocation. Strikingly, these subtelomeric gene clusters exhibited evidence of non-homologous repair with both endogenous and exogenous DNA sequences, most notably sequences derived from a killer toxin-encoding virus-like element (VLE) plasmid system. Additional searches of both short and long-read genomes further revealed extensive evidence of integration of VLE sequences in both subtelomeric and internal chromosomal locations. We furthermore identified several strains harboring active VLE plasmid systems. These results support recent evidence that VLE systems have ancient and broad associations with yeasts which can result in extensive genomic integration and can interact with other horizontally-acquired genomic features. Furthermore, we propose that the integration of such sequences in subtelomeric regions can exert influence on subtelomeric recombination and translocation dynamics. This work highlights the utility of long-read sequencing to resolve cryptic subtelomeric regions that can harbor diverse and complex signals of exogenous DNA integration.

402A

Exploring the biosynthetic potential of *Theobroma cacao* fungal endophytes of the *Bionectriaceae*. Librada Antonia Atencio[1], Hilda Castillo[2,3], Masiel Barrios[1], Indira Quintero[1], Luis C. Mejía[1] [1]Centro de Biodiversidad y Descubrimiento de Drogas, Instituto de Investigaciones Científicas y Servicios de Alta Tecnología (INDICASAT), [2]Universidad Tecnológica de Panamá, [3]Instituto de Investigaciones Científicas y Servicios de Alta Tecnología (INDICASAT)

Cacao (*Theobroma cacao*) production in tropical countries is threatened by various diseases. Fungal pathogens such as *Moniliopthora roreri* and *M. perniciosa* are the main causal agents of cacao diseases in Latin America. Fungi of the Bionectriaceae had been used as biocontrol agents of plant pathogens. This study explores the biosynthetic potential of two Bionectriaceae endophytes isolated from *T. cacao*, *Clonostachys rosea* TCF417 and *Waltergamsia zeylanica* TCF400, both exhibiting growth inhibitory activity against *M. roreri*.

Whole-genome sequencing was performed using Oxford Nanopore and Illumina technologies, resulting in assemblies of 52.9 Mb (*C. rosea* TCF417) and 31 Mb (*W. zeylanica* TCF400). Structural and functional annotations were conducted with Funannotate, and secondary metabolite biosynthetic gene clusters (BGCs) were predicted using the fungal version of AntiSMASH. To place these findings in a broader genomic context, comparative analyses were performed with 30 publicly available Bionectriaceae genomes using BiG-SCAPE. Across all 32 genomes, 1,523 BGC-containing regions were identified, primarily corresponding to T1PKS (591), NRPS (331), terpene (202), and NRPS-T1PKS hybrid (150) clusters. Network-based clustering revealed 209 gene cluster families (GCFs), of which 52 corresponded to previously characterized BGCs from the MIBiG database. For the most outstanding BGC categories, we found a total of 98 GCFs for T1PKS, 39 GCFs for NRPS, while for NRPS-T1PKS and terpene, a total of 22 GCFs were found for each one. The two cacao endophytes harbored 92 BGCs in total (*C. rosea*: 67; *W. zeylanica*: 25), indicating remarkable biosynthetic diversity. These results highlight the potential of Bionectriaceae endophytes as a source of novel bioactive metabolites for the development of sustainable crop protection strategies.

403A

Adaptation mechanisms of the human pathogenic fungus, *Fusarium oxysporum* MRL8996 Dilay Hazal Ayhan[1,2], Siyuan Wu[2,3], Serena Abbondante[4], Michaela Ellen Marshall[4], Eric Pearlman[4], Li-Jun Ma[2,5] [1]Molecular Biology and Genetics, Acibadem University, [2]Biochemistry and Molecular Biology, University of Massachusetts Amherst, [3]Molecular and Cellular Biology, University of Massachusetts Amherst, [4]Physiology and Biophysics and Ophthalmology, University of California Irvine, [5]Biochemistry and Molecular Biology, Massachusetts Amherst

Fusarium oxysporum is a cross-kingdom pathogen comprising diverse strains that cause disseminated fusariosis and keratitis in humans and vascular wilt diseases in plants. Our previous study documented that the wildtype strain MRL8996 exhibits higher thermotolerance than wildtype plant-pathogenic isolates such as Fol4287. To investigate mechanisms of adaptation to animal hosts, we conducted short-term evolution experiments with MRL8996, a keratitis isolate associated with contact lens use.

The ancestral strain was propagated into identical starting populations, evolved for ten passages under *in vitro* conditions (rich or minimal media at 28°C or 34°C adjusted to pH 5 or pH 7.4) and *in vivo* during mouse corneal infection with five biological replicates. The most distinct phenotypic variation is the loss of thermotolerance in 28°C-evolved populations.

Whole-genome sequencing revealed a striking genomic variation of the partial (28°C-evolved) or complete (34°C- and *in vivo*-evolved) loss of chromosome 12. Interestingly, upregulation of genes encoded in this fast-core chromosome was only detected at 28°C and pH 5, and these upregulated genes are involved in detoxification, oxidative stress defense, CAZyme-mediated plant host invasion, and heavy-metal resistance. Lacking active transcription in elevated temperature or pH suggests potential trade-offs between soil- and host-associated lifestyles.

Transposon insertion variants (TIVs) accounted for the majority of all detected mutations. SINE transposon Foxy5, the strain-specific and the highest expressed transposon in the ancestor, was the most active element, having twice the insertion frequency in accessory chromosomes than in core chromosomes. TIV numbers were more than 4-fold higher at 34°C than at 28°C. Recurrent mutations in *veA* and *velB*, components of the Velvet complex that regulate fungal development and secondary metabolism, were observed. Most TIVs occurred in promoter regions, consistent with altered gene regulation. Despite abundant variation, few mutations became fixed, indicating weak selection associated with modest phenotypic adaptation.

These findings demonstrate rapid genome remodeling in *F. oxysporum* MRL8996 during short-term evolution, driven by transposon activity. The concurrent loss of chromosome 12, rarely lost in other strains, highlights the evolutionary trade-offs accompanying this fungus's transition between environmental and host-associated niches.

404A

Integrative Sequence–Structure Mining of Fungal Polyurethane-Degrading Enzymes Through a One Health Framework

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Plastic has become deeply embedded in modern life, yet its accumulation in natural ecosystems—particularly polyurethane (PU), a chemically diverse and highly durable polymer—posed escalating environmental and public health risks. Although PU's industrial value stemmed from its resilient urethane, ester, and ether linkages, these same features made it a persistent pollutant for which conventional chemical remediation methods remained inefficient and environmentally burdensome.

In this study, we applied a systematic, bioinformatics-driven approach grounded in the One Health concept to discover and prioritize novel PU-degrading enzymes, with a particular emphasis on fungal lineages. We curated a comprehensive dataset of reported bacterial and fungal enzymes with activity on PU-associated bonds. Their physicochemical properties were characterized using ExPASy ProtParam, and high-confidence structural models were generated with AlphaFold. Comparative analyses using Datamonkey and MEME Suite identified conserved motifs and residues under positive selection that could underpin catalytic efficiency.

Guided by these molecular signatures, we performed large-scale sequence-based mining across NCBI, Swiss-Prot, EMBL-EBI, and especially the JGI MycoCosm portal, which provided extensive genomic coverage of ecologically diverse fungi. In parallel, structure-based searches using FoldMason identified protein architectures with active-site features favorable for urethane-bond cleavage.

By integrating evolutionary, structural, and sequence-level insights, we constructed a focused library of high-priority candidate enzymes enriched in degradation-associated motifs and stable folds.

405A

Genomic dynamics of *Magnaporthe oryzae* through sequential *in vitro* subculture and *in planta* infection cycles

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Magnaporthe oryzae (Mo), which causes serious diseases in diverse grass species, has seven indispensable core chromosomes and frequently carries dispensable mini-chromosomes. Mini-chromosomes can be highly variable, facilitating genomic instability, effector translocation, virulence and evolution. To understand the variability of genomes with mini-chromosomes, we designed an experimental evolution study using a rice field isolate, O-135, which contains a mini-chromosome. Five single spores of the O-135 isolate were independently subjected to 40 successive subculture cycles on oatmeal agar plates (*in vitro*) and 20 successive infection cycles on the YT16 rice cultivar (*in planta*). Single-spore isolates from the 20^{th} and 40^{th} cycles were isolated and subjected to whole genome sequencing and compared to the respective original starting parent. Phenotypes for both culture morphology and host virulence were well maintained in nearly all O-135 progeny isolates, except for one progeny isolate from the 40^{th} *in vitro* cycle that showed reduced sporulation and virulence. The whole genome comparison revealed an average of two mutations per isolate per generation, regardless of *in vitro* subculture or *in planta* infection. The mutation accumulation per megabase of chromosome was at a similar level between mini-chromosomes and the core chromosomes. However, the accumulated mutations were not uniformly distributed across the genome—high mutation rates were observed in repetitive sequences and in regions marked by histones with heterochromatic modifications, H3K9me3 and H3K27me3. Additionally, we observed multiple large sequence deletions and duplications in both core and mini-chromosomes of a few progeny isolates, identifying a structural variation hotspot on chromosome 1. In summary, we identified genomic regions that accumulated more mutations than expected during *in vitro* and *in planta* culture, but we did not observe the accumulation of significant mini-chromosome variation. This contrasts with observed mini-chromosome instability reported for other host infecting strains of *M. oryzae*, raising the possibility that differences in strains or mini-chromosome characteristics may cause differences in accumulation of mini-chromosome variation.

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Rapid shift in azole resistance of *Aspergillus fumigatus* during isolate processing

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Within the framework of the LatAsp (Latin American *Aspergillus fumigatus* Azole Resistance Survey in 12 countries, 20 laboratories), a large number of air samples were collected to assess the genetic diversity of *A. fumigatus* in Latin America and the percentage of azole resistance. During processing, some isolates showed discrepancies between their MIC profiles and their *cyp51A* genotypes. Repeating MIC assays yielded inconsistent results, with later assays showing higher MIC values than earlier ones. Several of these isolates were submitted for whole genome sequencing in duplicate, using DNA extracts from subcultures from earlier and later MIC assays. The initial hypothesis was that these isolates represented a population of strains, and different experiments corresponded to different strains from the population.

DNA extracts were sequenced using Illumina NovaSeq X. Sequencing reads were quality-filtered. Followed by read mapping using bwa against the reference genome, Af293. The sequences were also *de novo* assembled using SPAdes assembler. Relatedness of the genomes was estimated using whole genome SNP tree, average nucleotide identity of genome assemblies, and microsatellite genotyping (STR*Af* in the lab and *in silico* for genomes).

The sequencing replica results can be classified into three sets: i) showing nearly identical genomes including *cyp51A* sequence, ii) showing two completely divergent genomes confirming our initial hypothesis, and iii) showing highly similar genomes that deviated in their *cyp51A* sequences. This final set of replicas, having nearly identical genomes but differing in the *cyp51A* sequences, was further investigated. Closer analysis of the mapping data for the *cyp51A* gene region of these replicas showed 100% support for the absence of the resistance-associated SNP in the wildtype genome and 100% support for the SNP in the non-wildtype one. Some of these isolates had a similar genetic background, indicative of a clonal lineage. However, individual events involve different resistance-conferring mutations. In addition, this pattern has also been found with other genetic backgrounds besides the clonal lineage.

The results raise the question of whether closely related strains that differ in their susceptibility to azoles are distributed in the air together, or whether azole resistance can emerge so rapidly in the laboratory. Further experiments on these wildtype isolates could shed light on the rate of resistance development.

407A

Beyond the lab: The variable landscape of genomic instability in yeast isolates

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Previous work has shown that aneuploidy, a condition in which cells have an uneven chromosome content, may be favorable when growing cells under stressful conditions. In this work, we examine how the frequency of genetic instability events, focusing on aneuploidy and other large-scale structural variations, vary based on the genetic background of the budding yeast *Saccharomyces cerevisiae*. We designed an evolution experiment where diploid cells from a variety of wild and laboratory yeast backgrounds are passaged through strong bottlenecks on solid medium. Unlike liquid culture, solid medium minimizes selection generated by nutrient competition, thus allowing for nearly unbiased choosing of cells that move on to the next generation, regardless of their relative fitness. By using both long and short read sequencing data, we examined how aneuploidy and other structural variant frequencies change over time. In addition, when we measured genome instability in the ancestral and evolved strains, our results show a variable landscape of genetic instability frequencies dependent on the genetic background. Our work implies that studying laboratory yeasts alone provides a narrow understanding of how genetic instability emerges and evolves in nature. Understanding the effects of genetic instability is critical in the context of human disease, amidst its involvement in an increasingly dire landscape of treatment resistance acquisition in fungal pathogens and cancer cells.

408A

Integrated genomic and cytological approaches reveal mini-chromosome variation in *Pyricularia oryzae*

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The fungus *Pyricularia oryzae* (syn. *Magnaporthe oryzae*), the causal agent of blast disease in numerous grass species and major crops, possesses a genome consisting of seven indispensable core chromosomes and, in some strains, one or several extra mini-chromosomes. These mini-chromosomes are thought to contribute to effector gene mobility and facilitate gene exchange among strains belonging to different pathotypes. In this study, we aim to optimize genomic and cytological approaches to advance the exploration of mini-chromosome biology and characteristics. Long read sequencing can be routinely applied to generate high quality *Pyricularia* genome assemblies. Fluorescence in situ hybridization (FISH) with chromosome-specific probes enables reliable identification of individual core and mini-chromosomes. Using FISH with labeled mini-chromosomal DNA, we visualized mini-chromosomes in Pyricularia and observed variability in their numbers, ranging from 0 to 4 within a strain. Multicolor FISH using core chromosome-specific probes together with mini-chromosomal DNA can detect potential translocations from mini-chromosomes to core chromosomes. Cytological approaches complement

genome sequencing and assembly technologies, offering a robust strategy to study the genomic variability and stability of the mini-chromosomes in *Pyricularia*.

409A

Evolution of invasive *Phytophthora ramorum* populations in Oregon's coastal forests

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The Sudden Oak Death (SOD) epidemic within the coastal forests of Oregon and California, USA, is an oomycete invasion characterized by multiple introductions, clonality, and aberrant mutational patterns. It has altered coastal forest ecology through widespread host tree mortality. Continued pathogen spread has necessitated costly management programs and the quarantine of productive timber resources. Many micro-organism invasions are likely inconspicuous, but plant pathogens causing this level of damage are highly visible. These present opportunities to understand the evolutionary and ecological dynamics of fungal and oomycete invasions. Phytophthora ramorum, the causal pathogen of SOD, has escaped from the live plant trade into Oregon's wild forests as 3 genetically-distinct lineages: NA1, NA2, and EU1, which persist as 3 clonal populations. NA2 and EU1 populations are each dominated by a single clone. But many NA1 isolates diverge from its dominant clone in a pattern of cryptic diversity. This diversity is largely explained by NA1's high frequency of genomic runs of homozygosity (ROH), which accumulate in unique patterns in individual isolates. Recently-accumulated ROH were inferred using non-conserved SNPs called from whole genome Illumina sequences of NA1, NA2, and EU1 isolates. Genome analysis of forest and nursery populations from multiple US states and Canada revealed that 29% of NA1 genomes had at least 5 recent ROH tracts, compared to 7% of NA2 and 9% of EU1 genomes. A post-hoc Tukey test confirmed higher proportions of NA1 genomes were affected by recent ROH ($p<0.001$) when compared to NA2 and EU1. NA1 and EU1 were compared using RNA-Seq to identify expression differences in vitro and in planta related to ROH. Differential expression analysis of mRNA identified XRCC4, a DNA double-strand break repair protein, upregulated in all NA1 isolates. Potential triggers of ROH genesis were investigated by exposing isolates to biological stress. Isolates were sequenced and phenotyped before and after exposure to identify new mutations and their effects. ROH frequency is correlated with phenotype changes and ROH can persist in populations, yet the evolutionary consequences are not yet understood. These unique pathogen populations are used as a model to understand how somatic mutations accumulate in invasive micro-organism populations and, therefore, how these populations adapt to invaded environments despite low initial genetic diversity.

410A

Haplotype-level assembly for a hybrid species *Saccharomyces bayanus* reveal substantial introgression from multiple species

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Interspecies hybridization is prevalent in various eukaryotic lineages and plays important roles in phenotypic diversification, adaptation, and speciation. To study subgenome evolution in the hybrid yeast *Saccharomyces bayanus* (strain CBS380), we performed chromosome-level de novo assembly using Oxford Nanopore MinION long-read sequencing. Comparing *S. bayanus* with its parents, *Saccharomyces uvarum* and *Saccharomyces eubayanus*, we identified recombination events and loss of heterozygosity (LOH) in nine chromosome pairs. *S. bayanus* retains nearly all parental gene content and synteny but has acquired genes, primarily from *Saccharomyces cerevisiae*, enhancing maltose metabolism. Recombination and LOH patterns suggest an allotetraploid origin, likely aiding adaptation to maltose brewing environments and mitigating hybridization's maladaptive effects. This long-read sequencing study provides a key reference for hybrid yeast genome evolution.

411A

Evolutionary history of the transition of *Kluyveromyces* budding yeast from terrestrial to aquatic environments

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Fungi are known for their diversification on land but have repeatedly colonized and specialized to aquatic environments. The mechanisms of these transitions remain very poorly understood. We developed *Kluyveromyces* budding yeasts as a model for the study of evolutionary change in aquatic fungi. The *Kluyveromyces* genus consists of an aquatic clade, comprising *K. aestuarii, K. siamensis,* and *K. nonfermentans,* and a terrestrial clade that includes *K. lactis, K. marxianus, K. dobzhanskii,* and *K. wickerhamii.* Using comparative-genomic approaches, we traced a contraction in genome size and gene number in aquatic *Kluyveromyces*, and we identified over 700 genes with evidence for positive selection along the aquatic lineage. Phenotypic analyses revealed the loss of desiccation and cold tolerance in aquatic *Kluyveromyces*, supporting a model of a simplified lifestyle among these species. Furthermore, molecular analyses of the aquatic lineage established losses of function of the alcohol dehydrogenase *ADH1* and gains of coding and expression in its paralog *ADH2;* we interpret these changes under a model of a unique logic of ethanol metabolism in which the ancestral functions of these two loci were combined into one in aquatic *Kluyveromyces*. Together our phenotypic and genomic comparisons of marine and terrestrial *Kluyveromyces* aids in our understanding of fungal aquatic evolution.

412A

Genome evolution of homothallic and heterothallic members of the genus *Sordaria*

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Sexual reproduction promotes genetic recombination and enhances more adaptive progeny in fungi. In *Ascomycota*, the sex determinants are encoded by the mating-type (MAT) locus, which are the *mat A* (*MAT1-1*) and *mat a* (*MAT1-2*) idiomorphs. The two primary modes of reproduction are homothallism and heterothallism. The model organism *Sordaria macrospora* is homothallic, carrying both idiomorphs in a single genome, whereas heterothallic species typically harbor only one idiomorph. Homothallic *Sordaria* species have not been reported to produce asexual spores. This study investigates the evolutionary development of sexual and asexual reproduction in the genus *Sordaria* by integrating newly generated genomes from homothallic species, *S. fimicola* and the heterothallic species, *S. brevicollis mat A*; *S. sclerogenia mat A* and *mat a* with publicly available genomes. Using long-read de novo assemblies, we confirmed that *Sordaria* genomes contain seven chromosomes, with the MAT locus consistently located on the first chromosome. Comparative analyses reveal that *S. brevicollis* possesses a larger genome with reduced GC content, while *S. sclerogenia* resembles homothallic species in both genome size and GC composition. Phenotypic observations show that heterothallic *Sordaria* species are capable of asexual reproduction. Notably, *S. brevicollis mat A* strain exhibits rare but detectable self-fertilization, producing only occasional mature asci. We also found a substantial contribution of transposable elements (TE) to genome plasticity. Remarkably, in heterothallic species, GC content strongly correlates with gene density and TE distribution, indicating that active TEs may shape genomic divergence between heterothallic and homothallic *Sordaria* lineages. Overall, this research provides new insights into the genomic and evolutionary factors driving reproductive diversity within the genus *Sordaria*.

413A

Telomere-to-Telomere Reference Genome for *Septoria glycines* Highlights Shared and Species-Specific Virulence Factors Among Soybean Foliar Fungal Pathogens

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Septoria glycines, the causal agent of Septoria brown spot of soybean, is among the most widely distributed foliar fungal pathogens of soybean in the United States, yet genomic resources for this species have remained limited. The lack of a high-quality reference genome has constrained efforts to investigate pathogen evolution and virulence mechanisms. Here, we present the first chromosome-level reference-quality genome for *S. glycines* isolate SG026, assembled using PacBio HiFi long reads and Illumina short reads.

The 31.7 Mb assembly includes 14 telomere-to-telomere contigs, two single-telomere contigs, and a fully assembled mitochondrial genome, with 99.1% BUSCO completeness, representing a significant improvement over the fragmented short-read assembly available in NCBI. Genome annotation predicted 10,950 gene models, including 646 genes predicted to encode putative secreted proteins, of which 365 were predicted to encode putative effectors. Additionally, 39 biosynthetic gene clusters, including nonribosomal peptide synthetase (NRPS) and polyketide synthase (PKS) clusters, were detected.

Comparative genomic analyses of the *S. glycines* reference genome with the reference genomes of the other foliar fungal soybean pathogens, *Cercospora sojina* (frogeye leaf spot) and *C. kikuchii* (Cercospora leaf blight), revealed relatively low genome-wide synteny. Despite the lack of large-scale homology, ortholog analysis identified a conserved core set of orthogroups, including 256 predicted effectors with orthologs present in all three species. All three pathogens also possessed genes corresponding to four conserved biosynthetic gene clusters, including the cercosporin biosynthetic gene cluster. These findings suggest that although the genomes differ substantially, effector repositories and virulence-associated biosynthetic gene clusters are shared across all three species, likely as adaptations to modulate soybean defense mechanisms during infection. Notably, *S. glycines* also contained 58 species-specific effectors without any orthologs in *C. sojina* and *C. kikuchii*, indicating that portions of *S. glycines'* effector repertoire may be evolving independently to enhance pathogenic specialization. Together, the *de novo* reference genome of *S. glycines* and the comparative genomic framework substantially improve our understanding of the biology and evolution of *S. glycines* and provide critical information to manage foliar fungal pathogens of soybean.

414A

DNA damage tolerant colonies of a fungal plant pathogen acquire mutator phenotype

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Fungi contribute disproportionately to emerging diseases, partly due to their remarkable ability to adapt to new environments and fungicides. While it is known that DNA damage induces mutations that lead to adaptation to new environments, very little is known about this process in fungal pathogens. To address this gap, we investigated the response of the fungal plant pathogen *Fusarium oxysporum* to UV irradiation.

Upon UV exposure of spores, we observed a distinct subpopulation that failed to develop even after 48 hours. When separated by size, this arrested population displayed reduced plating efficiency and increased mutation rate. This subpopulation showed the most dramatic response to UV at the transcription and phosphoproteome levels ever reported in yeast or fungi. When sequencing the entire genome of colonies that survived UV we found that applying selection pressure results in higher UV-induced mutation frequency.

Unexpectedly, ~10% of the surviving colonies failed whole genome sequencing attempts. Strikingly, these colonies carried persistent DNA damage, with up to 60% of the lesions originally inflicted on the total spore population still detectable—even after regrowth. This raises a fundamental question: how can colonies with such extensive DNA damage sustain replication and propagation and what are the mutagenic and evolutionary implications of this phenomenon. Interestingly, daughters of UV survival colonies showed higher mutation density than their parents, indicating that DNA damage tolerant colonies are a mutators that can drive adaptation to stress.

415A

Contrasting environmental and clinical isolates of *Coccidioides*, the causative agent of Valley Fever

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Coccidioidomycosis (Valley fever) is an emerging, potentially life-threatening fungal disease with an estimated burden of over 200,000 cases in the U.S. each year. Humans become infected by inhaling wind-dispersed spores of *Coccidioides*—a soil-dwelling fungus frequently associated with desert mammals. Reducing exposure to environmental sources remains the primary means of disease prevention, as there is no vaccine for coccidioidomycosis and few therapeutic options. Yet whether all environmental sources of *Coccidioides* pose equal disease risk to humans remains unknown. In one possible scenario, colonizing the human lung could represent a selective barrier that only *Coccidioides* of certain phenotypic profiles are able to breach. Alternatively, the human host could be selectively indistinguishable from *Coccidioides*' native rodent niche, such that any strain would be capable of human infection.

A powerful approach to address this question is to compare isolates of *Coccidioides* derived from soil vs human tissue, yet the former are exceedingly rare owing to the practical difficulties of surveying for a microscopic fungus across thousands of acres of desert soil. Here, we overcome this challenge by leveraging a novel resource: 17 genomes of *C. immitis* from its endemic environment (California's San Joaquin Valley), generated through extensive soil sampling over the last decade. Combining these with previously sequenced genomes of *C. immitis* from infected patients in the same region, we conducted comparative genomic analyses to identify loci differentiated between these two groups and to detect signatures of differing selective pressures between soil and human infection settings. To date, we have identified 29 genes with significantly elevated FST between soil and host-infecting strains. These candidate genes include cell well protein PhiA (involved in conidia formation), two NADH-ubiquinone oxidoreductase subunits (linked to oxidative stress responses and pathogenicity in other fungal species), and a multidrug resistance protein. Ongoing analyses using McDonald-Kreitman and Hudson-Kreitman-Aguadé (HKA) tests will evaluate whether these candidate genes likely arose due to neutral processes or selection associated with host infection. These FST outliers represent compelling targets for functional analyses, potential drug development, and expanded mechanistic understanding of virulence in this emerging pathogen.

416A

Identification of genetic elements related to thermotolerance across the *Cryptococcus* clade

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As global temperatures continue to rise, fungal adaptation to these increased environmental temperatures could have serious clinical and ecological consequences. *Cryptococcus neoformans* is a major fungal pathogen belonging to the family Cryptococcaceae, which causes pneumonia and cryptococcal meningitis in individuals with compromised immune systems. Within the family Cryptococcaceae there are both pathogenic and nonpathogenic fungi, with the pathogenic fungi exhibiting higher heat tolerance than the nonpathogens. This is interesting because growth at high temperatures is necessary to cause disease in mammals. However, the genetic and evolutionary basis of the differences in this virulence-related trait are not well understood. To address this gap in knowledge, we have developed a genetic approach using insertional transposon mutagenesis (TN-seq). TN-seq generates random transposon insertions across the genome. We can then measure transposon insertion frequency before and after selection to identify genes that play a role in a given condition, such as high temperatures. I plan to integrate the TN-seq system into both pathogenic and nonpathogenic species of *Cryptococcus* in an effort to identify loci that are causal for the thermotolerance phenotype exhibited by pathogenic species. I will then conduct allele swaps between the pathogenic and nonpathogenic species of *Cryptococcus* at these candidate loci to test whether the thermotolerance phenotype is caused by allelic variation between these species. Identification of genetic elements related to thermotolerance is essential to understanding the evolution of virulence within fungi.

417A

Genomic diversity and rapid phenotypic adaptation following serial infection of a specialist fungal pathogen in a novel host

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The evolutionary trajectory of fungal pathogens making a host shift is difficult to study in nature while they occur, and often can only be inferred after they have occurred using comparative methods. Such host shifts are more easily explained between related hosts, when pathogens target similar host traits to establish the infection. The entomopathogenic fungus *Metarhizium acridum* specializes in infecting orthopteran insects. In this study, we investigate its potential to increase virulence towards a novel host from a completely different insect order, the beetle *Tenebrio molitor*, through repetitive serial passage. We evaluated the virulence of nine *M. acridum* isolates against *T. molitor* and observed variability in their initial infective capability. Three isolates with diverse phenotypic and genotypic backgrounds were chosen for serially passage through *T. molitor*. We observed significant increases in virulence to *T. molitor* in descendent lineages of two isolates of higher initial virulence, after ten passages within a novel host. Our findings demonstrate rapid increase in virulence towards a novel host through successive generations in some isolates. Transcriptomic analyses indicated that all lineages displayed a greater number of downregulated genes during the early stages of infection, but those lineages that exhibited successful increases in virulence showed a more pronounced downregulation of genes. However, the differentially expressed genes varied across experiments, suggesting that both standing genetic variation and isolate-specific gene expression plasticity play complex roles in facilitating host adaptation. Our study highlights the significance of genomic plasticity for understanding the evolution of virulence and host adaptation in fungal pathogens.

418A

Population-Specific Transcriptomic Rewiring Underlies Secondary Metabolic Diversification in *Aspergillus flavus* and the Domestication of *Aspergillus oryzae*

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The genomic signatures of microbial domestication remain poorly understood within the context of natural population variation. Here, we demonstrate that *Aspergillus oryzae*, the filamentous fungus used in soy sauce production, shares more recent ancestry with a predominantly northern, largely non-aflatoxigenic population of *Aspergillus flavus* (population C). Strikingly, *A. oryzae* isolates also overlap with the recently described, clinically enriched *A. flavus* population D, suggesting the possibility of multiple domestication events. Although *A. oryzae* exhibits reduced virulence compared to *A. flavus*, all isolates tested retained pathogenicity in a zebrafish infection model. At the transcriptomic level, *Aspergillus* populations are significantly differentiated, with distinct responses to population density, indicating that population-specific transcriptomes adapt differently to ecological conditions. These differences extend beyond gene content and are not always explained by phylogenetic relationships, suggesting that phenotypic diversification occurs through the rapid reorganization of transcriptomic architectures. For example, *A. oryzae* displays significantly elevated expression of a module enriched for carbohydrate metabolism. Population-specific variation is also evident among secondary metabolite (SM) gene clusters. While *A. oryzae* shows markedly reduced expression of specific SM genes, particularly those involved in aflatoxin biosynthesis, this trend does not extend across the entire secondary metabolome. Using machine- learning-based gene regulatory network inference, we identified population-specific transcriptomic differences linked to distinct transcription factors, with evidence for both cis- and trans-acting regulatory divergence, but no changes in global regulators such as *laeA*. Together, these findings provide new insights into the domestication of *A. oryzae*, its global significance, and the microevolution of fungal secondary metabolic pathways.

419A

Life cycle plasticity in *Cronartium ribicola,* the causal agent of white pine blister rust

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Rust fungi (*Pucciniales*) are highly complex plant pathogens capable of utilising two hosts and up to five unique spore types to complete their lifecycles. The inherent complexity of this group of plant pathogens has sparked an abundance of evolutionary discussion prompting researchers to study their lifecycles and host-pathogen interactions in an effort to better understand them and combat the outbreaks they cause. The genus *Cronartium* is notorious for its destructive effects on pines, and the introduced *Cronartium ribicola* J.C. Fisch., which causes white pine blister rust, has been especially damaging, driving the widespread decline of white pines across North America. Despite its traditional characterization as a genus of heteroecious rusts alternating between pine and angiosperm hosts, *Cronartium* also contains clonal, autoecious pine-restricted lineages with uniquely reduced life cycles, and whether these represent distinct species or variants within a single variable species complex remains unresolved. Research on these rusts has often split its attention between their heteroecious and autoecious forms, in the process missing a crucial facet of their biology. *C. ribicola* has prompted extensive research, yet a significant blind spot has remained regarding the autoecious white pine rusts. Although these lineages have long been known from Asia, particularly Japan, they have been poorly studied, and their relationship to *C. ribicola* has remained unresolved until now. An updated ITS and 28 S based phylogenetic analysis now shows that the autoecious white pine rusts *Cronartium yamabense* and *Cronartium sahoanum* cluster within the same strongly supported clade as *Cronartium ribicola* (97% bootstrap support). Additionally, autoecious rusts such as *Cronartium pini* and *Cronartium harknessii* fall within highly supported clades which include their heteroecious counterparts (100% bootstrap support for both clades). The intermixing of autoecious and heteroecious forms suggests that *Cronartium* may possess a deeper, genus-wide life cycle plasticity capable of generating uniquely short cycled autoecious lineages, potentially representing a synapomorphy of the group. Moving forward, greater attention must be devoted to understanding how these autoecious white pine rust lineages arise and incorporating them into the broader concept of *C. ribicola* biology, as the ability to produce autoecious forms may persist even in the introduced North American populations.

420A

Comparative genomics using long-read sequencing of clinical *Candida auris* isolates reveals transmission dynamics and drug resistance mechanisms

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Candida auris is an emerging, nosocomial, multidrug-resistant fungal pathogen that has been responsible for healthcare associated outbreaks worldwide. The use of short-read sequencing has allowed outbreak tracing, yet it does not fully resolve genome structure, telomeres, and rearrangements that may drive adaptation and persistence. In this study, we utilized Oxford Nanopore long-read sequencing on outbreak-associated *C. auris* isolates from two U.S. hospital systems, Northwestern Memorial Hospital (NMH) and UC San Diego Health hospitals (UCSD), in addition to the bio-banked reference isolates from the Centers for Disease Control and Prevention. We generated telomere-to-telomere assemblies of 121 isolates in total. These de novo chromosome-level assemblies were used to perform phylogenetic analyses, comparisons of genomic architecture, and the detection of single-nucleotide polymorphisms (SNPs). Phylogenetic analyses highlighted overrepresentation of Clade IV isolates from NMH and Clade III isolates from UCSD, adding to the previously reported geographical distribution of *C. auris*. Comparisons of genome architectures and SNP analyses suggested low genomic variation within patients and hospital-specific SNP thresholds. We found that the mean SNP numbers were 109 for NMH and 34 for UCSD suggesting a hospital-system dependent accumulation of SNPs. In addition, we identified frequent mutations in the *ERG11* gene concurrent with the previous reports identifying *ERG11* mutations leading to the elevated azole minimum inhibitory concentration. In addition, we uncovered significant structural genome rearrangements including large chromosomal inversions and translocations between the isolates both within a given clade and across clades. These differences in genomic architecture were not apparent with the analyses using short-read sequencing data. Notably, major reorganization of chromosome 1 was observed in a group of isolates, suggesting a potential region that can be used for transmission tracing. Taken together, our study suggests comparative genomics approaches using de novo chromosome-level assemblies as a standard for outbreak surveillance, resistance prediction, and mechanistic insight into genome evolution in healthcare-associated pathogens.

421A

Comparative genomics of plant associated fungi to assess endophytic genome signatures

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Beyond the scope of Epichloë fungi, the global diversity of grass endophytes contains many fungi that exist as plant pathogens and other non-endophytic lifestyles. The host ranges and life history strategies of these fungi are broader, resulting in a wider range of selective pressures acting on them compared to the Clavicipitacean endophytes. Here we present a phylogenetic placement of a wide range of primarily Ascomycete fungi isolated from endophytic niches within pre-dispersal grass seeds collected from 76 locations in Madagascar over a series of decades, then preserved at the Millennium Seed Bank, UK. We provide a focussed look at isolated fungi that are regularly documented as pathogens of plants and other grasses: Fusarium, Aspergillus, Alternaria and others, to examine whether the endophytic strains are phylogenetically constrained within a wider setting of closely related non-endophytes, or whether they are distributed throughout their genera. We then conduct comparative genomic tests between these endophytic strains and non-endophytic relatives. We use dN/dS to test for positive selection, then genome enrichment and gene family expansion to look for genomic signatures that are unique to endophytic strains. The results explore the phylogenetic grouping of endophytic strains within their genera and evolutionary processes that direct diverse Ascomycete fungi towards endophytism.

422A

A genomic view of hypermutation potential in *Candida auris* drug resistance

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Background: *Candida auris* is a globally emerging pathogen causing invasive, life-threatening infections, with up to 70% mortality. One major concern is its association to outbreaks with unusually high levels of multidrug resistance. A potential driver of drug resistance evolution is hypermutation, which is the accumulation of mutations at an elevated rate due to errors in DNA repair mechanisms, such as the mismatch repair (MMR) system. This elevated rate leads to the accumulation of beneficial mutations including conferring drug resistance and virulence. A global characterization of *C. auris* hypermutation is therefore essential to understanding the rapid emergence of multidrug resistance.

Methods: Phylogenies were resolved for over 900 clinical isolates with published drug resistance data, spanning all six clades of *C. auris*. Candidate hypermutants were identified by monitoring for signatures of elevated mutation rates, including elongated terminal branches, larger tip-to-root distances, and overrepresentation of private mutations along each branch. Variant analysis with functional impact annotation was performed to reveal DNA repair gene mutations which may induce hypermutation. Finally, drug resistance profiles were mapped to candidate hypermutators to assess the contribution of hypermutation to resistance.

Results: Candidate hypermutators were identified across all six clades. The largest cluster of hypermutators was in clade I, with all isolates harbouring frameshift mutations in the MMR gene *rad53*. Notably, these *rad53* mutants were associated with increased multidrug resistance. Conversely, candidate clade VI hypermutators exhibited *mlh3* and *msh3* frameshift mutations, but lacked associations with multidrug resistance. All other candidates had moderate to high-impact missense mutations in MMR genes but showed no association with elevated resistance.

Conclusion: This study offers insight into the prevalence of genes that can potentiate hypermutation and their associations with drug resistance across all six clades of *C. auris*. This work will be expanded to a global dataset of over 20,000 isolates, enabling a more comprehensive analysis of the prevalence of hypermutation, its genetic causes, and its impact on drug resistance. These findings will then be validated in vitro using CRISPR-Cas9 gene editing and experimental evolution. Overall, this study lays the groundwork for understanding the relationship between hypermutation and drug resistance in *C. auris*.

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Genome evolution of lichen-forming fungi associated with a major photobiont switch from a green algal to a cyanobacterial symbiont

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A switch to a different photoautotrophic partner by an obligate heterotrophic mutualist can significantly impact the evolution of symbiotically interacting organisms through processes such as Horizontal Gene Transfer (HGT), transmission of transposable elements between symbiotic partners, and duplication or reduction of genomic features (from genes to complete chromosomes). A major photobiont switch in the lichen-forming fungal class Lecanoromycetes (Pezizomycotina) from a green alga to the heterocystous cyanobacterium *Nostoc* is associated with the origin of the fungal order Peltigerales, which includes mostly cyanolichens. We detected multiple lineages of peltigeralean fungi with increased genome size following this major photobiont switch. An increase in genome size can be caused by genome duplication, acquisition of supernumerary-chromosomes, HGT events, and expansion of repetitive DNA. Transposable Elements (TE) are repetitive DNA sequences that can actively replicate and spread throughout the genome via their own enzymatic activity, having positive or negative effects on the affected organism. Bursts in TE activity have been reported after a major event such as the transition from one lifestyle to another (e.g., free-living to symbiotic). Unlike their *Nostoc* partners and other lichen-forming fungi, it has been nearly impossible to grow peltigeralean lichen-forming fungi in axenic culture. Therefore, we used 197 metagenomes (11 produced with long reads) of peltigeralean cyanolichens, from which we generated 197 metagenome-assembled genomes (MAGs) of the lichen-forming fungi. We compared these MAGs with 18 genomes of lichen-forming fungi associated with green algae, to assess the genomic changes associated with this major photobiont switch. Using maximum likelihood single-gene phylogenies, we inferred an ASTRAL species tree of these lichen-forming fungi. TE scanning across this phylogenomic framework revealed that at least 25% of the peltigeralean lichen-forming fungi genomes are composed of TE sequences, a trait not observed in closely related fungi outside of Peltigerales in symbiosis with green algae as their main photobiont. Classification of these TE sequences showed that Long Terminal Repeat sequences (LTR) are the dominant TE in peltigeralean genomes. Our results suggest that this major photobiont switch played a crucial role in the increase in genome size of peltigeralean lichen-forming fungi.

424A

Evaluating the role of bacterial endosymbionts in evolution and diversity of plant associated Mortierellomycotina fungal communities

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Endosymbiotic bacteria (EB) are increasingly recognized as dynamic partners of early-diverging fungi, although their presence is variable and often limited to only a subset of individuals within a species. Among these EB, Burkholderia-related endobacteria (BRE) are notably widespread and exert a strong physiological and developmental influence on their fungal hosts. This influence shapes metabolic activity, cell wall structure, transcriptional responses, and stress tolerance in the host. BRE also alter the outcomes of fungal sexual processes, including sporulation. This suggests that the presence of BRE may impact the genome structure and the long-term evolutionary trajectories of their fungal hosts.

Given these observations, we hypothesized that BRE-host isolates and non-host isolates within the Mortierellomycotina would display distinct patterns of genome organization, population diversity, and biogeographic distribution. To test this hypothesis, we: i) isolated Mortierellomycotina from over 300 sites across South Africa and California, USA using specialized culturing and enrichment techniques; ii) generated 455 metagenomes and assembled fungal draft genomes from these data; iii) applied SNP-based population genomic approaches to quantify genomic diversity patterns; iv) evaluated variability in genome and population structure between Mortierellomycotina BRE-host and non-host isolates at the intraspecific level.

Preliminary analyses indicate that BRE-host and non-host isolates of Mortierellomycotina fungi exhibit differences in their conservation of shared genomic content, and response to abiotic and biotic environmental variables in various habitats. Ultimately, this research seeks to advance our understanding of how intracellular symbioses emerge and persist, providing new insights into the genomic mechanisms through which microbial partnerships alter the evolutionary potential of both partners.

425A

Biofilm regulatory network divergence in *Candida* species is driven by cis-acting variations

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The *Candida* genus contains many medically important fungal pathogens, and biofilm formation is a key virulence trait across these species. In *Candida albicans,* biofilm formation is controlled by an interconnected gene regulatory network (GRN) containing nine core transcriptional regulators. Because biofilm capacity varies widely across *Candida* species, we investigated the genomic and epigenomic sources of this variation and the mechanisms underlying GRN divergence.

Using comparative genomics across twelve *Candida* species, we characterized divergence in core regulators and their target genes. We also constructed the biofilm GRNs for *C. dubliniensis, C. tropicalis*, and *C. parapsilosis*, and expanded the *C. albicans* network by integrating publicly available genome-wide binding datasets for six regulators with corresponding gene expression datasets.

We found that five of the nine core *C. albicans* regulators are conserved across large evolutionary distances, whereas three are either lost or highly diverged in non-CTG species through lineage-specific gene duplications, and one regulator appears to have arisen in the ancestor of *C. albicans*, *C. dubliniensis* and *C. tropicalis*. Despite this partial conservation, binding sites and target genes show only moderate conservation even among closely related CTG species. Moreover, conserved regulators often control distinct biological processes in different species – for example, Efg1 governs stress responses in *C. tropicalis,* whereas in *C. albicans* and *C. dubliniensis* Efg1 regulates phenotypic switching. These functional shifts are rarely attributable to changes in motif preferences and instead reflect extensive binding-site turnover.

Network-level architecture also diverges substantially. The *C. albicans* biofilm network is highly interconnected, with ~600 targets bound by at least four core regulators; genes involved in host interactions are especially enriched for such combinatorial regulation. Together, our findings show that biofilm GRN divergence in non-CTG species is largely driven by gene duplication-mediated changes to the regulator repertoire, whereas in CTG species it arises mainly from cis-acting variation that rewires regulatory connections and alters network interconnectedness.

426A

Pan-genomics of the Laccaria species complex reveal a two-speed evolution of the "mycorrhizal-toolkit"

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Laccaria bicolor (Maire) Orton is the preeminent model for investigating the molecular controls of ectomycorrhizal symbiosis, yet nearly all functional work has focused on a single strain, S238N, despite broad phylogenetic and geographic diversity within the genus. To resolve how symbiosis-related traits are distributed and evolve across Laccaria, we generated 112 new genomes spanning the genus, including 40 within the L. bicolor species complex, and used them to construct a Laccaria “super-pangenome.” This resource yields a fully supported species phylogeny and reveals that L. bicolor comprises multiple cryptic lineages. By mapping experimentally characterized colonization genes from S238N and genes upregulated during ectomycorrhiza formation onto the super-pangenome, we find strong evidence for a two-speed genome. “Free-living” genes are concentrated in a conserved, syntenic core, whereas symbiosis genes are disproportionately enriched in the accessory pangenome, show higher sequence diversity, are more frequently duplicated, and occur

in structurally dynamic, non-syntenic regions. Despite this genomic dynamism, a substantial fraction of the classical mycorrhizal toolkit remains broadly conserved across Laccaria, indicating deep retention of key symbiotic functions. Within this variable compartment, we also identify a previously uncharacterized gene with clear signatures of horizontal gene transfer from bacteria that is strongly induced during ectomycorrhiza formation and predicted to participate in ethylene biosynthesis, suggesting a novel route for fungal modulation of plant signaling. Variation in the presence, copy number, and genomic context of symbiosis genes is structured primarily by phylogeny rather than host origin, implying that host identity is not the dominant driver of diversification in the group. Together, our super-pangenome and two-speed genome framework provide a genome-scale view of how conserved and rapidly evolving regions jointly shape ectomycorrhizal symbiosis across Laccaria.

427B

ABAQS: A Comprehensive Scoring Method for Assessing Genome Annotation Quality

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As fungal genome resources continue to expand, estimating the accuracy and completeness of genome annotations (e.g., gene and protein models) becomes essential to ensure quality of downstream analyses and applications. It becomes critical in comparative genomic analysis to select genomes with comparable qualities of assembly and annotation. Without careful scrutiny, inconsistencies in annotation quality can undermine downstream studies. A robust quality metric should ideally capture multiple dimensions of genome annotation accuracy. However, in practice, most commonly used quality metrics are single-dimensional or consider only a few aspects of annotation quality, leaving a large space of high-scored but low-quality genome annotations unpenalized. For example, tools like BUSCO (Manni et al. 2021) and CEGMA (Parra, Bradnam, and Korf 2007) evaluate assembly and annotation qualities by detecting conserved gene content in assemblies or predicted proteomes. Such methods solely focus on genome and proteome completeness which is insufficient to generate a full picture of assembly and annotation quality.
To address this gap, we introduce the Accuracy-Based Annotation Quality Score (ABAQS), a comprehensive and minimal-data-driven method to summarize genome annotation quality as a single easily understandable numeric value. While developed primarily using fungal genomes from MycoCosm, ABAQS is broadly applicable across eukaryotes. ABAQS evaluates multiple factors, including genome completeness, gene model validity, and protein profile accuracy, outperforming metrics like BUSCO and PSAURON. We applied ABAQS to 1700+ eukaryotic genomes and showed its robustness and effectiveness in evaluating genome annotation quality, making it a valuable tool for researchers working with genomic data.

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428B

FLASH, a new statistical machine learning algorithm, can accurately predict and link novel genes to antifungal resistance in diverse clinical and environmental fungi

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The evolution of resistance to antifungal drugs in fungi, specifically to azoles such as fluconazole and polyenes such as amphotericin B, has been partially characterized in several clinical and environmental studies, but variation in the main target of these drugs, the ergosterol synthesis pathway, does not explain all resistance. We show that resistance phenotypes across diverse fungal lineages including *Candidozyma auris, Candida tropicalis, Aspergillus fumigatus* and *Nakaseomyces glabratus*, as well as the y1000 dataset of over a thousand species of yeast from both clinical and environmental isolates, can be accurately predicted directly from raw DNA sequencing data using a machine learning statistical algorithm called FLASH that bypasses the need for alignment. This analysis points to novel genes that are predictive of resistance in addition to ones previously associated with resistance, lending support to the hypothesis that mechanisms of resistance may be diverse and vary in different environments and lineages. This method can also be used to survey the landscape of predicted antifungal resistance from leaf and soil metagenomes.

429B

Characterization of global isolates of *Penicillium rubens* and *Penicillium chrysogenum* reveals differing strategies for maintenance of genome plasticity

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The filamentous fungi *Penicillium chrysogenum* and *Penicillum rubens* have impacted human life in tremendous ways through the discovery and production of penicillin. These species were not always considered separate, however, as *P. rubens* was only defined in the early 2010s. To explore the evolution of *P. chrysogenum* and *P. rubens*, we collected isolates from a diverse array of ecological niches, including both natural and built environments to study the impact of isolation source on genetic diversity and secondary metabolism. We generated whole genome sequencing for >80 isolates and downloaded publicly available data for an additional 25 isolates. Some isolates putatively identified as *P. chrysogenum* were instead found to be other species. In addition to the two species, which formed monophyletic clades in our phylogenies, we also discovered three additional clusters/clades possibly representing cryptic species. We also used single nucleotide polymorphisms to explore population structure in *P. chrysogenum* and *P. rubens*, with *P. chrysogenum* exhibiting population structure and *P. rubens* composing a single genetic cluster. Although isolation source was not correlated with population structure, we did identify lineage-specific "Starship" transposable elements, which were more common in *P. rubens* than *P. chrysogenum*. Surprisingly, we saw evidence for a higher recombination rate in *P. rubens* than in *P. chrysogenum*. We also used a subset of 25 isolates to explore differences among secondary metabolites produced by the species. The two species did not produce notably different abundances of shared metabolites,

although several individual isolates did produce unique metabolites, highlighting the variation within species. Our work sheds light on the complexities within the *Penicillium* genus, from which there is yet more to learn.

430B

Living with a killer: how coevolved *Saccharomyces cerevisiae* become killer toxin resistant

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Some yeasts are killers. They secrete toxins that kill neighboring cells but protect themselves with an intracellular antidote. Killers have an advantage in heterogeneous populations, although toxin production comes at a metabolic cost. Many *Saccharomyces cerevisiae* strains require two viral genomes to be killers. Along with sensitive cells in the environment, this is a complex genetic conflict: yeast and viral genomes alike are capable of adaptation and fitness tradeoffs abound at many levels. Our research dissects the molecular basis of adaptation in the face of these competing selection pressures to understand evolutionary outcomes.

We identified beneficial mutations that arose in coevolved killer and sensitive yeast, including mutations responsible for toxin-resistance. Through bulk segregant analysis, we identified a putative gain-of-function missense mutation in the HOG osmoregulatory pathway component *SSK1*. This dominant-acting polymorphism is sufficient to protect yeasts from the coevolved toxin, and other killer toxins, but comes with collateral sensitivity tradeoffs to other anti-fungal treatments. *SSK1* is a member of a two-component system, which are not found in metazoans, and has been implicated in fungal adaptation under other conditions. This data supports two-component systems and SSK1 in particular as appealing antifungal drug targets. *SSK1* is required for multiple drug resistance in *Candida auris*, and our ongoing work will address how this mutant allele might impact phenotypes of fungi associated with human pathogenicity and drug resistance.

Our preliminary data suggest the evolved *SSK1* mutation may affect protein-protein interactions, leading to crosstalk with the Cell Wall Integrity (CWI) pathway. We are currently pursuing the transcriptional and signaling impacts of the toxin-resistant allele, as well as profiling other evolved toxin resistant mutations. In future, we aim to understand how killer yeasts counter-adapt in the face of resistant competitors, and how viral genomes evolve as competing host population composition changes. Understanding how fungi adapt, defend themselves, and kill one another has important implications for health during climate change, as fungal pathogens emerge and drug resistance spreads.

431B

Phyletic Patterns of c-NHEJ Genes Are Reflected in Genomic Signatures in Fungal Genomes

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Double-strand break repair (DSBR) is a fundamental process that maintains genome stability through multiple pathways, including homologous recombination (HR) and classical non-homologous end joining (c-NHEJ). Phyletic pattern of DSBR pathways across hundreds of fungi revealed that a group of early-diverging fungi, Zoopagomycota, completely lacks the c-NHEJ pathway. In addition, a subdivision of Basidiomycota, the Ustilaginomycotina, lacks LIG4, a DNA ligase that is essential for c-NHEJ. We hypothesized that the absence of c-NHEJ would be reflected in in the genome as distinct genomic signatures. To test this hypothesis, we developed a comparative genomic pipeline that detects and classifies insertions and deletions as potential genomic signatures of different DSBR mechanisms. The analysis is based on pairs of genomes from different phylogenetic groups that either possess or lack c-NHEJ genes. We found that, genomes completely lacking c-NHEJ or missing only LIG4 exhibit a significantly higher proportion of variants that are consistent with unequal crossing over, single-strand annealing, replication slippage, and alternative end joining, whereas genomes predicted to retain c-NHEJ activity show more variants that are likely resulting from c-NHEJ. Furthermore, Genomes entirely lacking c-NHEJ tend to have the longest microhomologies, those missing only LIG4 show intermediate lengths, and genomes with a complete c-NHEJ pathway have the shortest. Together, these results suggest that the absence of c-NHEJ in fungal genomes is associated with distinct genomic signatures that are largely shaped by alternative DSBR mechanisms.

432B

Alternative evolutionary trajectories following RNAi loss in *Cryptococcus*

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Cryptococcus neoformans infections cause approximately 15% of AIDS-related deaths owing to a combination of limited antifungal therapies and drug resistance. A collection of clinical and environmental *C. neoformans* isolates were assayed for increased mutation rates via fluctuation analysis, and we identified two hypermutator *C. neoformans* clinical isolates with increased mutation rates when exposed to the combination of rapamycin and FK506. Sequencing of drug target genes found that Cnl1 transposon insertions conferred the majority of resistance to rapamycin and FK506 and could also independently cause resistance to 5-fluoroorotic acid and the clinically relevant antifungal 5-flucytosine. Whole-genome sequencing revealed both hypermutator genomes harbor a nonsense mutation in the RNA-interference component Znf3 and hundreds of Cnl1 transposons organized into massive sub-telomeric arrays on each of the fourteen chromosomes. Quantitative trait locus mapping in 28 progeny derived from a cross between a hypermutator and wild-type identified a locus associated with hypermutation that included *znf3*. CRISPR editing of the *znf3* nonsense mutation abolished hypermutation and restored small-interfering-RNA production against the Cnl1 transposons. We conclude that hypermutation and drug resistance in these clinical isolates results from loss of RNAi and accumulation of Cnl1 transposons. In further studies, two bioinformatic pipelines were developed and employed to identify other naturally occurring loss of RNAi isolates. These isolates do not display a hypermutator phenotype and have not accumulated a substantial transposon burden. By CRISPR genome editing we have confirmed that the RNAi loss mutations in these isolates indeed result in loss of RNAi function. Following a genetic cross of these RNAi loss isolates with a mating partner bearing a transposon burden, F1 progeny were obtained that exhibit a marked hypermutator phenotype. These studies illustrate distinct evolutionary trajectories following loss of RNAi, and alternative routes via which loss of RNAi can lead to hypermutator phenotypes.

433B

Phased nuclear and mitochondrial genome assemblies of *Cryptococcus* A/D hybrids using long-read sequencing and trio-binning

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Hybridisation between *Cryptococcus* lineages is common in both environmental and clinical settings and has been linked to altered virulence and antifungal susceptibility. However, the highly heterozygous, mosaic genomes of A/D hybrids are challenging to assemble and phase, limiting our understanding of their genome architecture and evolution.

Here we generated Oxford Nanopore long-read data for four *Cryptococcus* A/D hybrids and used a hifiasm trio-binning strategy informed by A and D parental references. Reads were assigned to the A- or D-derived haplotype and assembled separately, yielding two highly contiguous, near-complete haploid assemblies per hybrid.

We also identified mitochondrial contigs and processed them with Circlator to generate circularised mitochondrial genomes of ~29–33 kb with gene content and organisation consistent with previously described *Cryptococcus* mitogenomes.

These data provide a detailed view of how hybrid genomes are structured and reshaped following hybridisation and demonstrate that combining Nanopore long reads with trio-binning enables robust phasing of *Cryptococcus* hybrid genomes. These assemblies provide a resource for dissecting the genetic and mitochondrial basis of hybrid fitness, adaptation and pathogenesis in *Cryptococcus*.

434B

Malassezia parasexual hybridization, yeast-to-hyphae transition, and pathogenesis

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The lipophilic yeast *Malassezia* is the most abundant member of the skin mycobiome, representing over 90% of all commensal skin fungi. While primarily commensal, *Malassezia* has also been associated with skin disorders including seborrheic dermatitis (SD), atopic dermatitis (AD) and pityriasis versicolor (PV), with the hyphal form often observed in diseased lesions. However, the role of *Malassezia* hyphae in pathogenesis as well as the mechanisms underlying their formation remains poorly understood. Recent genomic studies have revealed natural *Malassezia furfur* hybrids arising from independent hybridization events, suggesting that genome plasticity and noncanonical reproductive modes contribute to its evolution. Here we defined the conditions that promote yeast-to-hyphae transition in natural diploid strains of *M. furfur*, and found that the presence of a complete mating type system is a requisite for hyphal differentiation. We used these conditions to interrogate the genetic requirements for hyphal formation in both intra- and interspecific crosses of *M. furfur* haploid strains. Interspecific mating resulted in the generation of diploid heterozygous hybrids, some of which exhibited tracts of loss of heterozygosity consistent with recombination, while intraspecific mating resulted in cell-fusion products that undergo recombination and a process of haploidization that seems consistent with a parasexual cycle. Functional analysis of the *M. furfur* mating-type (*MAT*) genes revealed a role for the *MAT A (*pheromone/receptor, *P/R*) locus in cell recognition and fusion, and a role for the *MAT B (*homeodomain*, HD)* locus in hyphal development, although *MAT B* appears to have lost its mating-type determination function, as a single *HD* allele is sufficient for hyphal formation. Lastly, both in vitro and in vivo skin colonization models demonstrated that *M. furfur* hyphae display a greater capacity to invade the epidermis when compared than yeast cells, triggering a stronger inflammatory host response, indicating their contribution in pathogenesis. In conclusion, this study unveils a novel role of the *MAT* loci and mating in *Malassezia* leading to parasexual reproduction and the formation of hyphae that mediate pathogenic interactions with the host.

435B

Human-mediated hybrid zones between amphibian-killing chytrid lineages produce second generation recombination

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Global biodiversity is under threat from introductions of non-native fungal disease. The pathogenic chytrid, *Batrachochytrium dendrobatidis* (Bd), causes chytridiomycosis – the infectious disease implicated in frog, toad, and salamander population declines worldwide. Where this fungus has been introduced, a single hypervirulent strain (Bd-GPL) proliferates through host populations. In the southern Atlantic Forest of Brazil, recent human introduction brought the globally invasive Bd-GPL strain into secondary contact with a distantly related, endemic strain, Bd-Brazil. In most anthropogenically mediated secondary contact scenarios such as this one, the epidemiological and evolutionary consequences of strain interaction remain unknown. We found that Bd, long considered obligately asexual, is capable of second-generation hybridization following the human induced contact of divergent lineages. Using whole-genome sequencing of fungal isolates cultured from wild-infected Brazilian frogs, we characterize the hereditary relationships among pathogen populations in this contact zone. Our analyses reveal regions of the Bd genome that are potentially driving adaptive variation among invasive and endemic strains. The patterns of hybrid inheritance we observe offer new insights into the genetic underpinnings of fungal reproductive isolation, the process which ultimately results in speciation of emerging fungal diseases. Finally, we compare our hybrid sequences to a newly discovered zone of Bd hybridization in South Africa and reveal parallel patterns of second-generation introgression. These newly observed patterns of hybridization we describe are of particular ecological and evolutionary concern because they demonstrate the ability of anthropogenic change to drive novel recombinant genetic variation in a deadly pathogen. These findings show how humans are actively creating new evolutionary trajectories for emerging diseases, such as chytridiomycosis, by creating novel mating opportunities between previously allopatric strains in different regions of the globe.

436B

PCR-based differentiation of *Aspergillus* oryzae and aflatoxigenic *Aspergillus* flavus targeting the aflatoxin biosynthesis gene cluster

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Aspergillus oryzae is an industrially important fungus widely used in the production of traditional fermented foods and beverages in Korea and other East Asian countries. *A. oryzae* belongs to *Aspergillus* section *Flavi*, which also includes *A. flavus*, a species known for producing carcinogenic mycotoxins. Although *A. oryzae* and *A. flavus* are particularly closely related species within *Aspergillus* section *Flavi*, they differ markedly in their ability to produce aflatoxin, a carcinogenic mycotoxin synthesized by the aflatoxin biosynthesis gene cluster (ABGC). To investigate the genetic basis underlying this difference, we conducted a comparative analysis of the ABGC across 30 strains of *Aspergillus* section *Flavi*. Our analysis revealed both large-scale and locus-specific deletions within the ABGC between the two species. Based on these species-specific deletion trends, we designed four primer sets targeting these unique deletion regions within ABGC to distinguish non-aflatoxigenic *A. oryzae* and aflatoxigenic *A. flavus*. Validation using both PCR and in silico PCR analyses showed that these primer sets successfully differentiated the two species just by comparing PCR amplicon sizes with high accuracy. The designed primer sets provide a rapid, accurate, and practical molecular tool for distinguishing *A. oryzae* from *A. flavus*.

437B

De novo chromosomal-level assemblies of *Coccidioides* species reveal inter-species and intra-species genomic rearrangements

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Coccidioides is a dimorphic fungal pathogen that causes coccidioidomycosis (Valley Fever) upon inhalation of infectious spores (arthroconidia) from the soil by humans. It is estimated that tens of thousands of individuals are diagnosed with Valley Fever every year in the Southwest and Pacific United States. The rising incidence of Valley Fever and the geographical expansion of *Coccidioides* highlight the importance of the characterization of the diversity of this pathogen. To better understand the genomic variations, we employed comparative genomics between two geographically distinct species (*Coccidioides immitis* and *Coccidioides posadasii*). Specifically, we used long-read sequencing to provide chromosome-level assemblies of commonly used *Coccidioides isolates* (*C. immitis* RS, *C. posadasii* C735, *C. posadasii* C735Δcts2Δard1Δcts3 and *C. posadasii* Cp1038). In this study, we included the attenuated *C. posadasii* C735Δcts2Δard1Δcts3 mutant strain to evaluate any potential genomic rearrangements due to serial passaging of the cultures during the deletion of three genes. We performed whole-genome alignments to compare chromosome-level architecture and identify structural variants, including inversions and translocations to assess genomic variations of the *Coccidioides* strains. We then generated a single nucleotide polymorphism (SNP) distance matrix to perform phylogenetic analyses to understand the evolutionary relationships between the newly assembled genomes and the existing *Coccidioides* genomes available in the NCBI database. Overall, the high coverage and contiguity of our assemblies allowed us to do chromosome-scale structural analyses with SNP-based phylogenetic resolution, which revealed translocations and inversions within and across *Coccidioides* species. In addition, our preliminary results show that there are no major rearrangements and only 41 SNPs between the *C. posadasii* C735Δcts2Δard1Δcts3 mutant strain and the parent *C. posadasii* C735 strain, suggesting that genome assemblies based on long-read sequencing can provide a fast and reliable tool to assess genome rearrangements during mutant strain construction. Furthermore, these assemblies serve as high-quality references for researchers to investigate how structural variations influence gene function and expression, virulence traits, and host adaptation in *Coccidioides*.

438B

Whole genome similarity provides a robust framework for classification of fungal species and their variants

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Fungal species definitions have evolved over time whereby morphological, ecological, geographic, sexual compatibility, and phylogenetic data have been considered to varying extent. Historically, these methods have been heterogeneously applied to define fungal taxa, and each method has drawbacks in terms of resolution, throughput, and/or culture requirements. More recently, mycologists increasingly rely on DNA sequences, with advancements in high-throughput sequencing consistently increasing the availability of whole genome sequences providing a growing resource for genomic investigation. Whole genome similarity has been used to delineate bacterial species for decades, but given differences in gene flow and reproductive isolation it is not clear that fungi, and eukaryotes more generally, share the same relationship between genome sequence divergence and speciation. In this work, we explore how whole genome similarity can be applied for delimiting fungal species and strains. Genomes from a diverse array of taxa across the fungal kingdom and encompassing over 1000 isolates are analyzed using the rapid whole genome comparison tool sourmash to compute average nucleotide identity (ANI) as a measure of whole genome similarity to establish if ANI permits accurate species and strain level identification. Taxonomic classifications based on whole genome similarity are compared to more conventional phylogenomic approaches like orthologous gene trees and SNP-based trees. Successful classification of fungi using ANI would permit isolate classification with greater speed and throughput compared to current phylogenomic methods, especially when coupled with rapid, scalable whole genome computations. Finally, we explore how whole genome similarity relates to speciation across the fungal kingdom by considering the magnitude of genome sequence divergence between closely related species, as well as genome divergence at higher taxonomic ranks as well as within species, and we consider if ANI might be applied for classification of novel taxa.

439B

Telomere-to-telomere pangenome of 60 *Aspergillus oryzae* strains enabled by a new robust DNA extraction protocol

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Long-read sequencing has made chromosome-scale fungal genomes technically accessible, yet most filamentous fungi genomes remain fragmented because obtaining sufficiently long, intact genomic DNA is still a major bottleneck. Existing extraction protocols typically rely on harsh mechanical or chemical lysis, compromising DNA length, purity, or yield and limiting routine production of telomere-to-telomere (T2T) assemblies at scale.

Here we present a simple enzyme-based extraction protocol that consistently produces high-molecular-weight DNA from *Aspergillus oryzae*. Using this method, we generated *de novo* T2T assemblies for more than 60 *A. oryzae* strains, representing the largest telomere-resolved genome collection reported for a filamentous fungus.

This dataset reveals a rich landscape of structural variation at chromosome and subchromosomal levels. These structural differences provide a framework to relate genome architecture to the diversity of morphological and metabolic traits observed across *A. oryzae* strains, and to address long-standing questions about its domestication and genomic distinction from the closely related species *Aspergillus flavus*.

The T2T assemblies enable detailed analysis of other genomic features, including the presence, conservation, and rearrangement of biosynthetic gene clusters, such as the highly carcinogenic aflatoxin, a defining aspect of the *A. oryzae* / *flavus* differentiation. They could also be leveraged to investigate the role of transposable elements or other gene transfer mechanisms in this structural diversity.

Finally, our protocol relies on inexpensive reagents and standard workflows, making it easily transferable to closely related *Aspergillus* species and possibly other filamentous fungi and opening the door to routine structure-resolved pangenomes.

440B

Signs of ongoing reproductive incompatibility in *Aspergillus fumigatus*

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Reproductive isolation is the inability of two populations to produce viable, fertile, sexual offspring due to biological barriers. In Aspergillus fumigatus, an important human fungal pathogen, studies have shown that antifungal resistant isolates cluster separately from sensitive isolates in phylogenetic analyses irrespective of geographic location, indicating a lack of current gene flow. Reproductive isolation could occur through prezygotic barriers, which prevent fertilization, or postzygotic barriers which act after a hybrid zygote has formed. Crosses within- and between-clusters are equally fertile, indicating a lack of prezygotic barriers.

To identify potential reproductive barriers, we used 102 isolates to perform 286 sexual crosses, 60 of which produced offspring. Of the 60 crosses, 13 showed unfit progeny with reduced mycelial growth, no sporulation, or both. We identified three main phenotypic segregation ratios: (a) 1:1 segregation of sporulating and non-sporulating offspring, (b)1:1:1:1 segregation involving differences in both sporulation and size, and (c) a small percentage (5–20%) of non-sporulators. These repeated phenotypic ratios do not appear linked to resistance background, equally found within and between cluster crosses. Surprisingly, these phenotypes are not found in either parent (both wild type), pointing towards a non-Mendelian mode of inheritance. We hypothesize that differential adaptation in phylogenetic clusters may lead to low-fitness hybrid progeny, which are outcompeted in nature.

To identify the genetic elements causing this segregation, we used BSA on a cross showing the 1:1 ratio of sporulating and non-sporulating offspring. We identified a 70-kb locus on chromosome 1 linked to the phenotype, containing 19 genes. Notably, in the brlA gene, a known regulator of asexual development, all non-sporulating offspring carried an identical deletion of ~45 bp, not shared with the sporulating offspring. This deletion is not found in either parent but only occurs in one parental genome. BSA analysis of the 1:1:1:1 ratio of offspring indicates a mechanism outside of brlA. Crosses of closely related species also show the same phenotype, but BSA also indicates a non-brlA mechanism. Our data shows for the first time a post-zygotic reproductive mechanism, that is surprisingly widespread in a sexually reproducing species. The importance of this mechanism will depend on the contribution of sexual and asexual reproduction in this species.

441B

Estimating the distribution of fitness effects of loss of heterozygosity (LOH) events using an engineered library of *Saccharomyces cerevisiae*

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Loss of heterozygosity (LOH) is a large contributor of genetic variation in natural populations or lab-evolved asexual diploids. However, its contribution to adaptation is uncharacterized because the full spectrum of its fitness effects remains largely unknown. To systematically investigate the distribution of fitness effects (DFE) of LOH, we engineered a diverse, barcoded library of heterozygous diploid *Saccharomyces cerevisiae* strains, each containing randomly induced LOH events. By employing competitive fitness assays and barcode sequencing (Bar-seq) across seven distinct environments, including various stressors from chemicals, temperature, and an *in vivo* host model, we quantified the fitness consequences of LOH events. Our results reveal that the DFE of LOH is predominantly neutral to deleterious, with a general trend of decreasing fitness correlated with larger cumulative lengths of LOH tracts. However, the fitness effects were variable and the fitness landscape was highly environment-dependent. While beneficial LOH events were rare and of small effect in standard rich media, they were common and conferred substantial fitness gains in novel or stressful conditions. A key finding was the

prevalence of antagonistic pleiotropy where over 75% of strains exhibited fitness trade-offs, gaining an advantage in one environment at the cost of fitness in others. This strength of trade-off was also observed to be correlated with longer LOH tracts. Overall, this work demonstrates that LOH plays a dual role in evolution. While it often imposes a genetic burden, it also provides a powerful mechanism for rapid, environment-specific adaptation, driving specialization by trading general robustness for niche-specific advantages.

442B

Genome-wide association of nutrient-use and pathogenicity in Botrytis cinerea

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Botrytis cinerea is a generalist necrotrophic fungal pathogen capable of infecting thousands of plant species, potentially requiring high metabolic flexibility to acquire nutrients, tolerate stress, and overcome host defenses. Despite the ecological and economic importance of host-generalist pathogens, the genetic basis of nutrient-use strategies and their relationship to virulence remains poorly understood. This project investigates how variation in nutrient-use diversity correlates with virulence traits. Using 96 whole-genome-resequenced *B. cinerea* isolates, we quantified in vitro growth and metabolic activity across 31 carbon sources, using fluorescence imaging and resazurin-based respiration assays. Genome-wide association (GWA) and phenotype correlations with existing virulence trait data identified co-localized loci underlying nutrient-use diversity and pathogenicity, indicating potential trait trade-offs. By linking metabolic flexibility to pathogenic fitness, this work aims to reveal the evolutionary pressures shaping trait variation in generalist pathogens and understand novel elements of pathogenicity.

443B

Comparative genomic approaches between cuticular wax composition of Populus species and germ tube length of Sphaerulina musiva enlightened promising genomic regions.

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Sphaerulina musiva (syn = Septoria musiva) is the causal agent of Septoria leaf-spot and stem canker disease of Populus species and their hybrids. This fungus is endemic to the range of Populus deltoides in the eastern USA and has been introduced to the Pacific Northwest where it can infect the native host Populus trichocarpa. Molecular interactions between the host and the pathogen remain poorly understood. Greenhouse, field, and lab experiments have revealed a correlation between cuticular wax composition and susceptibility to this disease. Indeed, Populus accessions with a greater abundance of alkenes in their cuticle controlled by KCS1 gene are more susceptible to leaf spot disease. Scanning electron microscopy, producing high quality images, and accurate measurements, showed that the germination of Sphaerulina spp spores was faster on wild type poplar accession than on KCS1 knock outs that do not produce alkenes. Alkene treatment of the S. musiva isolates grown on Petri-dishes significantly increases the germ tube length relative to untreated accessions. Furthermore, the growth of the germ tube under the alkene treatment is isolate-dependent and showed a great diversity among the isolates. Comparative genomics approaches on 108 Sphaerulina musiva isolates from the three different sub-populations in North America revealed different genomic regions associated with the germ tube length trait. The most interesting region is on chromosome 3. In this genomic region, there is a gene encoding an Opy2 protein that acts as a regulator of the filamentous growth pathway. Another genomic region on chromosome 6, showing a significant association, presents a relevant candidate gene with epoxide hydrolase function. We hypothesize that the protein encoded by this gene may oxidize epoxides transformed from alkenes by P450 monooxygenease.

444B

Global analysis of genotypic and phenotypic variation in an NIH collection of 160 *Candida albicans* patient-derived isolates with paired clinical metadata

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Candida albicans is a common member of the human mucosal microbiota and an opportunistic pathogen, capable of causing mucosal and systemic disease. To improve our understanding, diagnosis, and treatment of *C. albicans* disease, a key question remains: how the host and the fungus directly contribute to this opportunistic pathogenesis. At the NIH Clinical Center, a library of 160 *C. albicans* isolates has been curated in a collection that includes detailed clinical metadata. These strains were isolated from 34 patients across 16 anatomical sites, including multiple isolates collected temporally within the same patient, from both commensal and disease states. A subset of these isolates (n=69) underwent whole-genome sequencing, and all isolates were analyzed for phenotypic heterogeneity. Strains were evaluated for their growth, morphogenesis, antifungal susceptibility, expression of key virulence-related genes, recognition and killing by human neutrophils and macrophages, and induction of pro-inflammatory mediators by human epithelial cells. Interestingly, a wide range of phenotypes were observed, with no clear clustering based on anatomical site of isolation or disease state. Even within a given host, strains were not necessarily concordant for phenotypes or clonality. For example, one patient with APECED syndrome, an inborn error of immunity caused by AIRE deficiency that manifests with mucosal candidiasis and multiorgan autoimmunity, had two *C. albicans* isolates collected one day apart from the esophagus and the mouth. Despite the oral isolate being constitutively hyphal with reduced neutrophil killing and broad-spectrum antifungal resistance, the esophageal isolate had no altered morphogenesis or decreased susceptibility to antifungals. Ongoing work is continuing to analyze phenotypic and genotypic data to identify any potential genetic basis for these results. Collectively, this work aims to define the multifaceted elements that contribute to phenotypic variation and pathogenesis in the leading human fungal pathogen, *C. albicans.*

445B

Homothallic or heterothallic? A genomic investigation into the sexual capabilities of the ascomycete fungus *Clonostachys rosea*

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Sexual reproduction in filamentous ascomycetes is governed by the mating-type (MAT) locus, which determines whether species reproduce by outcrossing (heterothallism) or via selfing (homothallism). The ascomycete fungus *Clonostachys rosea* is a cosmopolitan mycoparasite and biocontrol agent, and has been described as homothallic. However, previous population genomic analyses reported rapid linkage disequilibrium (LD) decay, suggestive of outcrossing. To reconcile this, we sequenced and assembled 79 *Clonostachys* genomes, including 66 *C. rosea* strains sampled worlwide, to characterize MAT locus organization, infer reproductive mode, and examine genome-wide consequences of sexual strategy. We found that 52 *C. rosea* strains contain either *mat1-1* or *mat1-2* idiomorphs, consistent with heterothallism, whereas eleven strains carry an expanded MAT locus containing both *mat1-1* and *mat1-2* genes, indicating homothallism. Phenotypic assays confirmed that only homothallic strains produce perithecia in single culture. Phylogenomic and population structure analyses revealed that all homothallic strains form a well-supported monophyletic lineage, suggesting a single origin through unequal crossing-over. LD decay was slightly slower in homothallic strains (half-decay ≈1150 bp) than in heterothallic strains (≈1050 bp), supporting reduced recombination in the homothallic lineage relative to the outcrossing populations. Together, these results show that *C. rosea* contains both heterothallic and homothallic lineages, with heterothallism representing the ancestral state in *Clonostachys* and homothallism arising once in a derived lineage. We further propose that homothallism evolved recently in the evolutionary history of *C. rosea*, likely in South America, followed by intercontinental dispersal.

446B

Epigenetic regulation of *Starship* activity in *Pyricularia oryzae*

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Pyricularia oryzae (synonym *Magnaporthe oryzae*) is the causative agent of blast disease and a multi-crop threat to global food security. Many *P. oryzae* strains reproduce asexually and therefore require the creation of genetic variation without a sexual cycle. For instance, strains of *P. oryzae* have an association between genomic regions with increased genetic variation and specific histone modifications, but the mechanism and causality driving the association remains unknown. *Starships* are a recently discovered group of giant fungal transposons that mobilize genes as cargo and can horizontally transfer within and between species, thus making *Starships* a potential source of genetic diversity in asexual pathogens. To understand if *Starships* impact *P. oryzae* evolution, *Starships* were annotated and characterized in 13 diverse *P. oryzae* strains. Two distinct families of *Starships* and related *Starship*-like regions were identified that differed in their activity, sequence evolution, and epigenetic modifications. While many elements were enriched for heterochromatic histone modifications, one family largely lacked such epigenetic marks. *Starships* from this family have repeatedly transferred across the *Pyricularia* genus, exhibit higher rates of structural variation, and tend to be depleted of repeat-induced point mutations. However, contrasting histone modification profiles within this family suggests that *P. oryzae* genomes may be targeting *Starships* to repress their activity. Our findings demonstrate that the epigenetic modifications associated with *Starships* underpin the impacts of these giant transposons on fungal genome evolution and biology.

447B

Transposable elements drive evolution and alternative virulence strategies in *Colletotrichum lupini*

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Colletotrichum lupini is the sole species within its clade capable of infecting lupin, exhibiting remarkable genomic plasticity that underpins its host specialization. Through comparative genomic analysis with non-pathogenic relatives, we reveal how transposable elements (TEs) architect a distinctive "two-speed genome" that facilitates rapid evolutionary innovation and pathogenic adaptation.

Our analysis reveals a diverse TE repertoire distributed throughout the genome. Notably, many lineage-specific regions (LSRs), genomic compartments unique to *C. lupini* that serve as evolutionary hotspots for gene gain and loss, are associated with Starship elements. These recently characterized large mobile elements play a pivotal role in reshaping the genomic landscape by harboring and potentially mobilizing entire gene clusters, often related to fitness.

A striking example of this TE-driven innovation is a hybrid polyketide synthase-nonribosomal peptide synthetase (PKS-NRPS) gene cluster that bears signatures of horizontal gene transfer and resides within a Starship element. This species-specific BGC, compartmentalized within an LSR, represents a departure from canonical effector-based pathogenicity strategies. Instead, *C. lupini* employs an alternative virulence mechanism centered on secondary metabolite production, a strategy that may confer selective advantages in its specialized lupin niche.

Through TE-mediated compartmentalization, mobile elements can serve as architects of pathogen evolution, creating genomic neighborhoods where acquired virulence factors are insulated from purifying selection in the stable core genome. Our findings illuminate how the interplay between TEs, horizontal gene transfer, and genome architecture shapes host range determination and pathogenic potential in fungal plant pathogens. Ongoing functional characterization of candidate BGC-encoded metabolites will reveal whether this compartmentalized architecture directly contributes to host-specific virulence, providing insight into how genomic organization translates to pathogenic outcomes.

448B

Lineage-specific expansion of MoTeRs shapes subtelomeric architecture in *Magnaporthe oryzae*

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Magnaporthe oryzae is a filamentous ascomycete that infects more than 50 grass species and causes major diseases such as rice blast and wheat blast. Genomic plasticity in this species is tightly linked to transposable elements (TEs), which are highly enriched in subtelomeric regions that harbor diverse avirulence genes. Previous population-level studies have shown that TE insertion polymorphisms shape gene gain and loss, regulatory diversification, and the emergence of host-specialized lineages. Here, we focus on a family of telomere-targeted non-LTR retrotransposons known as MoTeRs (*Magnaporthe oryzae* Telomeric Retrotransposons). These elements are known to destabilize chromosome ends, promote ectopic recombination, and drive rapid evolution of subtelomeric sequences. To examine how MoTeRs activity and structural variation (SV) contribute to host diversification, we analyzed complete *M. oryzae* genome assemblies from eight host-specialized lineages. We found that Triticum- and Lolium-associated lineages show pronounced MoTeR accumulation at chromosome ends despite having lower genome-wide TE content. These lineages also exhibit a reduced overall SV burden compared with high-TE lineages, suggesting more localized rather than genome-wide structural remodeling. Mapping MoTeRs-associated SVs identified 81 *Magnaporthe* Effector Protein (MEP) genes impacted by rearrangement, linking MoTeR activity directly to effector diversification. Finally, analysis of Illumina assemblies from 29 additional lineages revealed strong lineage-specific patterns of MoTeR expansion and contraction across the species complex. Together, these findings support a model in which MoTeR-driven telomere instability and subtelomeric SVs generate highly dynamic genomic niches that facilitate effector diversification and contribute to the evolution of multiple host-specialized lineages within *M. oryzae*.

449B

Evaluation of a candidate MAT locus for *Linnemannia elongata* (Mortierellomycotina) using quantitative PCR methods

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Mortierellomycotina is a subphylum of fungi with beneficial, non-mycorrhizal interactions with plant roots. These ubiquitous organisms are sexual and produce meiospores (zygospores) with compatible partners or in a homothallic manner, depending upon the species. For the related Mucoromycotina (Mucoromycota), sexual compatibility is regulated by two idiomorphic high-mobility group (HMG) transcription factors (TFs), and involves the exchange of metabolic derivatives of beta-carotene, resulting in a functional, small organic pheromones called trisporic acids (TSAs). In the Mucoromycotina, the mating-type (MAT) locus structure underlying sexual compatibility is well described, yet the genetic source of compatibility in Mortierellomycotina has remained elusive to BLAST-based search approaches. Previously, we leveraged genomic data from *Linnemannia elongata* isolates with known compatibility interactions to identify candidate MAT loci for Mortierellomycotina. This candidate MAT locus contains either a HMG TF or homeodomain (HD) TF, and TF content correlates well with zygospore production data. We hypothesize that MAT locus TFs will be highly transcribed in sexual mating interactions compared to the same isolates cultured alone. To test this hypothesis, we collected mRNA and quantified MAT locus TF transcript abundance from two *L. elongata* isolates (JKU1 and JKU4) in monoculture and in coculture, using novel primers. Here, we present the preliminary results from these qPCR assays and discuss future directions for elucidating Mortierellomycotina sexual reproduction.

450B

Genomic characterization of Japanese *Syzygites* strains, mycoparasitic Mucoromycetous fungi

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Background: *Syzygites megalocarpus*, a mycoparasitic mucoromyceteous fungus, is monotypic and parasitic on the fruiting bodies of diverse basidiomycetes. This homothallic species was the first fungus in which sexual reproduction was observed. Previous studies have mainly reported on strains isolated from North America, whereas little is known about Japanese isolates. We previously collected 13 *Syzygites* strains from six locations in Japan and characterized their morphological, physiological, and molecular phylogenetic traits. NBRC 117016 and NBRC 117017 differed from *S. megalocarpus* in sporangiospore formation, pigment production, and ITS-based phylogeny. In this study, we sequenced the genome of the two Japanese strains and NBRC 9099 and then compared them with a CBS strain to characterise their genomic features.

Methods: Genomic DNA from strains NBRC 117016, NBRC 117017, and NBRC 9099 were sequenced using PacBio Revio platform. HiFi reads were assembled using Hifiasm, and the quality of the assembly was evaluated using QUAST and BUSCO. Gene prediction and functional annotation were performed using Funannotate. Genome similarity was assessed using FastANI, and orthologous genes were identified using OrthoFinder. SNP-based phylogenetic analysis was conducted using IQ-TREE, and variant effects were predicted with SnpEff. Pigment production was qualitatively identified by HPLC.

Results: Assemblies were of high quality, consisting of 140–242 contigs, with genome sizes of approximately 27 Mbp, and BUSCO completeness of 96.7–97.1%. NBRC 9099 contained 10,087 predicted genes, whereas the two Japanese strains had approximately 9,800, fewer than CBS 108947 (10,394). ANI analysis revealed 97.4% similarity between CBS 108947 and NBRC 9099, whereas similarity between CBS strain and the two Japanese strains were 92.1 - 92.2%, indicating lower genome-level homology. Phylogenetic analysis clearly separated Japanese two strains from CBS 108947 and NBRC 9099. About the two Japanese strains, OrthoFinder identified 376 unique genes, including those involved in osmotic stress tolerance such as *SRK1*/*SAC7*, and SnpEff revealed ~ 9,000 high-impact variants, including *TYR1* involved in pigment production. Correspondingly, L-DOPA, a precursor of eumelanin, was

detected in NBRC 9099, whereas it was not detected in NBRC 117017. These results showed the two Japanese strains have genomic features consistent with the suggested endemic species of Japan. This study was partly supported by a funding of the Japan Patent Office.

451B

Genetics of thermotolerance in *Neurospora discreta*

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In the western United States, *N. discreta* ranges from New Mexico to Alaska, providing a model for understanding how fungi adapt to climate differences and environmental change. Race tube experiments demonstrated that an *N. discreta* isolate from New Mexico had a significantly higher growth rate at 42°C than did an isolate from Alaska. A sexual cross between the two isolates demonstrated that the difference in thermotolerance was heritable and that multiple regions of the genome contribute to temperature growth rates. Whole genome sequences were obtained from both thermotolerant and non-thermotolerant progeny to identify potential genes associated with differences thermotolerance. Gene expression analysis revealed substantial differences in how the parental isolates responded to heat stress. Taken together, genomic sequence and expression data indicate that thermotolerance in *N. discreta* is the result of a mixture of genetic mutations and differences in gene regulation. These results therefore provide insights into how lineages within a species or species complex can adapt to environments with substantially different average annual temperatures. Many of the genes identified in this study have unknown functions and prioritizing the functional characterization of these genes is a crucial next step in understanding these adaptive differences.

452B

A telomere-to-telomere gapless genome assembly and mycotoxin potential of *Fusarium sporotrichioides* from Barley (*Hordeum vulgare*)

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Fusarium Head Blight (FHB) is a devastating disease that can cause significant yield losses in small grains. Several *Fusarium* species cause FHB, including *F. sporotrichioides*. Although a few genome assemblies exist for this species, a telomere-to-telomere assembly has yet to be produced, and the genome of this species isolated from barley remains unavailable. Here, we report the telomere-to-telomere genome assembly of *Fusarium sporotrichioides* isolate B322 from discolored barley spikes in McIntosh County, ND. The genome of F. sporotrichioides B322 is 38.0 Mb with an N50 of 8.9 Mb and a GC content of 47.95%. The assembly contains six contigs representing five chromosomes and one mitochondrial genome. Our genome assembly is 99.9% complete according to BUSCO analysis, with 2.18% repetitive content. Inoculations of strain B322 in barley spikes, wheat spikes, and oat kernels show very high HT-2 levels in oats (30.1 ppm) and elevated levels in barley (6.7 ppm) and wheat (6.8 ppm). For T-2, oats exhibit very high concentrations (60.4 ppm), barley shows high levels (5.4 ppm), but wheat has very low levels (0.638 ppm). This high-quality genome and improved trichothecene cluster for *F. sporotrichioides* provide an invaluable resource for exploring the genomic features of this pathogen and other *Fusarium* species responsible for FHB.

453B

Aspergillus fumigatus Af293/CEA10 community sequencing initiative

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The model *Aspergillus fumigatus* isolates Af293 and CEA10 have been the workhorses of fungal research for decades. As these strains have been independently cultured in laboratories across the globe, they have likely accumulated unique, unstudied mutations. Understanding the extent of this "lab-to-lab" genomic drift is crucial for reliably comparing findings on physiology, pathogenicity, and genetics across different research groups.

While community sequencing projects have been conducted for other model organisms, this effort is the first of its kind for a filamentous fungus. Our goal is to quantify the genetic divergence between parental Af293 and CEA10 strains currently in use globally and to create a foundational genomic resource for the entire *Aspergillus* community. All raw and processed sequencing data will be provided back to the contributing labs. This presentation will provide updates on the initiative's progress and details on how the community can participate by submitting their own Af293 and CEA10 isolates for sequencing.

454B

WTF: What the *Fusarium* is happening on winegrapes?

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Fusarium fujikuroi (*Ff*) is a well-known plant pathogen causing the "foolish seedling" bakanae disease in rice, which can lead to economically significant levels of plant lodging through the overproduction of gibberellic acid (GB). *Ff* can also produce fumonisin (FUM) mycotoxins, contaminating food and feed

supplies. Isolates of high-FUM producing *Ff* were collected from *Vitis vinifera* winegrapes from Alabama, Georgia, and North Carolina vineyards. *Ff* co-occurred across vineyards with the closely related, high-FUM producing species, *F. proliferatum* (*Fp*). Considering the novel host and high-FUM nature of the *Ff* isolates, and co-occurrence with *Fp*, we sequenced and assembled the genomes of six *Ff* and six *Fp* isolates to investigate if the winegrape isolates group phylogenetically as expected for these two species. A phylogenomic analysis based on single copy orthologs with several other *Fusarium* outgroup species indicated: 1) there are two metabolically distinct groups of *Ff*, which are composed of GB-producing or non-GB producing isolates, and 2) *Fp* is represented by an even more complex composition of several divergent lineages. Although the winegrape *Ff* isolates were clustered within a well-supported clade, the isolates exhibited relatively high divergence, indicating the presence of several populations. Therefore, we sequenced another 84 *Ff* isolates to investigate the overall population structure and genomic diversity. Analyses suggest there are at least 10 divergent *Ff* populations that are not constrained by geography or winegrape variety, that these populations exhibit high nucleotide diversity, and that mating type frequencies are supportive of clonal lineages. Additionally, we are carrying out a comparative genomic analysis to investigate whether there is evidence of gene flow between the two species using our genome assemblies. These preliminary data indicate the presence of large, non-syntenic structural variants, such as biosynthetic gene clusters and Starships, that may originate from *Fp* and other *Fusarium* species. Overall, this study highlights that there are two distinct groups of *Ff*, that the winegrape *Ff* are genomically diverse, and that the genomic diversity among the winegrape *Ff* isolates may be influenced by gene flow with other *Fusarium* species, such as *Fp*.

455B

Recent lineage diversification and gene flow shape climate adaptation in the Swiss needle cast pathogen of Douglas-fir

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Recurrent epidemics of Swiss Needle Cast (SNC) on *Pseudotsuga menziesii* var. *menziesii* in the Pacific Northwest have intensified alongside climate change, spreading from the Oregon coast into British Columbia. Using whole genome sequencing of the pathogen *Nothophaeocryptopus gaeumannii* (~200 SNC genomes), we identified highly divergent genetic lineages with different levels of suitability to climates. We resolved additional population structure within lineage 1, revealing sublineages 1c (coastal) and 1i (inland). Lineage 2 occurs sympatrically with lineage 1c on the coast, while lineage 1i is primarily associated with *P. menziesii* var. *glauca*, the Rocky Mountain Douglas-fir. Modeling of the demographic and evolutionary history indicated that lineages 1c and 1i diverged from a common ancestor about 20,000 years ago. This coincides with the divergence between the hosts of these lineages after the last glacial maximum. The hypothesis of complete reproductive isolation among these lineages was rejected: we find strong evidence of gene-flow between lineage 1c and 1i, with hybridization events likely happening on trees in the hybrid zone of Coastal and Rocky Mountain Douglas-firs. Similarly, introgressive hybridization from lineage 2 to lineage 1c suggests that genes with adaptive functions are likely transferred between these two sympatric lineages. Our genotype-by-environment association analysis finds that a few climatic variables explain a significant part of the genetic. Lineage 2 is adapted to cooler temperatures in cold months, while lineage 1c contains genomic signatures of adaptation to lower precipitations during summer months and the larger range of temperatures observed inland. These predictions were corroborated by phenotypic data and distribution modeling predicting that lineage 1c has a higher adaptive plasticity to temperatures than lineage 2 and that the current environmental tolerance range of lineage 1c will keep exceeding that of lineage 2. Our results demonstrate that recent diversification, hybridization, and adaptive introgression enhance climate adaptation in *N. gaeumannii* lineages.

456B

Losses of Deeply Conserved Genes in the Mitotic Exit Network Led to Multiple Rewiring Events in Budding Yeasts

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The mitotic exit network (MEN) is a signaling pathway first discovered in the budding yeast *Saccharomyces cerevisiae* where it regulates an essential cell-cycle checkpoint. Intriguingly, components of this pathway are widespread in eukaryotes, but the function has diverged to varying degrees even within fungi. To understand how the MEN has evolved, we identified orthologs of all MEN components across over 1,000 species of Saccharomycotina yeasts and found that the *S. cerevisiae* pathway composition is derived as a key intermediate step in the kinase cascade was lost in the common ancestor of Saccharomycetales and Saccharomycodales order. Furthermore, we discovered that a critical regulatory component has been lost in Phaffomycetales yeasts. This loss led to rapid evolution of the downstream gene with striking functional consequences as revealed by complementation analysis in *S. cerevisiae*. Our results demonstrated that both the composition and function of essential cellular signaling pathways can change drastically during evolution.

457C

The genetics of speciation in *Saccharomyces*

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New species are formed when they become reproductively isolated, usually as a consequence of a combination of isolating barriers. We investigated the potential genetic causes of reproductive isolation between two closely related species of budding yeast, *Saccharomyces cerevisiae* and *S. paradoxus*. First generation hybrids between these species are viable but infertile, with 99% of gametes failing to survive. We find that the primary reproductive barrier is chromosomal divergence, which results in mis-segregation of chromosomes during meiosis due to the rejection of recombination. All chromosomes in *Saccharomyces* yeast are essential, so a gamete missing a single chromosome is inviable. In this system, a lack of recombination is the cause of hybrid sterility. We then break this chromosomal species barrier by restoring recombination in the hybrid, allowing us to uncover evidence of genic incompatibilities between diverged alleles from the two parental species.

458C

Genomic insights into the melanized fungus *Exophiala pacifica* ETNP2018 reveals adaptation to the open ocean

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Despite exhibiting significant diversity and impact in marine biogeochemical cycling, few fungal genomes isolated from the open ocean have been published and characterized. Fungi in the open ocean must adapt to limited organic carbon availability in the water column and intense competition with other microorganisms. To investigate potential fungal adaptations to the open ocean, we isolated a novel species of black yeast-like fungus from the eastern tropical North Pacific (ETNP) oxygen minimum zone, named *Exophiala pacifica* ETNP2018. The genus *Exophiala* consists of pleomorphic, extremotolerant fungi characterized by their melanized cell walls. Here we present and analyze the draft genome of *E. pacifica* ETNP2018 and compare it with 26 representative *Exophiala* genomes spanning terrestrial, host-associated, polluted, and aquatic environments. *E. pacifica* ETNP2018 possesses a 40.3 Mb genome, placing it among the largest in the genus and significantly exceeding the average *Exophiala* genome size. Together with the only other aquatic species, *E. aquamarina*, it forms a distinct clade characterized by expanded gene content. The genome encodes an extensive repertoire of 406 CAZymes, including numerous extracellular glycoside hydrolases and auxiliary enzymes that enable degradation of both terrestrial polymers (e.g., β-1,4-xylan) and marine algal polysaccharides (e.g., laminarin, fucoidan, chitin), linking this fungus to multiple organic carbon pools. Secondary metabolic capacity is similarly enriched, with diverse biosynthetic gene clusters for siderophores, melanin, and carotenoids that likely underpin polyextremotolerance and protection from oxidative and radiative stress. *E. pacifica* ETNP2018 additionally encodes assimilatory nitrate reduction, nitrilase, and a fungal nitric oxide reductase (P450nor), indicating potential contributions to nitrogen cycling and N_2O production in oxygen-depleted waters. The presence of phosphoenolpyruvate mutase and a putative PhnZ-like oxygenase further suggests access to 2-aminoethylphosphonate, implicating this species in marine phosphonate and phosphorus cycling. Collectively, these features reveal an unexpectedly expanded, metabolically versatile genome in an oligotrophic oceanic fungus, contrasting with the streamlining often observed in marine microbes and highlighting a previously underappreciated role for black yeasts in open ocean biogeochemistry.

459C

A new reference graph-pangenome for *Aspergillus fumigatus* recovers gene expression of accessory genes absent from the reference strain

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Fungi harbour an incredible amount of genomics and phenotypic diversity, even within the same species. An example of this is the ubiquitous saprotrophic fungus *Aspergillus fumigatus*, which is responsible for the pulmonary infection aspergillosis, causing about 1.5 million deaths per year. Approximately 31% of the whole gene content of this species is considered accessory, exhibiting a presence-absence variation among isolates. This diversity also extends to phenotypes, with isolates displaying marked variation in their ability to withstand stresses such as iron starvation, hypoxia, and oxidative stress, and potentially making them more prone to infecting humans and/or more resistant to antifungals. Currently, the function of these accessory genes and their potential role in these phenotypic differences are not well understood. Part of this is due to the extensive use of a linear reference during bioinformatics analysis: a single strain of the species -here, Af293- is used as a guide for the mapping of genomics and transcriptomics reads, preventing the analysis of genes absent from this reference.

We propose here a new reference for *A. fumigatus*, called a reference graph-pangenome. Built using 26 genetically diverse long-read assemblies from a global distribution and environmental and clinical origin, the new reference graph-pangenome is almost the double of the current linear reference (55.6 Mb). It contains a total of 14,016 protein-coding genes, including 4,176 absent from the existing Af293 reference. To better characterize these accessory genes, we performed an RNA sequencing of seven different isolates across six different stresses. Using the graph-pangenome as a reference, we were able to recover up to 10% more reads aligned, leading to 984 additional genes expressed absent from the strain Af293. Furthermore, we identified up to 8% more differentially expressed genes in response to these stresses. In future work, we will perform a co-expression network analysis to assign putative functions to these non-functionally characterized genes. Altogether, this new tool provides not only a novel reference for the *A. fumigatus* community, but also establishes a reference pangenome framework for other fungal species, while offering novel insights into the function of accessory genes in virulence or antifungal resistance.

460C

Secondary Metabolism as a Source of Functional Diversity in *Colletotrichum*

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Colletotrichum is one of the most important genera of plant-pathogenic fungi, responsible for destructive diseases such as anthracnoses and rots on a wide range of crops, with significant impacts on global agriculture. The genus comprises over 340 species grouped into multiple species complexes, that display remarkable diversity in host range, geographic distribution, and infection strategies. A key factor underlying this diversity is the production of secondary metabolites, which contribute to fungal ecology, pathogenicity, and interactions with plant hosts, and are encoded by biosynthetic gene clusters

(BGCs). Despite their recognized importance, the biosynthetic potential of *Colletotrichum* and the biological roles of its secondary metabolic pathways remain poorly explored.

In this study, we performed a comparative genomic analysis of representative species from the main *Colletotrichum* species complexes to investigate the diversity, distribution, and evolutionary dynamics of BGCs across the genus. Our results reveal an extensive and highly heterogeneous repertoire of biosynthetic gene clusters, with substantial variation among species that does not strictly mirror phylogenetic relationships. In particular, pathways associated with polyketides and terpenes emerge as major sources of diversification, with several clusters showing lineage-specific patterns that may reflect adaptation to distinct hosts or lifestyles.

Beyond providing an evolutionary framework for secondary metabolism in *Colletotrichum*, this work lays the foundation for a broader, integrative platform to decipher the biological functions of fungal secondary metabolic pathways. By combining comparative genomics with functional, ecological, and host-interaction studies, such a platform will enable the identification of key metabolites involved in pathogenicity, niche adaptation, and host specialization. Ultimately, this knowledge will support the development of innovative strategies for disease management and deepen our understanding of how secondary metabolism shapes the evolutionary success of plant-pathogenic fungi.

461C

Pangeneric analysis of the oyster mushroom family illuminates the mechanisms of evolution of carnivorous mushrooms

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Wood-decaying saprotrophic mushrooms play a major role in the nutrient cycle. However, the decomposition of wood is limited by the availability of nitrogen present in the environment. Several wood-decaying fungi have developed mechanisms to overcome nitrogen limitation. One such mechanism is trapping and consuming microscopic invertebrates, including nematodes. Nematode-trapping fungi have evolved multiple times, including in the oyster mushroom family Pleurotaceae. In Pleurotaceae, each genus has developed unique nematode-trapping strategies. *Pleurotus* produces toxin droplets, which paralyze the nematode upon contact, whereas *Hohenbuehelia* produces adhesive structures to trap the nematodes. Despite its ecological significance, the genetic basis of nematophagy and its evolutionary relationship with other lineages in gilled mushrooms (Agaricales) remain understudied. Here, we use a comparative genomics approach to understand the gene family expansion/contractions as well as the mechanisms of selection of genes associated with nematophagy in Pleurotaceae. We sequenced new genomes from multiple species of *Hohenbuehelia* and *Resupinatus* (a close relative of Pleurotaceae containing non-nematophagous wood decaying mushrooms), and annotated 31 publicly available genomes from other members of the Agaricomycetidae. These data were used to reconstruct robust phylogeny for examining the evolution of nematophagy within the group and understand the evolution of genes associated with nematophagy, such as subtilisins, adhesive proteins, as well as biosynthetic gene clusters that may encode neurotoxins that immobilize nematodes.

462C

The Evolution of Host Preference Across the *Botrytis* Genus

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The genus *Botrytis* consists of over 35 necrotrophic fungal plant-pathogens that cause great economic impact to both the agricultural and floricultural industries. Previous work has established two major clades: a clade of eudicot generalist pathogens and another of monocot specialist pathogens. However, little is known about the evolutionary underpinnings of this clade division and the genetic differences underlying host preference within the genus. Primary metabolic genes are associated with growth and reproduction, while specialized metabolic genes mediate host-pathogen interactions. Both have the potential to influence the spread and virulence of a fungal pathogen. We compared the evolutionary histories of metabolic genes to identify correlations between host range diversification and the evolution of metabolic pathways in *Botrytis*. To make this comparison, we constructed a phylogeny of *Botrytis* using a multispecies coalescence model and whole-proteome sequence data for 20 species within the genus.

463C

Selfish genetic element promotes mitotic stability of a dispensable chromosome in the fungal pathogen *Fusarium oxysporum*

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Many fungal pathogens harbour dispensable chromosomes (DCs) which are non-essential for life. DCs often confer a selective advantage in certain environments such as the presence of the host, but can be spontaneously lost under non-selective conditions. How then is long-term persistence of DCs in natural populations achieved? Here we tested the role of a selfish genetic element known as Spore Killer (*Spok*) in mitotic stability of DCs in *Fusarium oxysporum* (*Fo*). *Fo* chromosome 12 (Chr12) was previously shown to be dispensable during *in vitro* growth of this fungal pathogen and to carry genes involved in the utilization of non-conventional carbon sources. Here we performed serial passaging of a human cornea isolate, MRL8996, and observed a high spontaneous loss rate of Chr12 on solid media containing glucose as the sole carbon source, but not on ribose, pectin or polygalacturonic acid. Furthermore, we found that the spontaneous loss rate of Chr12 on glucose medium was significantly lower in the tomato pathogenic isolate *Fol*4287 compared to MRL8996. Comparative genomic analysis of Chr12 in the two strains revealed that the mitotically stable version from *Fol*4287 harbours a *Spok* gene located within a giant Starship transposon, both of which are absent in Chr12 of MRL8996. Transformation-mediated transfer of the *Fol4287 Spok* gene into Chr12 of MRL8996 led to a significant reduction in mitotic loss rate, whereas no such effect was observed with a *Spok* allele carrying a loss-of-function mutation in the killer domain. Conversely, targeted deletion of the *Spok* element from Chr12 in *Fol*4287 resulted in a significant increase of loss rate. These findings suggest that selfish genetic elements such as Spore Killers can benefit the host genome by counteracting spontaneous loss of DCs under non-selective conditions, thereby contributing to the long-term persistence of conditionally beneficial DCs in fungal populations.

464C

Integrative Multi-Omics Analysis of *Candida albicans* Isolates from Distinct Human Niches

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Candida albicans is a commensal yeast residing in the human gastrointestinal tract but can transition from a harmless organism into an opportunistic pathogen under specific conditions. Previous studies have suggested that genetic and phenotypic variations in *C. albicans* clinical isolates may influence its ability to cause disease. In this study, we hypothesized that *C. albicans* residing in different body niches may adapt distinctly, which could impact their genotypes and phenotypes, including the pathogenic potential of the fungus. *C. albicans* strains were obtained from different body niches: fecal samples and gut mucosa of patients with ulcerative colitis or healthy individuals, as well as blood isolates from patients with candidemia. Phenotypic characteristics such as drug susceptibility to multiple antifungal agents, hyphal formation, biofilm production, and virulence were investigated. Our findings revealed distinct phenotypic traits among the *C. albicans* strains. Strains isolated from the mucosa of healthy individuals exhibited decreased biofilm formation and increased survival rates in the *Galleria* model, while strains from the blood of patients displayed reduced hyphal formation and lower larval survival rates. Genomic analyses, including genome-wide association studies and copy number variation analysis, are currently underway, and the results will be presented. Furthermore, metabolomic profiling to explore potential metabolic distinctions among isolates from different niches is also ongoing, and the results will be demonstrated. Overall, our data suggest that niche-specific environments may drive variation in *C. albicans* isolates

465C

Stage-specific transposase activation as mechanism of genetic variation in the clonally propagating pathogen *Fusarium oxysporum*

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Transposable elements (TEs) are key drivers of genome evolution and adaptation in eukaryotes. The clonally propagating fungus *Fusarium oxysporum* (*Fo*) is a major plant pathogen and an opportunistic pathogen in humans, offering a powerful model to investigate adaptive processes in the absence of sexual recombination. Previous studies revealed that rapid adaptation of the tomato pathogenic isolate *Fol*4287 during short-term experimental evolution is largely driven by DNA TEs. More than 80% of the TE insertions detected in experimentally evolved lines were caused by *Hormin*, a non-autonomous miniature element derived from the *hAT* superfamily TE *Hornet*. Here we investigated the expression dynamics of the *Hornet* transposase, likely responsible for *Hormin* mobilization, during different stages of fungal development. RT-qPCR and RNA-seq performed during synchronized growth in liquid medium detected a marked upregulation of *Hornet* transposase transcript levels during early stages of sporulation, with a peak in freshly produced microconidia. Consistent with stage-specific TE activation, experimental evolution on complete medium plates of a *Fol*4287 clonal line passaged as microconidia resulted in significantly higher TE mobility and phenotypic diversity compared to parallel lines passaged as mycelium. These findings suggest that TE activation during early stages of conidiation promotes genetic variation and environmental adaptation in this clonally evolving fungal pathogen.

466C

A supergene underlies evolution of a novel secotioid phenotype in the dimorphic mushroom *Lentinus squamulosus*

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How mushrooms evolve novel forms is still an open mystery in fungal evolutionary development. Forms adapted for enclosed spore production (e.g. puffballs), are hypothesized to evolve from ancestors with exposed spores by passing through a “secotioid” stage, where the mushroom looks ancestral except for a morphological difference that prevents spore release. Many secotioid forms exist, but *Lentinus squamulosus* (otherwise known as North American *L. tigrinus*) is the only known species with both an exposed form and a consistent and widespread secotioid form, making it ideal for studying early secotioid evolution. *Lentinus squamulosus* occurs across North America and is dimorphic – it has either exposed gills (“agaricoid”) or gills covered by a membrane (“secotioid”). Previous work showed that the dimorphism follows simple Mendelian inheritance (agaricoid is completely dominant to secotioid) but could not resolve the secotioid locus. To identify the locus, we used both a genome-wide association study (GWAS) and a population genomics approach. The GWAS was conducted on the offspring of a single cross using spores from a heterozygous individual collected in Massachusetts. For the population genomics, we sampled two populations from Massachusetts and two from Illinois. The results from both approaches identified the same region associated with the dimorphism. This region is over 2 Mb long (~5% of the genome), contains approximately 300-600 genes, and appears to be under strong selection. Roughly one third of the genes have known functions, many of which are involved in sexual development in other species. To investigate how this region can behave as a Mendelian locus, we generated new genome assemblies using Illumina and Nanopore data and assessed the region for large-scale rearrangement. We propose that the secotioid form of *L. squamulosus* evolved recently and is currently undergoing a selective sweep, suggesting a mechanism for secotioid evolution more broadly.

467C

Using TN-seq to identify genes required for yeast survival against amoeba predation

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Cryptococcus neoformans, *deneoformans*, and species within the *gattii* species complex are pathogenic yeasts that cause an estimated 147,000 deaths annually. One factor which enables these species to infect hosts is the ability to form large polysaccharide capsules. In the environment, *Cryptococcus* and *Kwoniella*, a closely related genus, use capsule formation as an adaptive method of protection against predators such as amoeba. Similarly, in vivo capsule formation shields pathogenic species from the host immune system by inhibiting phagocytosis. This allows fungal infection and dissemination through the host. Despite high conservation of capsule-associated genes across the two genera, there are vast differences in

pathogenicity among species with the group of pathogens nested within strictly environmental, nonpathogenic relatives. To investigate the difference in pathogenic capabilities between the pathogens and nonpathogens, and identify genes that are important in defending against phagocytosis, we selected eleven species across the two genera. We will use transposon mutagenesis and sequencing (TN-seq) in these species to identify genes required for defense against phagocytosis. TN-seq utilizes random insertional mutagenesis and sequencing to generate dense mutant libraries with multiple mutants of all nonessential genes. After mapping insertions in an initial library, we will mimic environmental predation by co-culturing each species with the amoeba *A. castellanii*, a natural predator of yeasts. Following co-culture, we will re-sequence transposon insertions. By comparing insertion frequencies to the initial library, we can identify genes required for survival during amoeba predation. This will be done in one representative strain for each of the eleven species we selected which will allow us to analyze our results at a species specific level as well as distinguish patterns that are unique to pathogenic species versus the nonpathogens. In addition, these results will refine our understanding of Cryptococcal virulence by identifying genes that are needed for survival in the presence of amoeba, particularly those that facilitate evasion of phagocytosis.

468C

Evolutionary Analysis of Pmk1 MAPK-dependent fungal pathogenesis across the Fungal Tree of Life

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Fungal pathogens are a significant threat to global agricultural, causing devastating plant diseases and yield losses. Some pathogens develop specialized infection structures to penetrate the plant cuticle while others have evolved mechanisms that exploit natural openings or wounds to enter plants. Among the evolutionarily conserved Mitogen-Activated Protein Kinases (MAPKs) in eukaryotes, the Pmk1-related MAPKs have been shown to play important roles in fungal pathogenesis, but is also functionally related to the well-characterised Fus3/Kss1 pathway in yeast, which regulates mating and filamentous growth. In the plant pathogenic fungus *Magnaporthe oryzae*, Pmk1 regulates appressorium formation, invasive growth and virulence. Pmk1 homologues have been identified in more than 33 fungal pathogens and shown to play a central role in regulating pathogenicity towards different host plant species, including major agricultural crops. To understand how phosphorylation-mediated regulation has evolved throughout the fungal kingdom, we used phosphoproteomics data that identified over 8000 phosphopeptides from ~2000 *Magnaporthe oryzae* proteins and performed comprehensive phosphosite conservation analysis across 1,143 fungal genomes spanning the entire kingdom Fungi. Our analysis shows striking patterns of phosphosite conservation, correlating with ecological niche and lifestyle adaptation in fungi. Most interestingly, we found a specific cluster of phosphosites conserved in plant pathogenic fungi but largely absent from saprophytes, animal pathogens, and species with other fungal lifestyles. Gene function enrichment provides evidence that these conserved phosphosites are overrepresented in genes within key regulatory hubs involved in signal transduction, transcriptional regulation, vesicular trafficking, cytoskeletal organization, and protein degradation, representing cellular processes that require synchronized regulation throughout host invasion. Our analyses suggest that the infection-related phosphorylation landscape is therefore evolutionarily conserved in plant pathogens, but has diversified according to fungal lifestyle to orchestrate the complex cellular reprogramming required for successful host tissue invasion. Kingdom-wide analysis therefore provides a comprehensive illustration of how post-translational mechanisms have evolved in plant pathogenic fungi and identifies conserved regulatory mechanisms that could be targeted for disease control strategies.

469C

Evolutionary Mechanisms Shaping Chromosome Architecture in *Fusarium*

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The genus *Fusarium* comprises ecologically diverse filamentous fungi that include major plant and animal pathogens. One interesting observation is the association with horizontally acquired accessory chromosomes (ACs) and host-specific virulence. Although it plays a central role in shaping genomic architecture and virulence among species, karyotype evolution—changes in chromosome number, structure, and organization—remains poorly characterized in Fusarium. In this study, we investigate genome and karyotype evolution in *Fusarium* through comparative genomics, synteny analysis, and structural variant profiling using genomes from a set of species representing the breadth of phylogenetic diversity within the genus. Our work focuses on detecting and validating chromosome fusion and fission events, identifying conserved syntenic blocks, and examining the contribution of centromere dynamics and transposable elements to chromosomal rearrangements. Preliminary evidence from published *Fusarium* genomes indicates substantial variation in genome size, GC content, and TE composition, reflecting repeated cycles of genome expansion and compaction. Such processes, coupled with inter-centromeric recombination and segregation errors, are likely major drivers of karyotypic. By integrating structural variation with phylogenetic analyses based on single-copy genes, we aim to elucidate evolutionary relationships that are not adequately resolved by sequence-based phylogenies alone. This research will generate a comprehensive assessment of chromosome-level evolution in *Fusarium*, providing insights into how genome dynamics influence adaptation, pathogenicity, and speciation. Understanding the mechanisms underlying karyotype diversification in *Fusarium* will not only enhance evolutionary and genomic frameworks for this complex genus but also support improved strategies for managing *Fusarium*-related diseases in agriculture and public health.

470C

A large-scale phylogenomic analysis of host-specificity of entomopathogenic fungi via automated data retrieval

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Entomopathogenic fungi (EPF) infect and kill diverse species of insects, making them valuable models for host-pathogen interactions, as well as important organisms in biocontrol applications and natural product discovery. Some EPF species have broad host ranges, while others are highly specialized and only infect select insect taxa. To establish a genomic basis for the EPF lifestyle, as well EPF host specificity, it is essential to first generate phylogenomic trees which elucidate the evolutionary relationships among species. Studies of EPF host range often depend on smaller, curated datasets, which have

EPF host data manually assigned; attempts at constructing a larger dataset are often quickly hindered by scale and complexity of gathering and curating genomic data with corresponding host metadata. To overcome these limitations, we developed custom scripts to automate the retrieval, curation, and processing of publicly available fungal genomes, and as well as associated host metadata. This automated approach dramatically increased our dataset size to allow for more rigorous articulation of EPF phylogeny and known host range. Here, we present a phylogenomic tree constructed from hundreds of genomes that met our quality thresholds from a diverse collection of EPF species. This foundational tree provides a framework for exploring relationships among diverse EPF and opens the door to further efforts draw connections between pathogen genomes and phenotypes.

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Lineage-specific rules for protein architecture at fungal scale

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The rapid growth of fungal genome data has made it possible to compare gene content across a wide range of species. However, we still lack a clear understanding of how proteomes themselves are organized and how they scale during evolution. In particular, it remains unclear whether the length of proteins and domain architectures follow shared rules across subsets of fungal lineages as observed in other comparisons within and between kingdoms . We seek to explore how evolutionary processes have shaped genome evolution and link functional potential and biological diversity. Here, we conduct a large-scale comparative analysis of fungal proteomes spanning multiple major subphyla, examining protein length distributions, domain organization, and their relationship to genome and total proteome size. Across fungi, protein length distributions are strongly right-skewed, but they differ systematically among subphyla in both typical length and the extent of long-protein tails. Proteome expansion does not lead to a simple increase or decrease in protein length. Instead, we observe nonlinear scaling patterns in which median protein length and length diversity change differently across lineages. Protein domain architecture shows a strong overall relationship between protein length and domain number, but this relationship varies among subphyla. Some lineages tend to encode proteins with higher domain density, while others favor longer sequences with fewer domains per unit length. At the genome level, these differences reflect distinct strategies of proteome organization, ranging from enrichment in short, domain-poor proteins to greater investment in long, multi-domain proteins. Despite these contrasts, the overall distribution of proteins across size classes remains broadly conserved, suggesting shared constraints that are modified by lineage-specific evolutionary pressures. Together, these results highlight protein length and domain organization as key features of fungal proteome evolution. By identifying general patterns alongside consistent lineage-specific differences across thousands of genomes, this study provides a quantitative framework for understanding how proteome architecture evolves and how it may contribute to functional and ecological diversification in fungi.

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Linking phenotype to genome: Integrating bioassays and genome comparison reveals candidate virulence factors in a highly pathogenic *Beauveria* isolate

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The cosmopolitan fungal genus *Beauveria* contains entomopathogenic isolates capable of infecting a wide range of arthropods. However, host susceptibility among different insect orders to *Beauveria* is enigmatic and raises foundational questions about pathogen-host relationship. To examine phenotypic variability in host-pathogen interactions, virulence of nine *Beauveria* isolates was assessed against nine different insect species from four orders: Lepidoptera, Coleoptera, Blattodea, and Diptera. All isolates exhibited varying degrees of virulence against the insect hosts with some displaying significantly higher virulence. Particularly, isolate *B. bassiana* ARSEF 2597 was the most virulent against insects from Lepidoptera, Coleoptera, and Blattodea, followed by ARSEF 1520, and ARSEF 3097; thus, indicating them as generalists, whereas *B. bassiana* isolate ARSEF 8028 exhibited the highest virulence towards *Aedes aegypti* larvae. The significantly higher hazard ratio observed for isolate ARSEF 8028 compared to the second most virulent isolate ARSEF 4305 against *A. aegyptii* larvae indicates host specificity and implies it is a specialist. In order to identify the genomic features associated with higher virulence of isolate ARSEF 2597, comparative genome analysis was conducted using six *B. bassiana* isolates used in our study. Genome assemblies were retrieved, and annotations were generated and functionally characterized. Unique genes specific to isolate ARSEF 2597 were identified through orthology analysis using Orthofinder and were analyzed further for potential roles related to virulence through functional annotation, secretome prediction, and secondary metabolite cluster analysis. Whole genome synteny alignments among isolates were generated using SyMAP to detect large-scale structural variations and synteny differences. This integrated comparative genomics approach identified isolate-specific genes, candidate virulence factors, and unique genomic regions that may contribute to the enhanced pathogenicity observed in the most virulent isolate ARSEF 2597.

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Uncovering Mitochondrial Plasmid Diversity in Fungi with GenomeOcean Embeddings

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Mitochondrial plasmids (MITP) are small, self-replicating circular DNA molecules found in the mitochondria of various eukaryotic organisms, including fungi, plants, and protists. Recent studies have revealed that mitochondrial plasmids have been observed in at least one species from six different phyla that harbor a DNA polymerase (dpo) and/or RNA polymerase (rpo) homolog, especially in plant-interacting species, including *Pleurotus* spp. and *Moniliophthora perniciosa*. Despite our growing knowledge of mitochondrial plasmids, their distribution, evolution, and functional significance remain poorly understood. Challenges in identifying these plasmids within genome assemblies—stemming from reliance on coverage and sequence-composition-based methods, coupled with a lack of comprehensive reference databases—contribute to this knowledge gap. However, the advent of large-scale genomic sequencing presents a valuable opportunity to systematically investigate mitochondrial plasmids across diverse organisms. Utilizing the extensive fungal genome datasets available at the Joint Genome Institute (JGI), we developed a tool to detect mitochondrial plasmids using sequence embeddings from GenomeOcean, a metagenomic foundational model developed at JGI. Our results demonstrate that GenomeOcean embeddings substantially improve performance over traditional embedding methods, such as tetranucleotide frequencies (TNF), in classical machine learning tasks. Classification was performed using linear discriminant analysis (LDA) and support vector machines (SVM). For SVM, we applied stratified 5-fold cross-validation, balanced by class and originating genome, to eliminate intra-genome leakage. The pooled confusion matrix across the test folds demonstrated that both

GenomeOcean embeddings markedly outperformed TNF. Specifically, the embeddings achieved a ~7-fold reduction in misclassification between mitochondrial plasmids and mitochondrial genomes, while simultaneously improving detection of true MITP sequences. LDA visualizations confirmed that GenomeOcean embeddings generate well-separated, linear decision boundaries for the three classes, whereas TNF-based representations do not. Our results establish that genomic embeddings capture non-linear sequence patterns critical for distinguishing mitochondrial plasmids from mitochondrial and nuclear DNA. Incorporating these embeddings into assembly pipelines should substantially reduce erroneous mitochondrial genome reconstructions and enable more accurate surveys of mitochondrial plasmid diversity.

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Deciphering the Role of Carbohydrate-Active Enzymes in the Pathogenicity and cell degradation of *Fusarium oxysporum* Infecting Banana Plant

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Banana wilt caused by *Fusarium oxysporum* is a major threat to global banana production, yet the enzymatic tools that this pathogen uses to colonize and degrade host tissues remain poorly resolved. Carbohydrate-active enzymes (CAZymes) are central to both host specificity and plant cell wall degradation, and comparative CAZyme profiling can provide insight into the evolution and virulence strategies of banana-infecting lineages. In this study, we analyze the CAZyme repertoires of 73 *F. oxysporum* strains to characterize their overall CAZyme composition and identify CAZy genes that may contribute to host specificity and banana cell wall degradation. Predicted proteomes were annotated using the dbCAN pipeline and InterProScan, and CAZymes were classified into the following families: GH, GT, PL, CE, AA, and CBM. We then compared CAZyme copy numbers across strains and focused on families known to target cellulose, hemicellulose, and pectin in banana cell walls. Preliminary analyses reveal that banana-infecting strains exhibit highly similar total CAZyme repertoires and PCWDE family abundances, whereas strains isolated from non-banana hosts display markedly greater variation. Ongoing work will link these candidate CAZy genes to genomic context and pathogenicity-related regions. Overall, our comparative CAZyme framework provides testable hypotheses about how *F. oxysporum* evolves host specificity and damages banana cell walls, with implications for resistance breeding and disease management.

475C

Elucidation of fluconazole resistance in the emerging fungal pathogen *Candida auris*

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Fungal infection diseases are estimated to cause approximately 3.8 million deaths worldwide each year. Developing antifungal agents is particularly challenging because fungal cells share many components with human cells. In addition to this difficulty, there is a growing global concern about antimicrobial resistance (AMR). To prevent the emergence of AMR and to inform the development of a new drug, elucidating the mechanisms of drug resistance is crucial.

Since fungal infections and drug resistance are social issues, we focused on a yeast pathogen, *C. auris*, which is persistent, causes severe diseases such as bloodstream infections, and contains a high proportion of isolates resistant to fungicides. We focused specifically on fluconazole resistance in this study, as it is a first-line drug. Fluconazole is an azole fungicide that inhibits *ERG11*, a key enzyme involved in ergosterol synthesis, an essential component of the fungal cell membrane. Thus, the canonical mechanism of fluconazole resistance relates to mutations in *ERG11*. In addition to this gene, overexpressed transporters play a role in drug resistance through their efflux activities.

Our institute, Medical Mycology Research Center at Chiba University, has deposited eight clinical isolates of *C. auris*. Although the Caur-R1 strain is resistant to fluconazole, no *ERG11* mutations were detected; therefore, an unknown mechanism of fluconazole resistance remains. This study aimed to uncover novel genetic and molecular mechanisms of non-canonical fluconazole resistance in *C. auris* Caur-R1.

Whole-genome sequencing was conducted on *C. auris* strains, and phylogenetic relationships were inferred from single nucleotide variants (SNVs). This analysis suggested that Caur-R1 was closest to a sensitive isolate Caur-S6, having 79 SNVs and 65 in/dels. The comparative genomic analysis between these two strains suggested that Caur-R1 harbors three SNVs and two in/dels, possibly causing loss of function due to protein truncation. We chose these mutations for the first candidates, as they can be verified by knockout of the genes. We conducted knockouts of the respective genes in Caur-S2 to investigate their functions and involvement in drug susceptibility.

476C

Evidence of actively-transposing *Starship* elements in *Sclerotinia sclerotiorum*

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Starships are a superfamily of large, mobile genetic elements that can serve as a mechanism of horizontal gene transfer in filamentous fungi. These transposable elements are characterised, in part, by their ability to move entire gene sequences, or “cargo genes”, which may affect fungal fitness. However, due to their novelty, the presence and potential roles of *Starships* in individual species remain largely unexplored.

Here, we present the first investigation of *Starships* in the globally important plant pathogen *Sclerotinia sclerotiorum*, using 29 long-read genome assemblies from strains spanning North America, Europe, and Australia. *Ab initio* gene prediction, functional annotation, and new *Starship* detection

pipelines were used to identify and characterise these mobile elements and their cargo genes. We identified 67 *Starships* and 97 *Starship*-like regions across these genomes, equating to a median of six elements per strain (SD=1.7). They formed 21 distinct haplotypes, with the “captains” grouped into 9 naves that fall within the *Phoenix* (n=5)*, Moya* (n=2) and *Serenity* (n=2) families.

Several of the identified elements were present at two or more loci, suggesting recent or ongoing transposition. We also identified two *Starship* navis-haplotypes with signatures of horizontal transfer from other fungal species. One haplotype was very similar to a *Voyager* family *Starship* previously identified in *Bipolaris zeicola*, and another was present in *Sclerotinia minor*, suggesting independent *Starship*-mediated transfer events.

Analysis of the cargo genes across the identified *Starship* elements in *S. sclerotiorum* revealed enrichment for selected functional categories involved in metabolism. Multiple matches to entries in the Pathogen–Host Interaction (PHI) database, as well as several putative effector genes, were also detected among the cargo genes. Collectively, this suggests that *Starships* may be mobilising genes involved in pathogenicity in *S. sclerotiorum.* This first study of *Starships* in *S. sclerotiorum* provides a compendium of these elements in a plant-pathogenic fungus, which can now be further evaluated. These results not only have implications for the evolution of the *S. sclerotiorum* genome but also add to the growing body of knowledge on the diversity and dynamics of *Starships* in filamentous ascomycetes.

477C

Unraveling a metabolic mystery: A novel galactose catabolism pathway in yeasts Emily J Ubbelohde[1], Marie-Claire Harrison[2], Antonis Rokas[3], Chris T. Hittinger[1] [1]Laboratory of Genetics, University of Wisconsin - Madison, [2]Standford Medicine, Standford University, [3]Vanderbilt University

Yeasts that metabolize galactose despite lacking canonical *GAL* genes upend the decades-old model of fungal carbohydrate metabolism. The *GAL* (Leloir) pathway in *Saccharomyces cerevisiae* is the established model for gene regulation, metabolic control, the evolution of environmentally responsive pathways, and is widely considered the sole mechanism for galactose utilization in Saccharomycotina. We recently identified a yeast lineage that entirely lack *GAL* genes, yet metabolize galactose robustly, raising questions about the identity, regulation, and evolutionary history of an alternative pathway. Machine-learning models suggested a relationship with sugar alcohol intermediates, so we tested the hypothesis that these yeasts catabolize galactose via an oxidoreductive pathway.

Across nine species HPLC confirmed extracellular galactose depletion, and enzymatic assays in three species demonstrated clear NADPH oxidation during growth on galactose, which suggests catabolism occurs via an oxidoreductive mechanism. We next generated transcriptomes for these species under three growth conditions to suggest candidate genes and guide functional experiments. Transcriptomic analyses revealed a previously uncharacterized, galactose-induced gene cluster known as the alt*GAL* cluster. The alt*GAL* cluster encodes a *GAL4*-like transcription factor and predicted oxidoreductive enzymes, which share homology to some filamentous fungal carbohydrate pathways, pointing to this cluster as a likely core component of this novel system.

To test this hypothesis functionally, we developed a transformation system in a yeast species related to *Candidiozyma auris* syn. *Candida auris* that has never been genetically engineered. We generated a complete cluster knockout, and preliminary growth and HPLC analyses revealed loss of robust growth on galactose, as well as a failure to deplete extracellular galactose over time. Together, these results provide the first functional evidence for a previously unrecognized oxidoreductive galactose catabolism pathway in Saccharomycotina yeasts and establish a foundation for uncovering the genes, enzymatic steps, and evolutionary origins of this alternative metabolic system.

478C

Investigation of a novel azole-resistance mechanism through sexual reproduction in *Aspergillus fumigatus* Momotaka Uchida[1], Saho Shibata[1], Takayuki Arazoe[2], Takashi Kamakura[2], Hiroki Takahashi[1,3,4] [1]Medical Mycology Research Center, Chiba University, [2]Applied Biological Science, Tokyo University of Science, [3]Molecular Chirality Research Center, Chiba University, [4]Plant Molecular Science Center, Chiba University

Aspergillus fumigatus is a pathogenic filamentous fungus that causes aspergillosis, with an estimated 340,000 deaths annually. Azoles are the most commonly used antifungals for treating this fungus, but azole resistance has been recognized in many clinical isolates, making treatment more difficult. A typical resistance mechanism involves mutations in the coding sequence or promoter region of *Cyp51*, which encodes the targeted enzyme involved in ergosterol biosynthesis. In addition, it has also been reported that mutations in the HMG-CoA reductase *Hmg1* could lead to azole resistance by increasing intracellular ergosterol levels. Yet, many azole-resistant isolates lack mutations in these genes, suggesting the presence of other unknown mechanisms.

Comparative genomic analyses, such as GWAS, are useful tools for linking phenotypes to the genes that determine them. However, direct comparisons possibly fail when the genetic determinants are distinctive to each isolate. Despite having long been considered an asexual species, reports show that *A. fumigatus* undergoes sexual reproduction and exhibits the highest known recombination frequency in progeny. This feature enables traditional genetic studies to identify a novel gene in this fungus, as in the well-established model species *A. nidulans*.

This study aims to identify a novel mechanism of azole resistance via genomic recombination, in parallel with evaluating sexual capacity in *A. fumigatus*. We set up crossings between azole-susceptible and -resistant isolates, with 17 susceptible (S-1 to S-17) and 10 resistant isolates without known mutations (R-1 to R-10), as well as a resistant *Cyp51*^{M220I} mutant (R-c), for a total of 95 combinations. Among them, 29 crossings successfully generated fruiting bodies called cleistothecia. We obtained F_1 progeny strains from 10 crossings involving 6 resistant isolates. The ascospore numbers per cleistothecia and the germination rate varied across crossing combinations, with significant mating-type distortions observed in two combinations. We then conducted comparative genomic analyses of two crossings, S-1×R-1 and S-1×R-c, with the latter as the control. Our newly developed pipeline successfully detected an 11-kb genomic region containing *Cyp51* in S-1×R-c. We also detected two regions covering 59 kb and 120 kb, respectively, in the S-1×R-1 cross. After refining the regions through contig-to-contig comparison, we identified 22 possible candidate genes.

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In genomes we trust: assessing genomic reliability within the family Nectriaceae

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The Nectriaceae include several of the most consequential plant and human pathogens, yet the genomic framework underlying their taxonomy and evolutionary interpretation remains uneven. To establish a reliable foundation for comparative and phylogenomic inference, we evaluated 1,530 publicly available genomes, integrating metadata completeness, taxonomic and geographic representation, and assembly quality. Essential metadata were missing from nearly one-third of accessions, and sequencing efforts were strongly biased toward a few agriculturally important Fusarium lineages, leaving many genera sparsely sampled or entirely absent. BUSCO and QUAST analyses revealed substantial variability in assembly quality, with widespread fragmentation and numerous genomes falling outside expected completeness thresholds. From a curated subset of high-quality genomes, we inferred a phylogenomic backbone using 763 single-copy orthologs and quantified gene- and site-level concordance. While major clades were broadly aligned with current taxonomic concepts, extensive gene-tree discordance was detected across the phylogeny, particularly along short internal branches. Discordance patterns were consistent with incomplete lineage sorting and gene-tree estimation error, although rapid radiation and structural genome features likely contribute as well. Comparison between MLST and genome-scale phylogenies confirmed broad congruence but highlighted conflicts in complexes characterized by dynamic genome evolution. Together, these results provide the first family-wide assessment of genomic reliability within Nectriaceae. Meeting the proposed quality and metadata standards will not only stabilize classification within this important family of fungi but also enable a more thorough integration of genomic data into biological interpretation. Ultimately, this approach will allow the field to move from descriptive taxonomy toward predictive frameworks that connect genotype, phenotype, and ecology.

480C

Non-coding sequence conservation correlates to the gene expression evolution and sheds light to the evolution of Sordariomycetes

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Gene regulation and expression directly shapes the phenotypic diversity in different lineages, which is typically studied with RNA-seq and CHIP-seq. But to enable meaningful comparisons among species, environmental and developmental variables need to be controlled in different fungal species, but it is often difficult to achieve. Since much of regulation and expression is controlled by regulatory elements in non-coding sequences, investigating the evolutionary history of such regions could provide us an alternative path to understanding the evolution of gene regulation and expression.

Here, we use *Neurospora crassa* as a model and identified the conservation of non-coding sequences in it and 256 other Sordariomycetes members. We found a positive correlation between the conservation in cis-regulatory regions and the conservation in gene expression. We also identified high conservation in accessible transcription binding domains. These observations highlight the relevance of conserved non-coding sequences. Our further findings of elevated conservation in the cis-regulatory regions of RNA polymerase genes in *Neurospora* suggest a potential requirement of strong regulation of gene transcription in this genus.

481C

Diversity of Major Surface Glycoprotein Copies Encoded Within the Expression Cassette of *Pneumocystis carinii*

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Pneumocystis are obligate fungal pathogens of the mammalian lung, causing *Pneumocystis* pneumonia in immunocompromised hosts. A unique feature of the otherwise highly reduced *Pneumocystis* genome is the Major Surface Glycoprotein (MSG) Superfamily to which ~10% of the genome is devoted. These proteins serve as the outer layer of the cell wall, mediating host interactions including concealing the exposure of its immunogenic b-glucan layer and attachment to alveolar epithelial cells.

Interestingly, the expression of the *MsgA1* subfamily appears to be exclusive to one copy per organism, despite the *Pneumocystis* genomes containing upwards of 100 unique members of *MsgA1*. This regulated expression pattern is due to the presence a singular, highly active promoter within the Upstream Conserved Sequence (UCS) of the transcribed *MsgA1*. The insertion of different *MsgA1* copies into this expression cassette is thought to occur through recombination events from the subtelomeric regions of the genome. This regulation of surface protein genes is hypothesized to serve as a mechanism of antigenic variation to evade the host immune response, similar to mechanisms observed in other eukaryotic pathogens such as *Trypanosomes and Plasmodium*.

In this study, we investigated the diversity of *MsgA1* genes within the expression cassette in a population of *Pneumocystis carinii*, a surrogate rodent model of human *Pneumocystis jirovecii* infection. PCR amplicons encompassing the UCS and downstream *MsgA1* were generated from *P. carinii* isolated from infected rats and sequenced. Our results reveal heterogeneity within *P. carinii* isolates, demonstrating the presence of multiple *MsgA1* subfamily genes within the expression cassette of individual cells in the population of *P. carinii*.

These results demonstrate a diversity of *MsgA1* gene superfamily copies present in the expression cassette of *Pneumocystis* and display the effects of genomic regulatory mechanisms that are hypothesized to contribute to immune evasion. Analyzing these amplicons using long-read sequencing will allow for further characterization of the specific *MsgA1* present in the expression cassette with calculations of the relative quantities within a given population, and are currently under analysis. Future studies, such as investigating the mechanisms of *MsgA1* recombination and potential MSG expression specific to certain stages of the *Pneumocystis* life cycle may reveal insights to the various functions of MSG that are yet to be deciphered. Recent techniques

described for gene-editing in *Pneumocystis* will allow for further functional assessment aimed at advancing our understanding of surface protein regulation and exploring its role in immune evasion of the mammalian host.

482C

Genomic insights into resistance-breaking strains of *Anisogramma anomala* causing Eastern filbert blight in the Pacific Northwest

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Eastern filbert blight (EFB), caused by the fungus *Anisogramma anomala*, is a major threat to hazelnut production in the United States. Native to the eastern U.S., the pathogen was introduced into the Pacific Northwest (PNW) in the late 1950s. For decades, EFB was managed using resistant cultivars carrying the single resistance locus 'Gasaway'. However, in 2022, a new strain of *A. anomala* capable of overcoming 'Gasaway'-mediated resistance was identified in Oregon, where 99% of commercial hazelnuts are produced. Subsequent reports across the PNW have raised concerns among growers. The origin of this aggressive strain, as well as the mechanisms enabling it to break resistance, remain unclear. In this study, we applied Oxford Nanopore long-read sequencing to assemble high-quality genomes from a small collection of *A. anomala* isolates, including the new strain able to overcome 'Gasaway' resistance. Sequencing produced long reads that were assembled into genomes organized into nine predicted chromosomes of total size of 344 Mb, which is one of the largest genomes among filamentous fungi. The expanded genome size reflects an exceptionally high proportion (90%) of repetitive DNA, primarily transposable elements (TEs). Unlike other TE-rich fungal genomes, nearly all TEs in the genome of *A. anomala* appear to be inactive remnants of ancient proliferation events. Nonetheless, TEs compartmentalize the genome of *A. anomala* into distinct TE-rich and TE-poor regions. Further comparisons between aggressive and non-aggressive isolates revealed highly similar genomes, with no clear presence/absence variations or mutations in predicted virulence genes associated with resistance breakage. However, further genome sequencing showed that aggressive isolates carried mutated alleles of the trehalose-6-phosphate synthase (*Tps1*) gene involved in trehalose biosynthesis. Based on previous studies of the rice blast pathogen *Magnaporthe oryzae*, we hypothesize that mutations in *Tps1* may indirectly affect repressors of transcription factors regulating expression of virulence genes in *A. anomala*. Overall, our findings suggest that the aggressive strain of *A. anomala* evolved locally in the PNW, and that *Tps1* mutations may underlie the pathogen's ability to overcome 'Gasaway' resistance in hazelnut trees.

483C

Investigating the repeated evolution of pathogenesis in *Aspergillus* section *Fumigati*

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Opportunistic fungal pathogens repeatedly evolve across all major clades of fungi. The ability of these fungi to cause disease is the culmination of multiple exaptations resulting in the convergent trait, pathogenicity. In clades like *Aspergillus* section *Fumigati*, pathogenicity evolved independently over relatively short evolutionary time scales. We utilized 36 closely related species of pathogens and non-pathogens from within *Aspergillus* section *Fumigati* to ask whether the independent evolution of pathogenicity is species-specific or conserved through a combination of growth and immunological assays and high-quality *de novo* assemblies. Growth at four different temperatures (30°C, 37°C, 40°C, 45°C) underlines the section's thermotolerance, with many species growing better at 37°C than 30°C. Conidiation and conidia germination, important ecological factors, display large outliers and no clear signal for pathogenic species. Similarly, survival with murine macrophages and the production of pro and anti-inflammatory cytokines support the independent evolution of pathogenesis across the section. The genomic data tells a similar story. Genome size, gene presence-absence, TE cargo, gene functional conservation, and gene gain/ loss rate all support the species-specific model of evolution. Finally, we consider potential ecological roles using predicted species occupation ranges and overlaps that may influence phenotypic and genotypic differentiation while still resulting in pathogenic potential

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Comparative Genomics Analysis Sheds Light on Pathogenicity Determinants in Cotton Fusarium Wilt Fungi

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Cotton Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *vasinfectum* (*Fov*), remains a major threat to cotton production, leading to significant yield losses across the United States. While races 1 and 2 are widespread and primarily associated with Fusarium wilt in Upland cotton, the emergence of race 4 (*Fov4*) has presented new challenges. Initially identified in California and later detected in New Mexico and Texas, *Fov4* is distinguished by its aggressiveness and the ability to cause disease in both Pima and Upland cotton without the need for nematode co-infection. Unlike other races, *Fov4* can infect plants even in neutral to alkaline soils and frequently induces severe root rot in addition to vascular wilt symptoms. Despite its impact, little is known about the molecular determinants of *Fov4* virulence. In this study, we performed high-quality long-read genome assemblies and annotation for *Fov4* and *Fov1* isolates collected from El Paso, Texas, enabling comprehensive comparative analyses with previously sequenced *Fov* races globally. Secondary metabolites prediction and ortholog analysis uncovered unique gene clusters of *Fov4* enriched in secondary metabolic pathways and pathogenicity. Functional analyses of select candidate genes demonstrated their critical roles in *Fov4* virulence. Overall, this comprehensive genomic characterization provides the insights into the evolutionary history of Texas *Fov*, sheds light on the underlying mechanisms of *Fov4* pathogenicity, and provides valuable insights into potential management strategies for cotton Fusarium wilt.

485C

Phenotyping of *Rhodotorula* tolerance to heavy metal contamination for bioremediation applications

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Rhodotorula is an extremotolerant generalist yeast that has been isolated from a wide range of locations, from food spoilage, soils, plants, dishwashers, to more extreme conditions such as the International Space Station (ISS). *Rhodotorula* has also been isolated from marine and terrestrial environments contaminated with various heavy metals. The ability of *Rhodotorula* to tolerate these environments may be associated with the production of carotenoids, protective pathways for oxidative stress, and the ability to form biofilms. These capabilities make *Rhodotorula* a potential candidate for bioremediation applications that can be naturally or engineered to detoxify or bioaccumulate contaminants from its environment.

Despite this potential, there is limited work on linking *Rhodotorula's* distinct tolerance to metals with genetic features. To fill this research gap, we aim to phenotype our collection of over 300 genome sequenced *Rhodotorula* strains to test their sensitivity to heavy metals. Using UCR's ExFAB biofoundry, we have applied automated workflows to replicate colonies, capture time-lapse of solid media growth, estimate pigmentation intensity and growth rates with newly developed software. The growth rates determined from the time series will permit research aims to (I) assess strain IC50s to heavy metals copper, iron, zinc, mercury, chromium, and cadmium, (II) identify strains with novel morphologies or tolerances, and (III) attempt to correlate genetic alleles or features associated with variance in tolerance.

486C

Developing Genomic, Taxonomic, and Biodiversity Resources for the USDA-ARS Entomopathogenic Fungi Collection

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The USDA-ARS Entomopathogenic Fungi (ARSEF) culture collection is the largest and most diverse collection of entomopathogenic fungi in the world. Housing over 12,000 fungi and protistan microorganisms isolated from insects and other invertebrates (i.e. spiders, nematodes, mites), ARSEF serves as a germplasm repository for development of microbial biocontrol agents and biopesticides targeting agricultural pests. The associated metadata on host-associations, host-substrates, and collection location information available on the USDA's collections SYMBIOTA portal (https://biocollections.ars.usda.gov/) can be utilized for biodiversity inventories as well as to guide initial selection of appropriate strains for development as biocontrol agents. The ARSEF also serves as a significant resource for elucidation of phylogenetic relationships among insect pathogenic genera and preserves germplasm of many Type or Ex-Type isolates and commercial biocontrol organisms. However, optimizing use of these fungi for biocontrol requires identification of strains with suitable host specificity and small non-target effects on other insects, wildlife, and humans. Additionally, some entomopathogenic fungi are difficult to grow and sporulate in pure culture, presenting challenges to mass production and deployment for biocontrol. In order to harness the full potential of these fungi, a better understanding of the genetic basis of virulence and host-specificity and a phylogenomic framework for predicting these traits is required. As ARSEF moves into the genomics era, we are synthesizing publicly available phylogenetic, genomic, and biochemical resources and creating a phylogenomic framework for predicting host-specificity and biocontrol phenotypes. Centralizing these genomic resources for entomopathogenic fungi will allow researchers to access genetic elements responsible for virulence from isolates that are not easily cultured for biotechnological development and facilitate genetic modifications of strains for improved virulence, host-specificity, and survival under field conditions. Ultimately, we aim to develop resources for taxonomic placement of new isolates and tools for prediction of host-specificity, virulence, and biocontrol phenotypes to more efficiently identify genetic factors underlying key phenotypes and facilitate their genetic manipulation to identify and improve fungal biocontrol agents.

487A

Deep Learning and Generative AI for Fungal Genomics

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Pathogenic and toxin-producing fungi pose a major risk to food security through yield loss and mycotoxin contamination, yet many genomic and proteomic features underlying virulence, toxin production, and host specificity remain poorly characterized. To address this, we are developing an AI-native methodological framework for plant pathogens that spans a wide range of multi-omic datasets, including genomes, transcriptomes, proteomes, and host–pathogen interactomes. We deployed resources for *Fusarium* and *Aspergillus* that leverage state-of-the-art tools to analyze these multi-omic datasets. First, we apply transfer learning with a convolutional neural network-based gene prediction framework to refine and harmonize gene model annotations across multiple fungal genomes, providing improved coding and regulatory annotations as browsable genome browser tracks. We then use AlphaFold and ESMFold to generate predicted 3D structures and provide structure-based search to enable hypothesis generation about novel effector virulence mechanisms and detoxification enzyme mechanisms. To quantify the impact of genetic variation, we used DNA language models (e.g., DNABERT) to score regulatory and coding variants, and protein language models (e.g., ESM) to score missense mutations likely to alter protein stability or function. Building on these foundations, we use multimodal methods (integrating structural and small-molecule features) to model the binding of different toxins, such as deoxynivalenol (DON), to putative detoxification or resistance proteins, including FHB7-like candidates, to identify alleles that could improve predicted affinity. We further employ protein diffusion models (e.g., RFDiffusion) to generate de novo binders targeting key effectors and toxins. Finally, we leverage multimer versions of protein prediction software (e.g., AlphaFold-Multimer) to screen potential host-pathogen protein-protein interactions between predicted effectors and crop targets, integrating these predictions with variant and structural scores in web-accessible tools. These AI-driven resources create a reusable, computable framework for exploring fungal biology and prioritizing host and pathogen targets for breeding, protein engineering, and functional validation to effectively reduce disease and mycotoxin contamination.

488A

High-throughput screening reveals mechanisms of environmental control of germination in a fungal thermophile

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Thermothelomyces thermophilus is a filamentous fungus isolated from self-heating compost. Unlike most of the fungal kingdom, this species exhibits a growth optimum at 45°C and is intolerant of temperatures below 30°C. To dissect the mechanisms of its thermophilicity, we first implemented a large-scale insertional mutagenesis approach. We first made thousands of *T. thermophilus* mutants and cultured them at different temperature extremes in standard medium. Phenotyping-by-sequencing identified dozens of disrupted loci that represented candidate determinants of thermophilicity, including several annotated in metal transport. We then validated a subset of screen hits with a directed, single-gene knockout paradigm. The results revealed a temperature-dependent regulatory logic for germination, the decision point by which a fungal spore initiates growth in replete conditions. Surprisingly, most of our *T. thermophilus*mutants germinated far better at 50°C than the wild-type in standard medium and showed markedly slower germination at lower temperatures. We hypothesized that *T. thermophilus* has evolved sophisticated regulatory machinery to block germination at high temperature unless conditions are suitable. As a proof of concept, we surveyed media conditions and established that elevated zinc dampened germination of wild-type *T. thermophilus* at 50°C but promoted it at lower temperature. We interpret these results under a model in which *T. thermophilus* integrates information about temperature and nutrients before it commits to exiting the spore stage to start vegetative growth.

489A

Fungal Factory for Terpenoid Production: Genome Editing of *Coprinopsis cinerea*

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Fungi of the phylum Basidiomycota offer a rich source of terpenes and terpenoids, a major class of natural products with great structural diversity. Many of these compounds possess olfactory, colouring or bioactive properties making them highly valuable for applications in the food, agricultural and pharmaceutical industries. For instance, illudane sesquiterpenoids are utilized in pharmaceuticals for their anti-tumor activity or the diterpene pleuromutilin forms the basis of several antibiotics (Gonzales del Val et al. 2003; Alberti et al. 2017).

In fungi, terpenoid biosynthesis is mediated by the mevalonate (MVA) pathway, with the diversity of terpenes and terpenoids based on the high variety of the corresponding terpene synthases (Zhang et al. 2020). Aiming to uncover novel terpenoids and enhance their production, we employ genome editing approaches to activate and optimize terpenoid biosynthesis in the basidiomycetous model organism *Coprinopsis cinerea*. Both CRISPR/Cas9 and the classical method of homologous recombination can be powerful tools for investigating and improving terpenoid biosynthesis. Using these methods, we disrupted three genes within the MVA pathway of *C. cinerea*—one putative transcription factor, and two prenyl-pyrophosphat synthases—to redirect metabolic flux towards the production of terpenoids of interest. The mutants were cultivated in different media, terpenoids were extracted (Orban et al. 2024) and compared to the parental (control) strains using UHPLC-MS/MS. CRISPR/Cas9 mediated mutation of *rox1*, a putative negative transcriptional regulator of 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase, and enzymes involved in the late sterol synthesis, resulted in slower mycelial growth, complete impairment of fruiting body formation and the loss of at least two compounds. In contrast, putative *squalene synthase* gene CRISPR/Cas9 mutants retained the ability to form fruiting bodies and exhibited elevated levels of putative sesquiterpenoids. Knockout of a putative geranylgeranyl pyrophosphate synthase-encoding gene by homologous recombination caused a significant growth defect, but, interestingly, led to higher accumulation of various compounds compared to the control.

These findings demonstrate that targeted genome editing can effectively redirect metabolic flux within the MVA pathway, enabling enhanced production of terpenoids and the synthesis of terpenoids not typically synthesized under native conditions.

490A

KERS Complex and Downstream Regulators Control Development and Secondary Metabolism in Aspergillus nidulans

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The KERS complex is an important epigenetic regulator in *Aspergillus nidulans*, integrating chromatin remodeling with transcriptional control and secondary metabolism. Composed of KdmB (histone demethylase), EcoA (histone acetyltransferase), RpdA (histone deacetylase), and SntB (chromatin-associated protein), the complex influences both primary and secondary metabolic levels. To distinguish KERS-dependent genes, RNA-seq was performed on Δ*kdmB*, Δ*ecoA*, Δ*rpdA*, and Δ*sntB* mutants and compared with the wild type at 20 hours (primary metabolism) and 48 hours (secondary metabolism). Differential expression analysis revealed broad transcriptional changes, with 500 to over 4,000 genes affected per mutant, including 204 genes commonly altered during primary metabolism and 545 during secondary metabolism; of these, 25 genes were consistently downregulated across all mutants at 20 hours and 104 at 48 hours. Among the strongest candidate genes is *kdrD*, which is consistently downregulated in all KERS mutants and directly bound by the complex. *kdrD* is identified to regulate fungal development, secondary metabolism, spore formation, and germination, and a loss-of-function analysis is underway to determine how its absence affects these processes and how this may relate to the defects observed in the KERS mutants. Overall, these results highlight how the KERS complex and its downstream regulator genes coordinate fungal development with secondary metabolite production in *A. nidulans*.

491A

Tracing host-specificity in *Magnaporthe oryzae* pathotype *Triticum*: Functional genomics of lineage-specific genes

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Magnaporthe oryzae comprises multiple host-associated lineages of major agricultural importance. Although bioinformatic comparative genomics has identified lineage-specific genes, experimental validation linking the presence or absence of such genes to host specificity remains limited.

We investigated the function of the gene *MoT6098*, found in all lineages except those that infect rice and predicted to encode a secreted trehalase protein. Using CRISPR/Cas9, we deleted *MoT6098* in the wheat-infecting isolate BR32 and the resulting mutants showed significantly reduced virulence, demonstrating that *MoT6098* is required for full disease development in wheat. As isolate BR32 is unable to infect rice, we questioned whether the deletion of *MoT6098*, mimicking the genomic situation of the rice-infecting isolates, would expand the host range of BR32. However, *MoT6098*-deletion mutants remained unable to infect rice, and *MoT6098* insertion into rice-infecting isolates did not alter aggressiveness.

We also examined the function of *MoTRE1*, another secreted trehalase. Consistent with previous work, deletion of Mo*TRE1* did not affect virulence. A double deletion of *MoTRE1* and *MoT6098* did not further reduce virulence compared to the *MoT6098* single deletion mutant, demonstrating that *MoTRE1* does not contribute to virulence. Interestingly, constitutive Mo*TRE1* expression in BR32 reduced virulence, suggesting a potential recognition of trehalase-derived sugars as danger signals that induce plant defence responses.

Our results show that *MoT6098* contributes to virulence in the wheat-infecting *M. oryzae* lineage, but its presence does not determine host specificity. This underscores the need for further functional studies beyond comparative genomics, to elucidate the genetic basis of host adaptation in the *Magnaporthe* species complex.

492A

Functional Assessment of *Sporisorium reilianum* a2 Mating Genes, *lga2* and *rga2*, in *Ustilago maydis*

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Mating-type genes are crucial regulators of sexual development and pathogenicity in most fungi. In the corn smut fungus *Ustilago maydis*, the a2 mating type allele governs both mating compatibility and proliferation of infection. Despite their importance, the conservation and functional divergence of these genes across closely related species remains poorly understood. We investigated the functional homology of the a2 mating type allele genes, *rga2* and *lga2*, from *Sporisorium reilianum* (SRZ) in the *U. maydis* FB2 strain background. Initial mitochondrial genome sequencing revealed significant variation between the *U. maydis* strains, enabling strain-level differentiation and providing a foundation for understanding potential functional divergence of mating-type components. We generated single deletion mutants (Δ*rga2* and Δ*lga2*) and a double mutant (Δ*rga2*Δ*lga2*) in *U. maydis* FB2 and analyzed their mating phenotypes when crossed with the MF34 strain. Wild type crosses of the strains produce mitotypes exclusively from the a2 (FB2) parent; however, the Δ*lga2* showed a significant deviation from this pattern, generating approximately 30% a1 (MF34). Critically, complementation of the Δ*lga2* with the SRZ *lga2* homolog fully restored the wild type a2 inheritance, indicating a possible functional conservation of this gene across these strains. In contrast, the Δ*rga2* substantially showed a1 mitotype preference, and the complementation with the homolog from SRZ did not rescue the phenotype. This suggests that *rga2* function may be species-specific rather than broadly conserved between these two species as for *lga2*. The effects of the mutants together with their complementation from the SRZ genes, will be discussed together with how they affect teliospore germination. Initial observations suggest reduced disease proliferation, tumor/spore formation and spore germination of the Δ*rga2* mutants. These findings may indicate *rga2*, despite its divergent mating-type function, may play a critical role in spore viability and pathogenic fitness. Our results demonstrate partial functional homology of *lga2* between SRZ and *U maydis*, while revealing that *rga2* has undergone functional divergence and is essential for spore germination and virulence. This work provides insights into how mating-type genes have diversified during evolution of smut fungi and highlights importance of species-specific genetic factors in fungal pathogenesis and sexual development.

493A

The dynamic idiomorph – a new role in heterokaryon incompatibility and diversification of mating-type regions

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The idiomorph region is defined within fungal genomes as a region where at a given locus two highly dissimilar stretches of DNA may be present in different isolates. This contrasts with standard loci where similar, although slightly divergent, alleles may be found. The term was first introduced in the early 1990s and has since been widely used to describe mating-type (MAT) regions in filamentous ascomycete fungi. Here, highly dissimilar stretches of DNA approx. 2-5 kb in size are present at the MAT locus in MAT1-1 or MAT1-2 isolates of heterothallic species, with these idiomorph regions typically containing between one to three ORFs. Thus, idiomorphs are primarily known for their role in sexual compatibility. In a first project involving pangenome analysis of the opportunistic pathogen *Aspergillus fumigatus*, an idiomorph-like locus was detected on chromosome 2 whilst searching for highly divergent allelic regions that might have a role in heterokaryon incompatibility. The idiomorphs consisted of highly dissimilar DNA regions of 2.0 or 3.2 kb in different isolates, bordered by common conserved flanking sequences. The different idiomorphs each contained at least one putative ORF. The functional role of the region was investigated by CRISPR-Cas9 mediated gene transfer using nit mutants. Different idiomorph combinations either allowed hyphal fusion or resulted in hyphal incompatibility i.e. genes present at this region were responsible for heterokaryon incompatibility, with the genes

termed *hetF1* and *hetF2*. Therefore, a novel role for idiomorphs in asexual heterokaryon fusion is now described. In a second project involving whole genome analysis of 292 *Aspergillus* species the structural organisation of the MAT locus and associated idiomorph region was investigated. A high diversity of six different MAT locus arrangements was discovered. Heterothallic species contained a typical idiomorph region with either MAT1-1 or MAT1-2 genes present. By contrast, homothallic species showed either incorporation of both MAT1-1 and MAT1-2 genes at the MAT locus, various forms of MAT gene incorporation elsewhere in the genome outside of the idiomorph region, or in one remarkable instance movement of both MAT1-1 and MAT1-2 out of the idiomorph region, leaving a locus with the conserved synteny of a MAT locus but without any MAT genes present. Results overall show that idiomorph regions can be highly dynamic and have roles both in sexual and asexual incompatibility in fungi.

494A

Fungal *Starships* as Hotbeds of Sub-functionalization: Divergent Roles of the LaeA Paralogs on *Starship Janus*

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Fungal *Starships* are a recently discovered superfamily of large, mobile DNA elements that not only encode a mechanism for their transposition but also carry a variety of other genes as genetic cargo. We have identified an intriguing pattern across several fungal species where paralogs of non-mobile core genes are repeatedly found in *Starships*. Yet we do not know how processes of phenotype evolution via gene duplication play out in the context of *Starships*: are *Starships* hotbeds of neo-functionalization or is sub-functionalization more common when compared with other related sequences in the non-mobile genome? Here, we test the hypothesis that paralogs carried as *Starship* cargo uniquely contribute to the evolution of phenotypic variation compared with other related loci. *Starship Janus* in *A. fumigatus* carries two paralogs of the master regulator LaeA whose activities profoundly impact fungal secondary metabolism, virulence and development. These two *Starship*-associated LaeA-like proteins (sLaeA and sLaeB) have canonical methyltransferase domains and are more closely related to LaeA than any other methyltransferase, suggesting they may also participate in global regulation of important phenotypes. We developed deletion strains of each *Starship*-associated paralog individually and in combination in both the wild type and *laeA* deletion backgrounds. Preliminary data suggests loss of *slaeA* reduces sporulation and is additive to the already reduced spore production observed in the *laeA* deletion background. Metabolomic analysis did not suggest further alterations to the chemical profiles beyond the extensive changes of *laeA* loss alone. Current efforts include assessing these strains with transcriptomics and proteomics to identify pathways that may be shared between LaeA and the *Starship*-associated paralogs to quantify the extent of neo- vs sub-functionalization. Although still preliminary, our data support the conclusion that *Starship*-associated LaeA-like proteins and their counterpart LaeA are similarly involved in fungal development but do not share a global regulatory role in secondary metabolism, supporting a model where duplicated *Starship* cargo contributes to fungal evolution primarily through the process of sub-functionalization.

495A

Transcriptomic variation explains *Suillus tometosus* zinc tolerance in the absence of population structure

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Soil zinc (Zn) excess, largely caused by anthropogenic activities such as mining and metal processing, can disrupt metabolism and reproduction of organisms inhabiting these sites, often leading to death. Some ectomycorrhizal fungi can tolerate elevated Zn concentrations and protect their plant partners against metal toxicity. However, the mechanisms of Zn tolerance are still unclear. To fill this gap in knowledge, we used a combination of *in vitro* Zn fungal assays, whole genome resequencing, and transcriptomics to investigate Zn tolerance in the ectomycorrhizal *Suillus tomentosus*.

Whole genome comparison of 48 isolates found no evidence of population structure in *S. tomentosus* based on Zn tolerance, but Zn-tolerant and -sensitive isolates showed regions of genomic differentiation, including in genes related to zinc ion binding. Global gene expression profiling in one Zn-sensitive (ZnS) and one Zn-tolerant (ZnT) isolate revealed distinct baseline profiles in the absence of Zn, with differential expression of genes related to signaling, transport and antioxidant activity. This points to ZnT having constitutive Zn tolerance. When exposed to low Zn toxicity, ZnS mounted a broad stress response characterized by downregulation of essential cellular processes and upregulation of detoxification enzyme genes such as glutathione S-transferases. ZnT, on the other hand, showed no transcriptional change at low Zn toxicity, but at higher concentrations this isolate downregulated Zn transporters and upregulated antioxidant genes. The ZnT response at high Zn involved fewer genes than that of ZnS at low Zn.

These results show Zn tolerance in *S. tomentosus* is a constitutive and polygenic trait, less defined by genomic signatures, but related to the expression of metal homeostasis genes, providing mechanistic insight into the molecular mechanisms of ectomycorrhizal fungal tolerance in metal contamination sites.

496A

Genome assembly, functional annotation, and secondary metabolite potential of *Akanthomyces lecanii*, a mycoparasite with biocontrol capacity of the coffee rust fungus *Hemileia vastatrix*

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The mycoparasitic and entomopathogenic fungus *Akanthomyces lecanii* is widely recognized for its potential as a biological control agent. It is commonly found in plantations of *Coffea arabica*, in Panama, parasitizing the coffee rust fungus *Hemileia vastatrix*. The isolate *A. lecanii* CAM33 was isolated from pustules of *H. vastatrix* in Chiriqui, Panama, and selected for genome sequencing and analysis due to its biocontrol capacity in a previous field study. This study aimed to sequence, assemble, and functionally annotate the genome of *A. lecanii* for comparative genomic analyses with other members of

the *Cordycipitaceae* and to explore its biosynthetic potential. The whole genome of the *A. lecanii* CAM33 was sequenced with Oxford Nanopore and Illumina technologies. The *de novo* genome assembly has a total size of 34.7Mb, with a N50 value of 5.23Mb and 16 scaffolds. Structural and functional genome annotation was performed using Funannotate. The genome annotation predicted 8157 genes, and 8025 mRNA. The analysis of secondary metabolite gene cluster with the antiSMASH tool identified a total of 35 biosynthetic gene clusters (BGCs) across seven scaffolds. These clusters were classified into several biosynthetic categories, including nonribosomal peptide synthetases (NRPS), polyketide synthases (PKS) and terpenes. These insights provide a genomic basis and key tool for future research on *A. lecanii* parasitism and its biocontrol potential of the *H. vastatrix*.

497A

FunDLM, a fungal-specific large DNA language model for genetic research

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Fungi play essential roles in human life, often fundamentally shaping our environment, health, food industry, and agriculture. For example, fungal pathogens are among the most destructive enemies of crops, causing extensive yield losses in staple crops such as rice and wheat and posing a serious threat to global food security. Despite their importance, the development and application of artificial intelligence (AI) genomic models to uncover biological insights remain largely lacking. Recent advances in large language models (LLMs), originally developed for natural language, have shown strong potential when applied to biological sequences, where DNA can be treated as a structured "language." In this work, we propose funDLM, a fungal-specific DNA language model designed to learn genome organization, evolutionary patterns, and functional signals directly from large collections of fungal genomic sequences. By focusing specifically on the fungal kingdom, funDLM aims to outperform more general models in tasks relevant to fungal genetic research, such as predicting the functional impacts of genomic mutations, identifying genomic features in fungal genomes, and determining genetic determinants of virulence and host interaction.

498A

Fungal standoff: Interaction-responsive gene expression in co-cultured white- and brown-rot fungi

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Fungal communities on wood are structured by interactions among species with contrasting decay strategies, yet the genetic mechanisms that underlie these interactions remain poorly resolved. White-rot fungi mineralize lignin through oxidative enzyme systems, whereas brown-rot fungi essentially bypass lignin and rapidly depolymerize carbohydrates using a radical-generating metabolism. When these decay modes co-occur on the same substrate, interaction outcomes range from rapid replacement to long-lasting coexistence, and alter lignocellulose utilization, decomposition rates, and carbon release. The white-rot fungus *Fomes fomentarius* and the brown-rot fungus *Fomitopsis betulina* frequently co-occur on birch in nature and, in laboratory co-culture, form a persistent interaction zone rather than rapidly replacing one another. This pairing, therefore, offers a model for linking interaction species genes to transcriptional pathways in a naturally relevant community context.

In this study, model species interactions were established in solid-state cultures, both in monoculture and in co-culture on birch wood substrate, and sampled at early- and late-colonization stages. Total RNA from each treatment was sequenced, and reads were mapped to species-specific reference genomes to obtain gene-level expression profiles. Differential expression analyses were compared between co-culture versus monoculture within each species and time point, with additional contrasts across colonization stage. Functional enrichment analysis was then performed for carbohydrate-active enzymes, oxidoreductases, transporters, stress and defense pathways, secreted small proteins, and secondary metabolite biosynthetic genes to reveal the underlying mechanisms of interaction.

By resolving which genes are specifically responsive to the presence of a fungal competitor, this work will help distinguish interaction-associated transcription from baseline decay pathways. Connecting these interaction-responsive gene sets to the colonization stage will provide mechanistic insight into how fungal encounters influence resource partitioning, decay outcomes, and carbon flow during community assembly. More broadly, the results will clarify how a naturally co-occurring white- and brown-rot pairing determines shared substrate space and informs the design of multi-species fungal consortia for lignocellulose bioprocessing and other biotechnological applications.

499A

Frequent and Specific Horizontal Chromosome Transfer Between *Metarhizium* Species *in Vitro* and in the Field

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Horizontal chromosome transfer has been reported in several fungal pathogens and typically involves accessory chromosomes. However, it remains unclear whether such transfers are regulated processes. Here, we show that a single accessory chromosome is transferred frequently and with remarkable specificity between different species within the fungal genus *Metarhizium*, both *in vitro* and in the field. In laboratory co-inoculation experiments involving multiple *Metarhizium* species, we observed highly specific transfers of the same accessory chromosome. Up to 20% of asexual spores in recipient species carried the horizontally transferred chromosome. These specific horizontal transfers occurred between all four *Metarhizium* species tested and always involved only this one accessory chromosome. Furthermore, genome analyses of 154 field isolates from around the world, representing 13 *Metarhizium* species, revealed more than 30 accessory chromosomes, the majority of which show signatures of past horizontal transfer. These mobile chromosomes share a core gene set enriched for predicted DNA-binding and chromatin-conformation functions. The high frequency and specificity of transfers, combined with the worldwide distribution and shared gene set of these horizontally transferred chromosomes, suggest the existence of a widespread, regulated mechanism of chromosome mobility in *Metarhizium*. Ongoing functional analyses of the shared gene set aim to elucidate the molecular basis of this process.

500A

FungiDB: Tools for Genomic-Scale Data Exploration, Integration, and Discovery

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Modern biological research—spanning biomedical, model organism, and biotechnology—is increasingly driven by Big Data: genome sequences, population-level diversity analyses, and an ever-expanding range of multi-omics datasets. Effectively collecting, integrating, and interpreting these data is vital to ensure FAIR (Findable, Accessible, Interoperable, Reusable) access and to accelerate discovery across diverse areas of life science research.

The Eukaryotic Pathogen & Vector Genomics Resource (VEuPathDB.org)—including FungiDB.org—offers a robust, sustainable platform for data mining and discovery, supporting thousands of researchers in the eukaryotic microbe research community.

At the Fungal Genetics Conference, the VEuPathDB team will be available at a help desk during all poster sessions to demonstrate database features, introduce new tools, and engage with the community to shape future priorities. Highlights include:

- **Comprehensive multi-omics data access:** Explore genes, genomes, annotations, population diversity, comparative genomics, epigenetics, transcriptomics, proteomics, metabolomics, phenotypes, AlphaFold structures, and more.
- **Integrated data analysis (in silico experiments)** with tools for pathway exploration, orthology-based functional inference, and subcellular localization.
- **AI-powered expression summaries:** A new experimental feature that automatically analyzes transcriptomics datasets for each gene and generates concise summaries with biological relevance scores. Experiment results are organized into thematic patterns for rapid interpretation.
- **AI-assisted gene function discovery** that leverages literature analysis to generate user-submitted comments and expand functional annotations.
- **Expanded support for oomycete and fungal phytopathogens**, in collaboration with Ensembl and backed by a three-year BBSRC award.
- **OrthoMCL-7 (OrthoMCL.org)**, featuring updated UniProt proteomes, OrthoFinder-based clustering, customizable phylogenetic trees, and improved visualization of protein superfamilies.
- **Commitment to sustaining open access:** VEuPathDB remains freely accessible; however, resource development and support requires support. A new subscription model helps ensure long-term sustainability, and most funding agencies allow budget allocations for website access and data dissemination.

FungiDB continues to evolve as a bioinformatics platform leveraging AI, community expertise, and new partnerships to transform large-scale biological data into actionable insights.

*Abstract submitted on behalf of the entire VEuPathDB team.

501A

Development of a high-throughput functional genomics platform for filamentous fungi

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Advances in sequencing technology that allow for rapid and inexpensive whole-genome sequencing highlight how few genes have been functionally characterized. This problem is particularly acute in fungal biology, where even in the best studied organisms upwards of half of genes are annotated as hypothetical. High-throughput tools to identify gene function exist for single-celled organisms, such as yeast and bacteria. However, filamentous fungi present a number of challenges to high-throughput gene characterization, including low transformation efficiency, multinucleate cells, and asexual fusion. We have developed tools to overcome these challenges and perform high-throughput functional genomics in filamentous fungi using the model fungus *Neurospora crassa* and the biocontrol agent *Trichoderma atroviride*. To assign functions to uncharacterized genes, we made libraries with tens to hundreds of thousands of uniquely barcoded insertional mutants and mapped the locations of these barcoded insertions using high-throughput sequencing. We then used these libraries to perform high-throughput screens to rapidly connect phenotype to genotype. Quantifying relative barcode abundance in the library before and after exposure to experimental conditions allows us to assign functions to hypothetical genes. This resource establishes a scalable platform for high-throughput functional genomics in filamentous fungi, enabling investigations of fungal biology that will contribute to improved medical applications, biotechnology, and sustainable agriculture.

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Assessing the role of the histone variant H2A.Z on histone PTM distribution and genome organization in *Neurospora crassa*

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Chromatin structure directly contributes to both genome organization and function, and is regulated by multiple factors, including histone proteins and their associated post-translational modifications (PTMs). In the most fundamental unit of chromatin, a histone protein octamer – two each of H2A, H2B, H3, and H4 – wraps 146 basepairs of DNA to form nucleosomes, which package both active euchromatin and silent heterochromatin. However, the canonical histones are occasionally substituted with histone variants, such as the highly conserved H2A.Z that replaces H2A in the +1 nucleosome of euchromatic genes for recruiting the transcription machinery. In addition to well-characterized roles in transcriptional regulation, H2A.Z may have other downstream effects on genome organization and function. Previous work in *Neurospora crassa* has shown that deletion of the gene encoding H2A.Z (D*hH2Az*) causes region-specific losses of the di-/tri-methylation of lysine 27 on histone H3 (H3K27me2/3), which demarcates transcriptionally silent facultative heterochromatin. H2AZ loss downregulates expression of the EED subunit of the Polycomb Repressive Complex 2 (PRC2) methylating H3K27. While the connection between H2A.Z and H3K27me2/3 is documented, H2A.Z loss may affect the deposition of other euchromatic histone PTMs or may alter three-dimensional genome folding. To this end, we examined the profiles of di- or tri-methylation of lysine 4 on histone H3 (H3K4me2 and H3K4me3) in an Δ*hH2az* mutant strain using Chromatin Immunoprecipitation-sequencing (ChIP-seq). Here, we will report our preliminary efforts to integrate our H3K4me2/3 ChIP-seq data with published H3K27me2/3 ChIP-seq and Δ*hH2az* RNA-seq datasets to comprehensively assess if H2A.Z loss globally impacts euchromatin or facultative heterochromatin composition. Moreover, H2A.Z loss could alter three-dimensional genome organization, particularly if the normally-segregated active and silent chromatin become amalgamated. We performed chromosome conformation capture with high-throughput sequencing (Hi-C) in an Δ*hH2az* mutant strain to examine if H2A.Z loss impacts genome organization, and we will present our preliminary results comparing wild type and Δ*hH2az* strain Hi-C datasets. Our findings will help elucidate if the conserved histone variant H2A.Z globally impacts genome organization and function.

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A high(er) throughput method for using CRISPR/Cas9 to delete genes from *Coccidioides posadasii*

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Coccidioides posadasii and *immitis* are human fungal pathogens endemic in the American southwest. *Coccidioides spp.* are thermally dimorphic, growing as a hyphae under environmental conditions and a large, endospore filled spherule structure under host conditions. *Coccidioides spp.* are the causative agents of coccidioidomycosis, or Valley fever. They are pathogens of growing concern as reported cases of coccidioidomycosis have increased 20-fold in the last two decades. Despite their importance as pathogens, *Coccidioides spp.* are understudied, especially compared to other human fungal pathogens with similar infectious burdens. The reasons *Coccidioides spp.* are understudied are multifactorial, including requirement of high biocontainment, technical difficulties in lab-based culture growth, and a historic lack of genetic tools. Previous methods of genetic manipulation have been technically challenging, time-consuming, and inefficient. Updated methods to delete genes in *Coccidioides* are required to help identify genes important to virulence, the dimorphic shift from environmental to pathogenic phase, and resistance to anti-fungal drugs. We have developed reproducible and affordable methods of making high-quality protoplasts, or competent cells, using enzymes used in the wine-making industry. We also developed a high-efficiency method to delete genes from *C. posadasii* using homology-directed repair and CRISPR/Cas9 guides. To test the effectiveness of these methods, we selected 12 genes with hypothesized roles in anti-fungal resistance, virulence, and the dimorphic transition from hyphea to spherule. We deleted the genes and replaced each gene with a hygromycin resistance markers. We found that the transformation methods were highly efficient with 90% of hygromycin resistant colonies showing presence of the hygromycin gene. 60% of colonies showed evidence of gene deletion and integration of the repair construct in the targeted location. The 12 deletion mutants had varying phenotypes including changes to growth rate, virulence, and ability to make spherules. Overall, the protocols presented here will increase the efficiency and speed of research occurring in the *Coccidioides* field and open opportunities for novel studies and discoveries.

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Extracellular vesicle-mediated delivery of genetic material for transformation and CRISPR/Cas9-based gene editing in *Pneumocystis murina*

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Pneumocystis pneumonia (PjP) remains a critical cause of morbidity and mortality in immunocompromised individuals. This disease is caused by the obligate fungal pathogen *Pneumocystis jirovecii*. Despite significant clinical relevance, critical aspects of *Pneumocystis* biology, pathogenesis, and antifolate drug resistance remain poorly characterized, primarily due to the persistent inability to culture these fungi *in vitro*. Unlike other fungi that readily grow in defined media, *Pneumocystis* species are obligate biotrophs, requiring a mammalian host to replicate, which severely limits functional genetic studies and the exploration of novel therapeutic targets.

The absence of robust genetic manipulation tools has impeded our mechanistic understanding of *Pneumocystis* biology and the development of novel therapeutic strategies. Herein, we describe a novel method for the stable transformation and CRISPR/Cas9-mediated genetic editing of *Pneumocystis murina* utilizing extracellular vesicles (EVs) as a delivery vehicle. Building upon our prior investigations demonstrating EV-mediated delivery of exogenous material to *Pneumocystis*, we engineered mouse lung EVs to deliver plasmid DNA encoding reporter genes and CRISPR/Cas9 components. Our initial findings demonstrated successful *in vitro* transformation and subsequent expression of *mNeonGreen* and $Dhps^{ARS}$ in *P. murina* organisms. Subsequently, we established stable *in vivo* expression of *mNeonGreen* in mice infected with transformed *P. murina* for a duration of up to 5 weeks. Furthermore, we designed and validated a CRISPR/Cas9 system targeting the *P. murina Dhps* gene, confirming its *in vitro* cleavage efficiency. Ultimately, we achieved

successful *in vivo* CRISPR/Cas9-mediated homologous recombination, precisely introducing a *Dhps*^{ARS} mutation into the *P. murina* genome, which was confirmed by Sanger sequencing of *P. murina* populations across all tested animals.

Together, these results establish a foundational platform for genetic manipulation in *Pneumocystis*, demonstrating that EVs can deliver functional DNA with high efficiency in this previously genetically intractable fungus. The performance of EV-mediated delivery in this system suggests that similar approaches could enable genetic manipulation in other organisms that historically resist transformation.

505B

The target specificity of a heterochromatic histone deacetylase complex in *Neurospora crassa*

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Aberrant levels of deacetylation can hinder the proper transcriptional patterns of genomic DNA and can result in epigenetic deficiencies that are associated with human diseases, including cancer. Nuclear DNA is wrapped around an octamer of histone proteins to form nucleosomes, the fundamental unit of chromatin. Chromatin is divided into transcriptionally active euchromatin and silent heterochromatin, the latter subdivided into temporarily silent facultative heterochromatin and permanently silent constitutive heterochromatin. Post-translational modifications (PTMs) of histone proteins maintain proper chromatin function, with the trimethylation of lysine 9 on histone H3 (H3K9me3), catalyzed by the DCDC (DIM-5/7/9, CUL4, DDB1^{dim-8} complex), marking constitutive heterochromatic loci. Further, lysine residues on histone N-terminal tails are often post-translationally acetylated or deacetylated by histone acetyltransferases (HATs) or histone deacetylases (HDACs), respectively. The filamentous fungus *Neurospora crassa* encodes a constitutive heterochromatin-specific histone deacetylase complex – the HCHC complex (composed of the proteins HDA-1, CDP-2, HP1, and CHAP). While previous research examined region-specific acetylation levels in HCHC mutants, the global HCHC specificity is unknown. To identify possible HCHC deacetylation targets on histones H3/H4, we performed Chromatin Immunoprecipitation-sequencing (ChIP-seq) in strains lacking the genes *hda-1* or *cdp-2*. Here, we report the enrichment of H3K4ac (the acetylation of lysine 4 on histone H3), H3K14ac, H3K18ac, H3K27ac, H3K36ac, H3K56ac, H4K5ac, H4K8ac, H4K12ac, and H4K16ac in WT and HCHC mutant strains. We show that the HCHC deacetylates multiple histone H3/H4 lysines, with H3K27ac being the most notable target. We are also examining the global histone acetylation changes upon DCDC loss, since the HCHC complex requires DCDC-catalyzed H3K9me3 for targeting. Lastly, since histone acetylation and deacetylation are closely linked, we hypothesized that a HAT specifically acetylates constitutive heterochromatin. Using a candidate approach, we will report our preliminary data of double mutant strains where genes encoding the non-essential histone acetyltransferases HAT-1 or HAT-8 are individually deleted in an HCHC deficient background. Our research provides insight into the global acetylation changes that occur upon loss of a heterochromatin-specific HDAC complex and could motivate further study of HAT–HDAC dynamics on constitutive heterochromatin.

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Fitness effects of multi-fungicide resistances in *Aspergillus fumigatus*

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Aspergillus fumigatus is an opportunistic human pathogenic fungus mainly found in plant-degrading material. Although not a plant pathogenic fungus, within this substrate it is exposed to multiple classes of agricultural fungicides. It has been shown that exposure to agricultural triazoles leads to cross-resistance of clinical triazoles, but the evolutionary impact of exposure to other fungicide classes is unknown. Recent genomic surveys have shown correlations of variants assumed to lead to resistance between DMI, QOI, SDHI, and MBC fungicides, suggesting ongoing adaptation to current agricultural practices. To identify the full diversity of genetic resistances, we used literature to identify target genes and screened for common (>0.3%) non-synonymous variants across 1500 available A. fumigatus genomes. We identified 12 resistance variants, spread across seven genes, with five resistant variants being shown for the first time. Natural isolates that contain one resistance likely contain multiple, resulting in multi-fungicide resistance phenotypes. On the other hand, certain resistance variants like sdhB H270Y and sdhC S105I were never found together, suggesting negative fitness costs of some combinations, particularly alleles of the same gene or in members of the same protein complex.

To test the impact of these resistance variants, we introduced point mutations with CRISPR/Cas9 using co-transformation of a transient hygromycin resistance plasmid. All predicted resistance variants caused resistance, including newly described alleles to the aminopyrimidines class of fungicides. We did not observe a fitness cost for single mutants or naturally co-existing combinations. However, by constructing combinations not observed to occur in nature, we show significant fitness reductions. For instance, multiple variants in the SDH complex are not tolerated (more than 40% growth reduction), and likewise within beta-tubulin, the target of MBCs (more than 70% growth reduction). Our results indicate that natural selection is shaping a combination of resistance variants in different genes and for different fungicide classes and provides additional evidence that the continued use of non-triazole fungicides are indirectly selecting for triazole resistance.

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Investigating fungicide mode of action using high-throughput functional genomics

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Fungi are responsible for diseases that result in the deaths of over a million individuals each year and devastating crop infestations that threaten global food supplies. To develop future clinical and agricultural treatments for fungal diseases and improve the efficacy of current treatments, we must understand what cellular processes these treatments target and how fungal cells respond to a given treatment. VM Agritech recently developed a novel fungicide formulation, called Curezin®, that combines copper and zinc as active ingredients. To determine the fungal genetic response to the fungicide, we utilized a combination of functional genomics and transcriptomics approaches. Screening of a library of over 300,000 *R. toruloides* mutants revealed genes necessary for resistance to treatment with copper and zinc individually and in combination. Intriguingly, genes that were identified by this functional genomics approach were rarely differentially expressed in *R. toruloides* during exposure to the respective metal stress condition. Conversely, genes differentially expressed in response to metal exposure rarely caused fitness defects when mutated in the functional genomics screen. Molecular genetic analysis of genes identified via both the functional genomics and transcriptomics approaches demonstrated that both methods can identify genes important

for the genetic response to metal stress. One of the few genes identified by both methods was a transcription factor that is partially responsible for the transcriptional response to copper and zinc exposure. Our findings demonstrate the power and complementarity of combining functional genomics and transcriptomics approaches in identifying a more comprehensive map of the genetic mechanisms necessary for responding to fungicides. Additionally, our work sheds light on mechanisms of synergy in the activity of two antimicrobial metals within fungal cells.

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AI-Driven *Fusarium* Databases: identify key genetic variation and target to combat agricultural threats and aid development of crops resistant to disease and mycotoxin contamination

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Fusarium is a major threat to agriculture because collectively its species cause disease and mycotoxin contamination of diverse crops. In the U.S., mycotoxins are estimated to cause annual losses of over one billion dollars to the agricultural economy. In addition, new epidemics and species of *Fusarium* with novel pathogenic and/or toxigenic abilities continue to emerge, causing additional economic threats. Understanding interactions of *Fusarium* and plants at the protein level, including impacts of single nucleotide polymorphisms (SNPs), has tremendous potential to improve resistance of crops to disease and mycotoxin contamination. Advances in Artificial Intelligence (AI)-driven computational approaches are accelerating development of disease-resistant crops by enhancing the ability to analyze large-scale datasets to more rapidly identify protein-protein interactions (PPIs) that impact plant disease. However, progress has been limited by fragmented data and inadequate tools to visualize and assess how structural and missense variation can impact the outcome of PPIs and in turn affect crop disease. To address these limitations, we developed the *Fusarium* Protein Toolkit (https://fusarium.maizegdb.org/), an interactive, AI-driven database that contains models of all predicted proteins from 22 *Fusarium* species. The Toolkit also includes tools to explore predicted 3D protein structures, functions, and effects of missense mutations on protein structure. Currently, we are developing two additional interactive databases that will allow users i) to query, visualize, and explore SNP variation at a population genomic level (https://fusariumsnps.maizegdb.org/) and ii) to determine which pairs of *Fusarium* and crop proteins are most likely to interact (https://fusarium.maizegdb.org/multimer). These databases use AI language models and computational biology to predict PPIs that impact disease. We anticipate that the three databases can be used to accelerate development of crop varieties with enhanced resistance to *Fusarium*-incited disease and mycotoxin contamination by identifying targets for plant breeding and genetic engineering programs.

509B

Functional characterization of peptidase G1 in *Cryphonectria parasitica*

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The chestnut blight pathogen to study fungus virus interactions *Cryphonectria parasitica* and its associated hypovirus serve as an important model system. Among the genes showing significant differential expression in RNA-Seq analyses using the wild-type strain EP155/2, isogenic hypovirus *CHV1*-713-containing strain UEP1, the Cell Wall Integrity (CWI) deletion mutant, and their sectored progenies, peptidase G1 was selected the significantly differentially expressed genes (DEGs). This protease contains a catalytic dyad consisting of glutamate (E) and glutamine (Q), displays optimal enzymatic activity under acidic pH conditions, and is known for its resistance to pepstatin, a microbial protease inhibitor. To analyze the function of peptidase G1 in *C. parasitica* wild type, a peptidase G1 gene deletion mutant was generated using homologous recombination. The successful construction of the mutant was confirmed by PCR and Southern blot analysis. Morphologically, the null mutant exhibited a reduced number of spores, slower growth, and darker pigmentation compared to the wild type. The mutant exhibited hypersensitivity to under ROS stress. In addition, compared to its growth on PDA, the growth rate of the null mutant was restored when cultured on PDA supplemented with skim milk or water agar containing only agar. Future research will investigate the virulence of the null mutant on chestnut trees and examine the changes that occur after introducing the hypovirus into the null mutant.

510B

Topological Consequences of Large-Scale Genome Rearrangements in *Neurospora crassa*

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A species' population can accumulate vast genetic diversity through syntenic changes that rearrange the DNA linear order. Structural variants (SVs), such as translocations, cause syntenic changes that often compromise genome function, such as misregulating transcription, leading to genetic diseases such as human cancer. However, syntenic changes may also be harmless, implying that some SVs minimally impact genome function or that compensatory mechanisms preserve cell viability. Currently, it is unknown if syntenic changes can impact genome organization. Previous work has shown that heterochromatic histone post-translational modifications (PTMs), such as tri-methylation of lysine 9 on histone H3 (H3K9me3) and di-/tri-methylation of lysine 27 on histone H3 (H3K27me2/3), are major determinants for organizing eukaryotic genomes. In *Neurospora crassa*, silent regions enriched with H3K9me3 interact over Megabases of linear distance to form chromatin loops, while subtelomeric H3K27me2/3 mediates telomere tethering at the nuclear periphery. To assess if translocations impact genome organization and function, we performed chromosome conformation capture with high-throughput sequencing (Hi-C) and histone PTM Chromatin Immunoprecipitation-sequencing (ChIP-seq) on three Neurospora strains that each harbor a single, large translocation; all genomics data was mapped to refined translocation strain reference genomes for more accurate reporting. We found that translocated DNA simply integrates into acceptor sites, highlighting fungal genome plasticity. Moreover, while most translocation breakpoints occurred in heterochromatin, H3K9me3-enriched regions can be bifurcated without appreciable changes in H3K9me3 levels. In contrast, subtelomeric translocations influencing H3K27me2/3 levels also compromise subtelomere bundling, suggesting subtelomeric DNA is essential for fungal genome organization. Our work clarifies how SVs can impact eukaryotic genome organization and function.

511B

Investigating Drug Resistance in *C. neoformans* using Diploid TN-seq

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Cryptococcus neoformans is a yeast that causes disease in immune compromised patients. Clinical *C. neoformans* isolates display a range of resistance to the antifungal drug fluconazole, however, many of those isolates lack the major genomic changes known to drive fluconazole resistance in other fungal pathogens. To explore how sequence variation contributes to drug resistance phenotypes, I will implement and perform transposon mutagenesis and sequencing (TN-seq) in diploid *C. neoformans* strains that are produced by mating two *C. neoformans* isolates with opposite fluconazole resistance phenotypes. When transposon mutagenesis is initiated in a cell that is diploid rather than haploid, one allele of a gene is disrupted by a transposon while the other remains intact. Thus, performing saturation transposon mutagenesis in diploid strains will allow us to generate a library of mutants that are functional hemizygotes, expressing only one or the other parental allele of a gene. Growth of this mutant library under stress, for instance fluconazole, followed by sequencing the transposon landscape before and after stress will reveal conditionally important alleles of both essential and non-essential genes. Currently, I have built a diploid strain that is the product of mating two identical reference strains of *C. neoformans*. I am using this strain to optimize TN-seq for diploid strains of *C. neoformans* and provide insight into the impact of diploidy on gene essentiality landscapes. I plan to implement TN-seq in diploids that are the product of mating reference strains with clinical or environmental isolates that have a range of fluconazole resistance phenotypes to assess allelic contributions to fluconazole resistance. This will allow me to map causative alleles for fluconazole resistance with high resolution and provide insight into the genetic underpinnings of drug resistance in fungal pathogens.

512B

Establishing gene essentiality and incorporation in *Candidozyma auris*

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The emerging pathogen *Candidozyma auris* is a critical public health threat that forms tenacious biofilms that encourage the continual spread of infection, as well as exhibiting superbug-like resistance to frontline antifungals. This high degree of multidrug resistance and nosocomial persistence highlights an urgent need to identify new therapeutic targets. However, this is a challenge since *C. auris* is a recently emerged pathogen, and its genetics and evolutionary potential are largely uncharacterized. Through our work, we find that *C. auris* has an unusual propensity to incorporate foreign genetic material randomly throughout its genome in a transposon-like fashion. We have leveraged this to generate random insertion collections within a *C. auris* population using an antibiotic cassette marker. This allows us to identify genic regions of essentiality through analysis of the distribution of marker inserts. Simultaneously, this method generates a knockout library, which can be applied to antifungal challenge to identify conditionally essential genes for antifungal resistance. Additionally, we interrogate the mechanisms of random gene integration in *C. auris*. Together, this work allows us to both classify therapeutic targets that are essential for *C. auris* survival and antifungal resistance, and investigate the evolutionary potential of this pathogen through a gene-incorporation strategy.

513B

Unraveling the regulatory network of Zeb2 in zearalenone biosynthesis

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Zearalenone (ZEA) is a polyketide mycotoxin synthesized by various *Fusarium* species. Its biosynthesis involves three key enzymes: two polyketide synthases (Pks4 and Pks13) and an alcohol oxidase (Zeb1), which function sequentially. Zeb2 acts as a positive transcriptional regulator of the genes encoding these key enzymes. However, the broader genetic network and molecular mechanisms interacting with *ZEB2* in ZEA biosynthesis remain largely unknown. To explore this, we performed RNA-seq analysis on wild-type, *ZEB2*-overexpressing, and *zeb2* deletion mutant strains. We identified twenty genes that were upregulated in the *ZEB2*-overexpressing strain and downregulated in the *zeb2* deletion strain, suggesting their regulation by Zeb2. Among these Zeb2-regulated (*ZR*) genes, only *zr32* was found to affect ZEA production, as its disruption led to reduced ZEA synthesis. Notably, *ZR32* encodes nonribosomal peptide synthetase 15 (Nrps15), whose functional characterization is currently underway to determine its precise role in ZEA biosynthesis. Further investigations are currently underway to determine whether the genes upregulated in the ZEB2OE strain are directly regulated by the Zeb2 transcription factor or are secondarily induced by ZEA accumulation. Overall, our findings provide new insights into the regulatory function of Zeb2 in governing secondary metabolite production, including ZEA.

514B

Roles of aneuploidy in *Aspergillus fumigatus* unstable adaptation and antifungal resistance

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Aneuploidy causes genome plasticity and enables adaptive responses that confer drug resistance in eukaryotes ranging from fungal pathogens to human cancer cells. *Aspergillus fumigatus* is a soil-resident fungus and the most common etiologic agent of invasive fungal infections globally. Aspergillosis has an alarmingly high mortality rate due in part to treatment failure resulting from widespread antimicrobial resistance and an ability of the fungus to dynamically

adapt to changing environmental and host conditions. Our understanding of these treatment failures is hindered by gaps in knowledge about how this organism adapts to resist antimicrobial stress. We find that exposure to FK506, an antifungal and immunosuppressive compound produced by *Streptomyces*, selects for whole-chromosome aneuploidy in this fungus. These chromosome duplications also reduce susceptibility to clinically-used azole antifungals, and whole-chromosome and segmental aneuploidies arise independently directly in response to azole exposure and confer resistance. Transcriptomic analysis revealed that Chr7 disomy leads to marked induction of the normally-silent neosartoricin biosynthetic gene cluster in response to FK506 exposure, and we demonstrate that constitutive genetic induction of this cluster recapitulates in the euploid background the response to FK506 in the aneuploid state. We further show that this response is independent from the production of neosartoricin, suggesting a role for cross-pathway metabolic changes resulting from the activation of this gene cluster or from the aneuploid state. In the aneuploids that arose in response to voriconazole, we find that a single duplication of the left arm of Chr2 is sufficient to confer a high level of resistance, and that the expression of known azole resistance genes is not altered in this strain. In both FK506-resistant and voriconazole-resistant aneuploids, culture in the absence of drug leads to aneuploid chromosome loss and rapid reversion to a euploid, drug-susceptible state. These results uncover a novel mechanism of rapid and reversible antimicrobial stress adaptation in *A. fumigatus* and demonstrate the utility of aneuploid strains to identify novel genetic contributions to antifungal adaptation.

515B

***Aureobasidium pullulans* as a genetic model for phyllosphere commensalism and enhancing crop resilience**

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Aureobasidium pullulans is a ubiquitous dimorphic Dothideomycete fungus important for crop protection and for industrial fermentation. While *A. pullulans* controls many fungal and bacterial crop diseases without antimicrobial agents, its use remains mostly limited to organic production due to inconsistency in effectiveness. Despite its current insufficiencies, *A. pullulans* exhibits frequent recombination, ease of isolation, and substantial genetic and phenotypic diversity allowing for precisely examining the genetics of host-microbe interactions in plant commensals with potential for enhanced efficacy. We assembled a panel of 210 genome-sequenced isolates of *A. pullulans* from Europe and North America and performed a variety of *in* and *ex planta* screens to identify traits and their genetic bases that contribute to colonization and disease control. We identified nutrient utilization and stress tolerance as traits significantly associated with colonization ability on lettuce. Genome-wide association implicated a SNP in the 5' UTR of a nuclear-localized putative E3 ubiquitin ligase as linked to a growth tradeoff for nitrate versus ammonium assimilation. Deletion of the gene and subsequent rescue demonstrated *in vitro* and *in planta* phenotypes. While the SNP did not predict gene expression at the locus in response to nitrate/ammonium availability, its location in the 5' UTR suggests that regulation may occur after transcription. The protein's nuclear localization further suggests that ubiquitination of transcription factors may contribute to the phenotype. Our approach of leveraging natural variation allows us to link colonization-relevant traits to specific loci and dissect genetic mechanisms, providing a framework for crop disease prevention via optimized inocula and host-strain matching.

516B

Genetic Manipulation of the Dermatophytes *Trichophyton mentagrophytes* and *Trichophyton rubrum*

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Superficial fungal infections caused by dermatophytes affect a substantial number of humans and animals worldwide. These specialized fungi are uniquely adapted to infect keratinized tissues, yet the molecular basis of their pathogenicity and infection strategies remains poorly understood. To advance functional studies in these medically important pathogens, improved genetic tools are needed. Here, we present a robust genetic manipulation platform for dermatophytes based on a CRISPR/Cas9 microhomology-mediated homology-directed repair strategy. This system enables efficient generation of targeted gene knockouts for dissecting secondary metabolite biosynthesis and virulence-associated traits. As a proof of concept, we disrupted a selected non-ribosomal peptide synthetase gene in two prevalent agents of dermatophyte infections, *Trichophyton mentagrophytes* and *Trichophyton rubrum*, using an *in vitro*-assembled ribonucleoprotein complex together with a 80 bp microhomology repair template, successfully generating targeted knock-out mutants These mutants were validated through whole-genome sequencing using long-read Oxford Nanopore technology following high-molecular weight DNA extraction. This CRISPR-based toolkit provides a powerful foundation for functional genomics in dermatophytes and opens new avenues for uncovering the molecular mechanisms underlying infection and pathogenicity.

517B

Mating hormone biosynthesis and mating type determination in *Phytophthora* are controlled by a conserved gene cluster

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Phytophthora is a genus of oomycete pathogens that resemble fungi but are distinct, diploid eukaryotes in the stramenopile group. Collectively, *Phytophthora* species cause blights, rots, wilts, and damping-off diseases on thousands of crop, forest, and wildland hosts, resulting in tens of billions of dollars in global losses each year. In many species sexual oospores serve as the overwintering inoculum for epidemics while asexual sporangia drive within-season spread. Some species are heterothallic, requiring two mating types to interact to form oospores, while others are self-fertile (homothallic). Heterothallics use two acyclic, diterpene hormones, alpha1 and alpha2, to trigger the sexual cycle. Despite the identification of mating hormones more than a decade ago, their biosynthetic pathway and genetic basis of mating type have remained elusive. Using phased genome assemblies, multiomics, and biochemistry, we defined the alpha2 biosynthetic pathway and a conserved gene cluster that helps establish mating type. Two cytochrome P450 monooxygenases (CYPs) are required to make alpha2. CYP1 is expressed specifically in A2 strains, whereas CYP2 is expressed in both mating types. Heterologous expression in engineered yeast showed that CYP2 first converts phytol to a hydroxyphytol intermediate and then CYP1 yields alpha2, based on metabolomic analysis and mating assays. Cas12a-mediated knockout of CYP1 in an A2 strain abolishes alpha2 production and leads to A1 phenotype, demonstrating that the A2-specific expression of CYP1 is a key determinant of mating type. CYP1 forms a co-regulated cluster with two START–FYVE domain proteins. Allele-specific RNAseq indicates that their expression is *trans*-controlled, consistent with a second unlinked regulatory locus. Comparative genomics across 96 Peronosporales species shows that this cluster is conserved in heterothallic *Phytophthora* and downy mildews but often damaged in homothallics. Nearly all species use differential expression of the cluster to encode mating type. However, clade 2 species establish

mating type using a hemizygous locus, based on our reanalysis of GWAS data from *P. capsici.* That prior study used an A1 reference genome that lacked this gene cluster, obscuring interpretation. By remapping that data onto an A2 genome we identified a hemizygous copy of the cluster in A2 strains, but absent from A1s. Work to identify what regulates expression of the gene cluster in *P. infestans* is ongoing.

518B

SIR2 is Required for Spore Development and Infection Capability in *Pyricularia oryzae*

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Silent Information Regulator 2 (SIR2) is a conserved NAD^+-dependent histone deacetylase that plays a central role in telomere maintenance, heterochromatin formation, and transcriptional silencing. Despite its importance in model organisms, the function of SIR2 in filamentous phytopathogenic fungi remains largely unexplored. Here, we investigated the biological role of SIR2 in *Pyricularia oryzae*, the causal agent of rice blast disease. Deletion of *SIR2* resulted in striking developmental and pathogenicity defects: the mutant exhibited a severe reduction in conidiation and complete loss of virulence, which we found to be caused by the failure of conidia to germinate. Transcriptome analysis revealed widespread gene-expression alterations in the *sir2* mutant, with strong enrichment for subtelomeric and stress-responsive genes. In parallel, chromatin profiling revealed marked changes in the distribution of the activating histone mark H3K27ac, indicating that SIR2 is required to maintain proper epigenetic landscapes at telomeric and gene-regulatory regions. Consistent with this, multiple subtelomeric gene clusters became aberrantly activated, suggesting a breakdown of telomere-proximal silencing normally maintained by SIR2. Moreover, genes involved in conidiation, germination, and early infection stages showed pronounced expression changes, reflecting the broad regulatory disruption caused by loss of SIR2 rather than simple repression. These chromatin and transcriptional abnormalities point to a global loss of epigenetic stability, potentially altering higher-order chromosome organization and telomere-associated regulatory circuits. Together, these findings demonstrate that SIR2 is essential for fungal development, chromatin integrity, and infection capability. Our study strengthens the broader understanding of how telomere-linked chromatin regulation influences genome function and pathogenicity in *P. oryzae*.

519B

Energy Trade-Off via Ribosome Biogenesis Control Underlies Early Plant Penetration in Plant Pathogenic Fungi

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Pathogenic fungi encounter multiple host defenses, necessitating tightly regulated gene expression to establish early infection. In plant-pathogenic fungi, early penetration of plant tissue is a critical step in pathogenicity and requires specific transcriptional regulators. To identify factors involved in this process in *Fusarium graminearum*, we screened a transcription factor (TF) knockout library and identified mutants defective in penetration. Among them, *FgIEC1* emerged as a key regulator, as the Δ*Fgiec1* mutant showed only slight growth defects but completely failed to penetrate a nitrocellulose membrane. Chromatin immunoprecipitation sequencing (ChIP-seq) revealed that Fglec1 directly regulates subunits of the INO80 chromatin-remodeling complex, including *FgINO80* and *FgIES4*. Knockout mutants lacking these INO80 complex components also displayed impaired penetration, supporting their critical roles in penetration. To further investigate the biological pathways associated with *FgIEC1*, we performed integrated transcriptomic and proteomic analyses under nitrocellulose conditions mimicking early penetration. Gene ontology enrichment showed that ribosome biogenesis is actively downregulated during penetration. However, Δ*Fgiec1* and INO80 subunit deletion mutants exhibited elevated ribosome-related gene expression and protein abundance. These findings indicate that Fglec1 directly regulates INO80 complex and affects ribosome biogenesis during early host entry. Collectively, this study identifies Fglec1 as a central regulator of penetration and provides new mechanistic insight into the pathogenicity of *F. graminearum*.

520B

Assessment of exudate-triggered responses driving *Fusarium solani* species complex dominance in the soybean spermosphere

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Members of the *Fusarium solani* species complex (FSSC) can predominate in the soybean spermosphere, but the mechanisms behind this early ecological advantage are still unclear. This study aimed to assess whether soybean seed exudates act as chemical signals that trigger transcriptional responses enhancing *Fusarium* dominance. Two isolates, *F. solani* Cherokee5 and *F. falciforme* Progeny4, capable of suppressing seedling emergence in soybean, were sequenced and assembled into genomes of 58.07 Mb and 62.65 Mb, with 95.3% and 97.2% BUSCO completeness, respectively. Cherokee5 included 41 biosynthetic gene clusters, 715 carbohydrate-active enzymes, and 460 predicted effectors, whereas Progeny4 contained 44 biosynthetic clusters, 815 CAZymes, and 509 effectors. To test our seed exudate hypothesis, transcriptomic profiling of *F. falciforme* after treatment with soybean seed exudate identified 1,259 differentially expressed genes ($|\log_2 FC| \geq 2$, padj < 0.05). Genes encoding transporters, oxidoreductases, and biosynthetic pathways for fusarubin, gibepyrone, cyclosporin, and sansalvamide were significantly upregulated. Several putative antimicrobial peptide-containing proteins also showed significant upregulation, suggesting enhanced competitive ability in the spermosphere. Overall, these findings show that soybean seed exudates stimulate secondary metabolism and secretion pathways in *Fusarium.* This may help uncover molecular mechanisms that support the predominance of members of the FSSC in the soybean spermosphere and early infection during seed germination.

521B

The US NIAID Genomic and Bioinformatic Resources for Fungal Research

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The National Institute of Allergy and Infectious Diseases (NIAID), through the Division of Microbiology and Infectious Diseases (DMID) has a comprehensive Genomics Program and funds extramural research to generate and analyze genomic and transcriptomic datasets and to advance cutting-edge 'omics' technologies. These technologies enable studies to address a wide variety of biological questions related to pathogen evolution, fitness, virulence and interactions with its host and microbiome to support the development of novel therapeutics, diagnostics and vaccines. This Program is in line with NIAID's mandate to quickly launch a research response to newly emerging and reemerging diseases and biodefense. Here, we provide an overview of NIAID/DMID's Genomics Program. (1) the Genomic Centers for Infectious Diseases (GCIDs) apply and develop innovative sequencing technologies for high-throughput pathogen and host genomics. The GCIDs have made important contributions to understanding of pathogen evolution and outbreak investigations, including for several fungal species: they used comparative and functional genomics to understand Cryptococcus, Aspergillus, and Histoplasma host-pathogen interactions, and have supported data analysis through the TERRA genomics workspace. (2) The Centers for Structural Biology of Infectious Diseases (CRSTAL-IDs) provide accurate and effective determination of 3D protein and protein-ligand structures; a significant proportion of human pathogenic fungal protein structures in the Protein Data Bank have been contributed by the CRSTAL-IDs. (3) The Systems Biology for Infectious Diseases Program use large scale data types for computational modeling of host-pathogen interactions and are developing predictive computational models of resolving and persistent Candida bacteremia from multi-omics datasets. (4) The Bioinformatics Resource Centers (BRCs) provide computational workbenches for access to multi-omics data, bioinformatics analysis tools and cutting edge AI technology, including for pathogenic fungi genome assembly and annotation, phylogenetic trees of curated sequences, and sequence-variation and metadata-driven sequence analysis. NIAID/DMID leverages these centers and other NIAID research programs in a concerted response to advance genomics and to drive discoveries in fungal evolution, diagnostics, therapeutics, and vaccine development.

522B

Development of a Genetic Transformation System for *Pseudopithomyces toxicarius*

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Pseudopithomyces toxicarius produces sporidesmin A, the mycotoxin responsible for sporidesmin-induced liver disease (SILD), also known as facial eczema, in ruminants. Although well-documented in New Zealand, recent surveys revealed *P. toxicarius* as the dominant *Pseudopithomyces* species present in dairy pastures located in the Eastern Cape, South Africa. From genome analyses and comparisons, a putative sporidesmin A biosynthetic gene cluster (BGC) was identified, but functional validation has not been possible due to the lack of a transformation system for this genus. *Agrobacterium*-mediated transformation (ATMT) is a widely used tool for targeted gene manipulation and functional genomics in filamentous fungi, but species-specific implementation remains challenging and often requires optimisation.

Here, we describe the development of the first ATMT workflow for *P. toxicarius*, adapted from a protocol established for *Cephalotrichum gorgonifer*. Isolates showing a characteristic sporidesmin-associated peak (m/z 474.0632) in UPLC-QTOF-MS/MS profiling were prioritised for transformation to link genetic manipulation with confirmed toxigenic potential. Using a custom pCBCT vector carrying a synthetic green fluorescence protein (sGFP) and the hygromycin resistance gene (hph), we optimized key parameters, including the duration of co-cultivation of *Agrobacterium* with *P. toxicarius*, substitution of freshly harvested spores with germinating conidia as starting material, and refined hygromycin selection thresholds. These adjustments made it possible to recover randomly integrated, hygromycin-resistant and mitotically stable transformants, although the efficiency was lower than reported in the original protocol.

This study established the first genetic toolkit for *P. toxicarius*. The successful transfer of a protocol originally developed for *C. gorgonife*r and its adaptation with minor modifications suggests that ATMT methods may be applicable across other fungal lineages. These results will enable the targeted manipulation of the sporidesmin A BGC and support functional studies on toxigenicity, secondary metabolism, and ecological adaptation of the fungus.

523C

Meta-analysis of *Pleurotus ostreatus* Transcriptomes: Global Transcriptomic Architecture and Transcriptional Effort Allocation in Basidiomycetes

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mRNA synthesis in fast-growing filamentous fungi represents a major energetic investment, accounting for up to 30% of total cellular ATP consumption. In the basidiomycete *Pleurotus ostreatus*, this investment is distributed across more than 12,000 genes, sustaining both homeostasis and the organism's ability to respond to environmental and developmental cues. Traditional transcriptomic studies typically focus on identifying genes that are differentially expressed between two specific conditions, often neglecting broader features of the transcriptome, such as its global gene expression distribution or overall transcriptomic landscape.

Here, we operationally define *transcriptional effort* as the proportion of total mRNA synthesis dedicated to a given gene or functional gene set. Using this concept, we characterize the global transcriptomic profile of *P. ostreatus* and investigate how transcriptional effort is allocated across individuals, developmental stages, and environmental conditions. Our analyses address several key questions: What is the baseline transcriptomic architecture of a growing filamentous basidiomycete? Which genes dominate the transcriptional landscape in actively expanding colonies? Which genes maintain stable

expression across diverse growth conditions? What fraction of total transcriptional effort do these core genes represent? How is transcriptional effort partitioned among major functional gene categories, including those involved in primary metabolism, secondary metabolism, and informational processes? Finally, can distinct transcriptional effort profiles be associated with particular biotic or abiotic stress conditions?

Overall, this study proposes a novel framework for the analysis of large-scale basidiomycete transcriptomic datasets, offering new insights into the regulatory, adaptive, and ecological strategies that underpin the survival and phenotypic plasticity of filamentous fungi.

524C

Adaptations in the polyextremophilic yeast, *Naganishia friedmannii*. Surprises from comparative genomics and a series of freeze thaw RNA Seq experiments.

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We have developed a system to study Eukaryotic adaptations to extreme environments using the polyextremophilic yeast, *Naganishia friedmannii*, a distant relative of *Cryptococcus neoformans*. This particular strain of *N. friedmannii* was isolated from a high elevation, high UV, xeric, volcano in South America, where the daily temperature fluctuations range from approximately -10 °C to 26 °C. It is the dominant microbe that survives in this harsh environment and serves as a promising eukaryotic model for understanding stress tolerance under combined environmental extremes. To understand adaptations of this fungus to such extreme conditions we sequenced its genome and compared it with closely and distantly related yeasts, many of which also live in extreme environments. We found that *N. friedmannii* has expanded carbohydrate metabolism and a limited DNA repair repertoire, promoting a high mutation rate. In addition, we exposed liquid cultures of *N. friedmannii* to freeze-thaw cycling in the laboratory and sequenced RNA extracted from cultures growing at different temperatures within the daily thermal cycle ranging from a high of 27°C to a low of -10°C to analyze its transcriptional response under extreme temperature fluctuations. Cultures grown at optimal constant temperature of 17°C were used as controls. We identified stress related genes that are upregulated in the warmest condition we tested, constant 26°C, which had the most divergent transcriptional profile compared to all other conditions. We also found large differences in RNA profiles between the cultures experiencing "Heatshock Recovery" and those growing at 0°C or below, and "Heatshock Recovery" and "Coldshock Recovery" profiles were nearly identical.

525C

Dissecting the root-fungal interface in 3D reveals spatially distinct signalling landscapes

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Plant rhizospheric interactions represent intricate relationships that determine plant fitness and are crucial for interrogating host-pathogen dynamics, with significant fundamental and translational implications. Most fungal-plant interactions occur in soil – a disordered and granular 3D environment – and hence remain challenging to unravel due to complex regulatory networks. Our current body of evidence characterising these molecular dialogues largely stems from experimental systems employing soil or *in vitro* 2D flat plates, hydroponics and gnotobiotic systems. Soil itself features widely varying visco-elasto-plastic material properties, and its inherent opacity precludes direct visualisation of the infection progression in complex diseases such as wilts and root rots. Here, we introduce the first such optically transparent, 3D granular growth matrix to recapitulate complex properties of the soil microenvironment, which enables direct, cellular-level visualisation of the plant-fungal interface in vascular wilt disease by *Fusarium oxysporum*. Our mechanically tunable 3D matrices support long-term co-culture of plants and fungi with compatibility to classical molecular and physiological assays for unravelling the early signalling events and inter-kingdom crosstalk. By leveraging the optical transparency of this matrix, we track fungal development in response to host signals *ex-planta* with unprecedented 3D resolution, to report pioneering evidence of hyphal reprogramming preferentially towards the root tips during the early stages of infection. Crucially, we integrate spatiotemporal transcriptomic analyses and discover distinct pathogen-host *ex-* and *in-planta* modules during early signalling, which are likely associated with biomimetic soil-like environments. Together, our findings establish an integrable and versatile 3D platform offering an unprecedented view of the pathogen infection processes, which enables fundamental discoveries into the biological regulation of growth and infection. These insights hold immense potential for advancing our understanding of host immune responses and adaptation of filamentous pathogens, as well as open avenues to decipher drought and disease-resistance mechanisms with major agricultural benefits.

526C

Genomic and transcriptomic profiling of *Hanseniaspora guilliermondii*'s ethanol stress response

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Hanseniaspora guilliermondii (*Hg*) is a non-conventional yeast with recognized potential as a bioflavorant, particularly in winemaking. Nonetheless, its low ethanol tolerance, a trait in the *Hanseniaspora* genus, is a recognized "Achilles-heel" that reduces their competitiveness during fermentation and limits their aromatic contribution to the final product. Leveraging the previous disclosure of the first *Hg* genomic sequence (of the UTAD222 strain) (Seixas et al., 2019), we explored transcriptomics, chemogenomics and comparative genomics to disclose the molecular mechanisms by which this species responds to and tolerates ethanol stress. RNA sequencing performed on ethanol-stressed *Hg*UTAD222 cells (exposed to 6% v/v for 1 hour) uncovered massive alterations in the transcriptome of this yeast, resulting in 305 up- and 392 down-regulated genes, compared to transcript levels observed in non-stressed cells. A substantial portion of the differently transcribed genes under ethanol stress play a role in lipid metabolism, pointing to a substantial membrane restructuring in response to ethanol toxicity. Results from phenotyping of a mutant library obtained by chemical mutagenesis of *Hg*UTAD222 with EMS (ethyl methanesulfonate) corroborated this idea, being identified at least one mutant strain hyper-susceptible to ethanol that was found to harbor disruptive modifications in UFD2, a gene encoding a putative ubiquitin ligase predicted to regulate unsaturated fatty acid biosynthesis. This represents the effective

first ethanol-resistance gene identified in the *Hg* species. This is the first reported study so far to examine stress tolerance in *Hg*, which remains poorly characterized especially from a molecular point-of-view. The results elucidate essential aspects of this yeast's physiology and stress response mechanisms, unlocking *Hg*'s potential in the industry as a bioflavorant and as a cell factory for production of add-value molecules where low ethanol tolerance can be a bottleneck.

527C

A CRISPR activation platform to identify genes involved in trisomy-associated antifungal drug resistance in *Candida albicans*

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Candida species are among the leading causes of invasive infections and the fourth most common cause of hospital-acquired bloodstream infections. There are limited therapeutic options for treating *Candida* infections and other fungal diseases, and treatment is further hindered by the evolution of resistance to antifungal therapies. One way in which *Candida* rapidly adapts to antifungal drugs is via large genome rearrangements, including aneuploidies such as trisomies. These chromosomal trisomies are frequently identified in drug-resistant *Candida* strains, and are responsible for conferring resistance and tolerance, although the precise mechanism by which these trisomies lead to resistance is not always understood. We are using a novel approach to dissect the genetic mechanisms underlying aneuploidy-linked antifungal drug resistance in *Candida albicans*. Using CRISPR activation (CRISPRa)-based overexpression techniques, we have generated chromosome-wide libraries of overexpression strains to identify key genes that, when overexpressed, confer increased resistance to diverse antifungal agents. We have further developped multiplexing Cas12-CRISPR tools to validate genes singly and in combinations, that are involved in trisomy-mediated drug resistance and tolerance. Together, this work is exploiting novel functional genomic approaches to characterize the fundamental genetic mechanisms by which important fungal pathogens adapt and evolve resistance to antifungal drugs.

528C

Understanding how Candida albicans, a fungal species, is adapted for survival in the human gut microbiome

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The World Health Organization (WHO) has classified fungal pathogens like *Candida albicans* and *Candida auris* as critical priority pathogens due to their capacity to cause severe, often lethal medical complications. Understanding how fungal pathogens evolve is essential to reveal how they differ from their close relatives and to anticipate emerging threats. I aim to address a simple yet important question: why does *C. albicans* successfully colonize the human gut, while its close relatives including *Candida dubliniensis* cannot? To identify the adaptations that enable *C. albicans* to dominate this niche, I performed a systematic screen of 70 diverse fungal species under conditions that mimic the gut. This revealed a key discovery: the ability to utilize host-derived sulfur compounds acts as a powerful ecological filter. The metabolism of these compounds in fungi is entirely unexplored.

Comparative analysis across 1,000 fungal genomes spanning 500 million years suggests that this metabolic capability arose through small modifications of existing genes and regulatory elements, highlighting a lineage-specific adaptation that enables *C. albicans* to thrive in the gut. These findings provide new insight into the molecular and evolutionary mechanisms by which fungi colonize host niches and interact with microbial communities, establishing a framework for understanding gut-specific adaptations in fungal pathogens and informing strategies to combat fungal infections.

529C

Paralogs of the *Candida albicans TLO* (telomere-associated) gene family form interconnected functional networks through incomplete redundancy

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Gene families form through a process of repeated gene duplications. Although most cells containing duplicated genes are culled from the population, paralogs that enhance organismal fitness may be retained in a population. Retained paralogs are often thought to increase gene expression, encode a specialized function, or acquire novel function. While the function of gene duplicates has been studied in detail, little work has explored how repeated duplication impacts paralog redundancy and may restrict the emergence of new paralogs or novel function. Here, we constructed a panel of single deletion mutants for each of the 14 members of the *Candida albicans* telomere-associated (*TLO*) gene family to determine the degree of molecular and biological redundancy among paralogs from a lineage-specific expansion. Tlo proteins function as interchangeable subunits of the Mediator transcriptional regulatory complex and have the potential to alter gene expression and an array of cellular responses. Approximately 80% of the phenotypic assays performed with single gene deletion strains displayed redundancy. However, mutants for all 14 paralogs displayed non-redundant functions ranging from carbon utilization to *in vivo* virulence. Loss of single *TLO* genes produced similar trends in redundant and non-redundant changes in gene expression that disproportionately affected cell cycle regulation, morphology, and the response to stress. Importantly, sequence divergence between paralogs correlated with the frequency of altered phenotypes in single *TLO* mutants, indicating the acquisition of non-redundant function with increased evolutionary distance. Double mutants lacking two *TLO* genes produced both positive and negative synergistic phenotypes, suggesting that crosstalk or coordinated regulation is common among paralogs. Together, this study demonstrates that recently emergent paralogs acquire non-redundant functions despite often retaining redundancy with other gene family members to create a highly interconnected functional network.

530C

Staying Dry: The Peptidic Architecture of Fungal Hydrophobicity

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Maintaining highly hydrophobic cell surfaces is critical for fungal survival under fluctuating environmental conditions, yet the molecular mechanisms responsible for this property remain incompletely understood. Emerging evidence indicates that the majority of ascomycetes employ a conserved class of hydrophobic non-ribosomal peptides—termed WAter-Repellent Peptides (WARPs)—as metabolite-like contributors to the stability of aerial mycelium. These peptides show substantial diversity in length and structural features across lineages, reflecting an evolutionarily adaptable scaffold that nevertheless converges on a shared biophysical role. Impaired WARP biosynthesis correlates with rapid collapse of aerial hyphae when exposed to water, highlighting their importance for maintaining surface integrity. Taken together, these observations point to a widely distributed strategy in which many ascomycetes rely on small, specialized peptides, working in concert with established cell-wall components, to sustain a highly hydrophobic outer surface. This framework offers a refreshed perspective on fungal surface biology and the molecular systems that support it.

531C

Environmental impacts on gene expression in the amphibian gut fungus *Basidiobolus*

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Basidiobolus is a genus of fungi with a complex life cycle of contrasting environmental niches and unique hyphal and spore morphotypes. It spends part of its life cycle as a free-living filamentous saprobe on plant detritus and exists in the anaerobic digestive systems of herptiles in a single-cell growth stage. *Basidiobolus* genomes exhibit a strong signal for biologically active specialized metabolites, which are predicted to provide an adaptive advantage. To elucidate functional variation and gain insight into the ecological relevance of this genus, we subjected three phylogenetically distinct strains of *Basidiobolus* to different pH levels and both aerobic and anaerobic conditions—abiotic factors that reflect the variation encountered in free-living environments and the herptile gut. In-vitro manipulation of oxygen and pH induced morphologies associated with these life stages, and fungal biomass was further assessed via RNA-seq to evaluate gene expression. Preliminary data indicate that aerobic conditions resulted in greater variation of specialized metabolism within and between species compared to anaerobic conditions, suggesting anaerobic conditions may promote a more conserved metabolism within the gut environment. Additionally, the anaerobic low-pH condition showed an overrepresentation of upregulated chitin CAZymes and serine- and metalloproteases, supporting the hypothesis that *Basidiobolus* is metabolically active in the herptile gut microbiome. We will present data and analyses exploring differential gene expression in response to environmental variables relevant to the life history of *Basidiobolus*, providing insights into the functional diversity of this complex fungal genus.

532C

Saturation mutagenesis of fungal *pyrE* to identify variants that endow resistance to DHODH inhibitors without impacting fitness

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The use of demethylation inhibitor fungicides as plant protective products have driven the expansion of azole-resistant populations in the human fungal pathogen, *Aspergillus fumigatus,* reducing the effectiveness of first-line therapeutics. In the wake of this problem, new antifungals with novel modes of action have been developed. The dihydroorotate dehydrogenase (DHODH) inhibitor olorofim is in the late stages of clinical development. However, in parallel, an analogous DHODH inhibitor, ipflufenoquin, has been developed as a fungicide and has been approved for use in several territories, including the USA. This puts at risk decades of clinical antifungal development. We have recently shown that mutations at several sites in *pyrE,* the gene that encodes DHODH, can provide cross-resistance to both olorofim and ipflufenoquin in *A. fumigatus*. The G119 codon position is one of these key sites. Here we used a rapid *in vivo* saturation mutagenesis approach coupled with competitive fitness assays to understand which G119 variants are most important for cross resistance.

We deployed selection-free CRISPR-Cas9 to generate a pooled library of all 64 possible codon variant strains at the G119 position, achieving a transformation rate of between 8 and 32%. Competitive profiling of the library in the absence of drug revealed that high fitness correlated with substitution of glycine with other small, uncharged amino acids. Variant fitness was modulated in the presence of DHODH inhibitors, with some strains exhibiting high fitness in either olorofim, ipflufenoquin or both. Worryingly, several variants, including G119C, G119A and G119S showed cross resistance to both inhibitors while displaying no fitness defect in the absence of drug. We also demonstrate that this approach enables evaluation of fitness in complex growth substrates, including soil microcosms, allowing us to determine minimum selective concentrations of ipflufenoquin. Furthermore, we reveal that selection for ipflufenoquin resistance in soils can be mitigated by exogenous pyrimidine supplementation.

533C

Role of pyrimidines transport in the adaptation to the novel antifungal olorofim in *Aspergillus fumigatus*

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Aspergillus fumigatus is an environmental fungus that can infect humans and cause life-threatening or debilitating lung diseases. Azole drugs are the first-line treatment, but the number of human infections caused by azole-resistant *A. fumigatus* have increased over last years. The development of new antifungals with novel mechanisms of action has been proposed as the most promising intervention to stop and contain the emergence of antifungal resistance. Olorofim acts by inhibiting the de novo synthesis of pyrimidines in a fungal-specific manner and will reach the clinic in the following years. Therefore, a thorough understanding of processes impacting olorofim's effectiveness is urgently needed. Supplementing exogenous pyrimidines (uracil and uridine) upon olorofim exposure completely restores *A. fumigatus* growth. However, when decoupling them, uracil alone is enough to reverse olorofim's

activity in all *A. fumigatus* strains tested in the study while uridine only restores growth in a subset of them. By using functional genomics, genome association analyses and transcriptomics we have identified the amino acid starvation-responsive transcription factor CpcA as a regulator of uridine uptake in *A. fumigatus*. These findings indicate a pre-existent genomic variability in uridine uptake regulation in natural populations of *A. fumigatus* strains that might impact olorofim's performance upon clinical deployment.

534C

Spore Whiteout – Depigmentation of *Apiospora arundinis* Conidia

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The genus Apiospora, known for its impressive capacity to produce diverse secondary metabolites, is globally distributed and predominantly comprises plant-associated pathogens, endophytes, and saprobes. Apiospora arundinis AAU773 contains 38 predicted PKS biosynthetic gene clusters, eight of which are conserved across 13 species. Among these conserved clusters, the non-reducing PKS6 gene has been identified as a strong candidate for spore-pigment biosynthesis based on its domain architecture and predicted functional annotation. Using a CRISPR/Cas9 microhomology-mediated homology-directed repair strategy, a PKS6 knock-out mutant was generated that exhibited complete loss of pigmentation in the normally dark brown conidia characteristic of Apiospora. Additionally, synteny analysis of the genomic region surrounding the PKS6 cluster shows that eight genes are conserved across the 16 analyzed strains (including 9 different species) of *Apiospora*. These findings establish PKS6 as essential for conidial pigment formation in Apiospora and highlight a conserved gene cluster likely involved in pigment biosynthesis across the genus.

535C

Dissecting the homeodomain *MAT* locus and engineering novel tripolar and bipolar mating systems in *Cryptococcus amylolentus*

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Sex in fungi is governed by the mating-type (*MAT*) locus, which exists as bipolar, pseudobipolar, or tetrapolar systems. The significance and impact of *MAT* on sexual reproduction, however, remain understudied. Furthermore, the evolution of fungal *MAT* loci shares features with the evolution of sex chromosomes in plants and animals. Pathogenic *Cryptococcus* species harbor a bipolar system with a large contiguous *MAT* locus, whereas closely related species, such as the non-pathogen *C. amylolentus* possess a tetrapolar system with unlinked *P/R* and *HD* loci. The *HD* locus encodes homeobox domain containing proteins that play an important and evolutionarily conserved role in sexual reproduction. Here, we explored the roles of *HD* genes in sexual reproduction and determined the implications of a tetrapolar to bipolar *MAT*transition. With a CRISPR–Cas9 system we developed for *C. amylolentus*, we generated gene deletion mutants and demonstrated that a single compatible Sxi1-Sxi2 pair is necessary and sufficient for mating. By relocating the *HD* genes to the *P/R* locus, we found that the artificially generated bipolar configuration led to defective sexual development, which could be partially restored through additional rounds of sexual reproduction. Transcriptomic profiling further revealed that a Sxi1-Sxi2 heterodimeric complex drives expression of genes required for DNA replication and ergosterol biosynthesis during sexual reproduction. These findings provide the first experimental demonstration of a tetrapolar-to-bipolar transition in a tetrapolar mating species, illuminating *MAT* locus evolution and homeodomain protein functions in *Cryptococcus*.

536C

Can genes “knock-in/out” resolve the “loss-for-gain” paradox of brown-rot fungal wood decomposition?

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Fungi have evolved two distinct strategies, white- and brown-rot, to degrade lignocellulose in woody biomass. White rot fungi (WRF) utilize the lignin-modifying enzymes (LMEs) to deconstruct lignin to access carbohydrates, whereas brown rot fungi (BRF) shed the LME activities while emerging from the WRF ancestral branches. Genome sequencing projects have shown that not only the LME genes but also many functional CAZy genes involved in lignocellulose degradation have been lost in BRF. Gene losses, however, created more efficient carbohydrate utilizers and faster wood decomposers in BRF. This long-lasting “loss-for-gain” paradox of BR needs to be resolved. Especially, genes responsible for brown-rot need to be identified, and the underlying forces driving the massive gene-loss event need to be explained.

It is proposed that BRF employs a delignification system that relies on Fenton chemistry to produce reactive oxygen species (ROS) for the rapid deconstruction of lignocellulose and the sequential, selective removal of carbohydrates. The redox cycling driven by lignin-like hydroquinone compounds is documented *in vitro* as capable of mediating the production of Fenton reagents of H_2O_2 and Fe^{2+}. Although the biochemistry is plausible in explaining brown-rot efficiency, the fungal genes responsible for these processes have not been identified, and the Fenton paradigm remains incomplete. Besides the unidentified Fenton genes, LME gene loss remains mysterious, too. Genomic analyses have suggested that both the LME and Fenton-like genes can be found concurrently within a single white-rot fungal system, but it is not clear what drove the BRF adaptation thrived through the evolution involving the loss of LME genes.

To answer the above questions and resolve the “loss-for-gain” paradox, we developed a set of genetic toolkits in the typical brown-rot species, *Gloeophyllum trabeum*, for accurate genome editing and rapid mutant screening, enabling knock-in or -out of genes of interest. Genetic manipulation procedures were tailored for the heterokaryotic strain, which has a complex genome structure, but can most likely maintain the complete genetic inventories essential for wood decay. These tools allowed us to investigate the distinctive genetic inventories that brown rot has adapted. The suspicious benzoquinone reductase (BQR) genes that drive Fenton recycling were deleted, the LME genes were retro-expressed, and the wood decay phenotypes of the mutants were assessed to interrogate their genetic functions. With these research, we revealed the allele-specific roles of BQRs played in balancing quinone detoxification and wood degradation, and the incompatibilities of LMEs with the recently adapted, Fenton-dominated system in BRF. The tools established in these studies will be continuously employed for genotypic studies for discovering the distinctive mechanisms adapted in BRF.

537C

How does genetic background influence phenotypes?

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Genetic background profoundly influences how organisms tolerate stress and evolve, yet we lack a systematic understanding of how background shapes both essential gene requirements and adaptative routes. This gap limits our ability to predict pathogen behavior and design targeted interventions. The fungus *Cryptococcus neoformans* is an ideal organism to study this question: it is genetically diverse, phenotypically variable, and genetically tractable. Clinically, it is a major opportunistic pathogen that causes life-threatening lung infections and meningitis, responsible for more than 147,000 deaths annually, particularly among individuals with HIV/AIDS. Here, we test the hypothesis that specific cellular pathways are more susceptible to genetic background effects on gene essentiality and stress adaptation. We leverage a lab-developed genome-wide CRISPR/Cas9 mutagenesis screening platform capable of disrupting all non-essential *C. neoformans* genes (~5,500) in a single transformation. We are constructing barcoded CRISPR/Cas9 gene disruption libraries in eleven genetically diverse clinical and environmental isolates and conducting pooled fitness screens under heat and antifungal stress. Barcode sequencing will enable quantitative comparisons across backgrounds to identify (i) universally required genes, (ii) background-specific genetic determinants of stress tolerance, and (iii) pathways enriched for background-dependent requirements. By illuminating how background shapes pathogenic potential, this work will reveal fundamental principles of microbial evolution and directly inform precision antifungal strategies.

538C

Functional characterization of a SAM-dependent methyltransferase implicated in sectorization of MAPK mutants in *Cryphonectria parasitica*

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Sectorization in *Cryphonectria parasitica* is a distinctive morphological trait that has been previously shown to arise in mutants defective in the cell wall integrity (CWI) MAPK pathway, including *CpBck1* (MAPKKK) and *CpSlt2* (MAPK). Although previous studies have shown that disruption of this pathway can result in sporadic sector formation, the genetic factors underlying the transition to the sectored phenotype have remained unclear. To identify candidate genes associated with sectorization, we performed comparative transcriptomic analyses of *CpBck1* and *CpSlt2* null mutants and their sectored progeny. Among 73 genes consistently altered in both mutant backgrounds, 22 were also responsive to CHV1 hypovirus infection, indicating an overlap between virus-induced transcriptional changes and MAPK pathway disruption share common targets in regulating morphogenesis. From these, a gene encoding an S-adenosylmethionine (SAM) dependent methyltransferase emerged as a candidate regulator. Because SAM-dependent methyltransferases function in epigenetic modification, RNA and protein methylation, and secondary metabolite biosynthesis, the differential expression of this gene suggests that chromatin or metabolic changes may contribute to the establishment of sector morphology. To test this hypothesis, we have generated targeted mutants of this methyltransferase. These experiments aim to reveal whether this enzyme modulates metabolic pathways to integrate MAPK signaling and CHV1-responsive programs during the development of sectorization.

539C

CpFre1-mediated iron homeostasis in relation to hypovirus infection and sectorization

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The chestnut blight fungus *Cryphonectria parasitica* and its hypovirus Cryphonectria hypovirus 1 (CHV1) is a model system for investigating mechanism of hypovirulence. In our previous study, null-mutants of cell wall integrity (CWI) pathway genes (*CpBck1* and *CpSlt2*), which were affected by CHV1 infection, exhibited a distinctive phenotype marked by sporadic sectorization. Transcriptomic analysis revealed that the putative ferric reductase gene (*CpFre1*) was specific to both sectorization and CHV1. *CpFre1* was most similar to *Fre7* of *Saccharomyces cerevisiae* and is predicted to encode a membrane-bounded ferric reductase responsible for converting ferric (Fe^{3+}) to ferrous (Fe^{2+}) for cytosol uptake. Compared to the wild type EP155/2, the *CpFre1*-null mutant exhibited severe growth inhibition, abnormally intense pigmentation, and a markedly thickened cell wall. The growth defects were partially rescued by ferrous (Fe^{2+}) supplementation, supporting that *CpFre1* encoded gene for ferric reductase and is required for efficient ferric reduction for uptake. Stress response of the *CpFre1* null-mutant revealed an increased sensitivity to osmotic stress, whereas responses to oxidative and cell-wall perturbing stresses were unaffected. The Wild-type and even *CpFre1*-null mutant exhibited lower pathogenicity than the CHV1-infected hypovirulent UEP1. These findings demonstrate that *CpFre1* is a key regulator of iron acquisition, cell-wall organization, and pathogenicity.

540A

Genomic features of parasexual reproduction among two genetically distinct hybrid groups of *Cryptococcus neoformans*

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Hybridisation is an important source of genetic and phenotypic diversity in fungi, yet the mechanisms shaping their genomes remain unclear. *Cryptococcus neoformans* is a major cause of fungal meningitis and comprises two varieties: *var. grubii* (VNI) and *var. neoformans* (VNIV), which occasionally hybridise to produce AD hybrid strains that are typically diploid or highly aneuploid. To investigate parental origins and recombination, we analysed 197 *C. neoformans* genomes, including 13 newly sequenced isolates, with assembly, haplotyping, phylogenetics, and population genetics approaches. We identified 73 hybrid isolates forming two groups that we named H1 and H2, which have distinct ecological origins and parental ancestry. H1 comprised exclusively clinical isolates, had limited admixture, yet had high nucleotide diversity, consistent with older hybridisation events. H2 primarily consisted of environmental or laboratory-derived crosses, and exhibited extensive admixture between VNI and VNIV lineages. Both groups displayed pervasive aneuploidy, chromosome/chromosome-arm wide loss-of-heterozygosity, and evidence of crossovers. Furthermore, mosaic haplotype inheritance revealed patterns similar to those observed following parasexual reproduction in distantly related fungi such as *C. albicans*. Phenotypic assays of the hybrids revealed substantial variability in growth and virulence in *Galleria mellonella*, highlighting functional impacts of hybrid genome plasticity. Our study provides

new insights into the genomes of *Cryptococcus* following hybridisation, with potentially evidence of parasexual reproduction. This unique strategy may shape genome evolution, population diversity, and pathogenic potential of *Cryptococcus*.

541A

Uncovering Cultivable Fungal Diversity from Cabbage Microhabitats Through Selective Media Strategies

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Culture-based methods remain essential for linking fungal taxa to ecological function, yet many members of plant-associated microbiomes remain difficult to isolate due to media-driven biases. To evaluate how medium composition shapes the cultivable fungal community of Brassica rapa, we compared seven media—½-strength R2A, R2A agar, soil extract agar (SEA), DG18, Czapek-Dox, 10% PDA, and water agar—using soil, rhizosphere, and root endosphere samples. Across all media, 93 species were recovered based on colony phenotype and ITS rDNA sequencing. Diversity differed markedly by medium: 10% PDA and water agar yielded the broadest taxonomic range, while SEA and DG18 facilitated the recovery of several slow-growing or previously uncultured taxa. Notably, taxa considered rare or difficult to cultivate were isolated when low-nutrient or minimally selective media were applied, highlighting the importance of medium choice in shaping observed diversity. These results demonstrate how selective media influence the assembly of cultured fungal communities and provide a foundational framework for expanding the cultivable fraction of the Brassica rapa fungal microbiome. This work supports future functional and genomic studies aimed at understanding ecological roles, host interactions, and metabolic capabilities of cabbage-associated fungi. (This work is supported by RDA, Korea: RS-2025-02653099)

542A

A 150-Year Record of Foliar Fungal Endophyte Dynamics in Norway Spruce (*Picea abies*) Across Finland

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Herbarium specimens offer valuable insights into plant history, yet their potential to reveal past plant-associated microbiomes remains underexplored. Here, we reconstructed the temporal and spatial dynamics of foliar fungal endophytes in Norway spruce (*Picea abies*) by sequencing the ITS region from herbarium needles collected in Finland (1861–2023). Fungal communities were analyzed across 50- and 20-year intervals and between southern and northern regions, considering temperature and precipitation effects. Ascomycota dominated the community, with *Lophodermium, Ceratocystis, Yarrowia, Saccharomyces*, and *Heterobasidion* as the most abundant basidiomycete genera. *H. parviporum* appeared in one 50-year interval, while *Lophodermium piceae* increased significantly during 2001–2023 ($P < 0.05$). Community composition differed significantly between 1951–2000 and both 1851–1900 and 1901–1950 ($P < 0.05$). The southern region showed distinct composition and lower alpha diversity than the north ($P < 0.05$). Functional predictions indicated a rise in Saprotroph, Symbiotroph, and mixed guilds during 1901–1950 ($P < 0.05$). Although temporal and spatial factors strongly structured communities, no direct correlations with historical temperature or precipitation were found. These findings demonstrate that herbarium specimens are powerful tools for reconstructing long-term microbial dynamics and reveal time and geography as key drivers of foliar fungal community structure.

543A

ColonyPy: A python-based image analysis pipeline to quantify tolerance and heteroresistance in *Cryptococcus neoformans*

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Clinical outcomes in cryptococcal meningitis patients infected with fluconazole-susceptible *Cryptococcus neoformans* isolates vary widely and can include treatment failure, relapse, prolonged therapy, and high mortality. These discrepancies suggest that conventional drug susceptibility testing may overlook subtle phenotypes that survive above the Minimum Inhibitory Concentration(MIC) in *C.neoformans*. Two phenomena are increasingly recognized in susceptible isolates of pathogenic yeasts. Tolerance, defined as slow-growing subpopulations that survive above the MIC, and heteroresistance, where ~1% of cells grow at concentrations above the MIC. These phenomena are frequently observed with fluconazole (FLC), a fungistatic drug central to cryptococcal meningitis therapy. Importantly, tolerance and heteroresistance differ from resistance. In *Candida albicans* tolerance is common whereas *C.neoformans* exhibits both tolerance and heteroresistance. To simultaneously measure tolerance and heteroresistance, we developed ColonyPy, a Python-based image analysis pipeline using open-source libraries. ColonyPy detects and quantifies colony number and size within the zone of inhibition (ZOI) in disk diffusion assays, and above the MIC in agar dilution plates. Using ColonyPy, we quantified tolerance and heteroresistance in 40 *C.neoformans* clinical isolates using disk diffusion with FLC 25ug disks. Culture plates were photographed at 48, 72, 96 and 120 hours post-inoculation on Sabouraud dextrose agar (SDA), Yeast peptone dextrose agar (YPD) and Müller-Hinton agar (MHA) with 2% glucose incubated at 37°C. The number and size of colonies within the zone exhibiting ≥80% inhibition (ZOI 80) were analyzed using the ColonyPy program. This analysis revealed that: (1) clinical isolates of *C. neoformans* varied in their levels of tolerance and heteroresistance; (2) tolerance and heteroresistance levels differed significantly across media types; (3) YPD showed the highest levels of both tolerance and heteroresistance; and (4) both tolerance and heteroresistance stabilized after 96hrs, defining a reliable measurement time point. Taken together, our results demonstrate that tolerance and heteroresistance are distinct, stable phenotypes in *C.neoformans* that are influenced by nutrient source. Our robust assay that differentiates between tolerance and heteroresistance will facilitate future studies to elucidate underlying mechanisms of these phenomena and their role in treatment outcomes.

544A

Insights into the Taxonomic Diversity and Genome Complexity of the Fusarium Bean Root Rot Complex

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The *Fusarium solani* species complex (FSSC) contains more than 20 phylogenetic clades. Pathogens causing bean root rot belong to Clade 2 of this species complex (FSSC 2). In recent years, three of the four causal species of bean root rot have been identified in Michigan: *F. virguliforme*, *F. cuneirostrum* and *F. brasiliense*; with the latter two previously thought to be endemic to South America. Because FSSC 2 species are cryptic, their true diversity and distribution are poorly understood, and accurate identification remains challenging. The main objective of this study was to better understand the taxonomic and genomic diversity of FSSC 2 present in dry bean hosts throughout Michigan. A total of 110 isolates collected between 2014–2025 from 7 counties were identified using multilocus sequence typing (MLST) of the *ITS*, *TEF1, RPB2, TUB1,* and *IGS* loci. Phylogenetic analysis revealed greater species diversity than previously documented, identifying species such as *F. crassipitatum,* previously unreported in the region. Whole-genome sequencing was used to examine genome plasticity in representative isolates from the bean root rot complex that were selected from the MLST phylogeny. Genomes were sequenced using Oxford Nanopore technology, and comparative genomics paired with synteny analyses were performed to assess genomic diversity within the complex. This approach resulted in the construction of new chromosome-level reference genomes for the species in FSSC 2, showing structural variation, including chromosomal rearrangements consistent with high genomic flexibility within FSSC 2. Overall, this work strengthens current understanding of the taxonomy and genome complexity of the bean root rot complex, and provides a foundation for improved surveillance and disease management strategies.

545A

Diversity and Functions of Zoosporic Pathogens of Invertebrates

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Invertebrate pathogenic fungi have canonically been thought to predominate in several ascomycete orders (e.g. Hypocreales, Onygenales, Orbiliales) and in the non-dikarya order Entomopthorales (e.g. “insect destroyers”). The role of other non-dikarya fungi as insect pathogens has often been overlooked but emerging evidence suggests that taxa in Mucormycota, Glomeromycota, Chytridiomycota, Kickxellomycota, and Zoopagomycota also function as pathogens of insects and other invertebrates (nematodes, aphids, mites) and may have potential for development of biocontrol. As many of these fungi are near obligate pathogens that are difficult to culture, environmental sequencing and metagenomic studies have revealed their diversity and abundance in natural ecosystems. Metabarcoding studies of a nematode suppressive soils in our laboratory have identified several taxa in Mortierellomycota, Kickxellomycota, and Chytridiomycota that represent hub taxa in co-occurrence networks and/or are enriched or unique to suppressive soils, suggesting they may play key roles in controlling nematode populations. An isolate of the Kickxellomycota genus *Ramicandelaber,* originally isolated from a cyst nematode, was identified in the USDA-ARS Entomopathogenic Fungi (ARSEF) collection and cultured for genome sequencing and functional assays of nematode parasitism. The ARSEF collection houses over 12,000 isolates of fungi isolated from invertebrate hosts and contains the largest collection of Entomopthoromycota in the world as well as other early diverging phyla. Mapping of host-metadata from the ARSEF onto fungal phylogenies has identified multiple independent origins of nematode parasitism or symbiosis among early diverging fungi. Other recent research using metadata from the ARSEF collection and literature reviews has updated a checklist of arthropod pathogenic Entomopthoromycotina in North America (Canada, Mexico, USA) and highlights key gaps and priorities for new collections. Our research underscores the value of environmental sequencing in understanding ecological roles and the importance of preserving and leveraging biocollections of zoosporic fungi for research on genomics, taxonomy, host-interactions, and mechanisms of symbiosis.

546A

The Macroevolution of Filamentation Morphology Across the *Saccharomycotina* Yeast Subphylum

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Saccharomycotina yeasts are a highly diverse and widely distributed subphylum of ascomycete fungi that exhibit diversity in their asexual growth morphologies; the human commensal yeast *Candida albicans* exhibits both unicellular and filamentous growth, whereas the baker’s yeast *Saccharomyces cerevisiae* grows unicellularly by budding. Filamentous growth (and the production of hyphae) can comprise linear and branched budding cells that do not undergo cell separation, termed pseudohyphae, or of tubular filaments with septa that perforate allowing movement of organelles, termed true hyphae. We integrated phenotypic, genomic, metabolic, and isolation environment data from 1,154 strains from 1,051 *Saccharomycotina* species to examine the variation and evolutionary history of filamentation across *Saccharomycotina* and determine the extent to which genes, metabolic traits, and environments can predict filamentation types. We found that 63.37% of strains can form filaments; 6.56% can form true hyphae, 42.40% can form pseudohyphae, and 14.39% can form both true hyphae and pseudohyphae. We found that the distributions of species that can produce true hyphae or that can filament were more strongly correlated with the yeast phylogeny than the distribution of species with pseudohyphae. Ancestral state reconstruction suggested that the ability to form true hyphae and/or pseudohyphae evolved several times in yeast evolution. We infer that most yeast ancestors likely produced pseudohyphae or lacked filaments, while the *Saccharomycotina* last common ancestor likely produced pseudohyphae but not true hyphae. Machine learning models trained on genomic and metabolic features predicted filament morphologies with about 70% accuracy. Connecting the evolution of *Saccharomycotina* morphologies to their genomic, metabolic, and ecological characteristics will enrich our understanding of the evolution of the diversity of lifestyles observed in this lineage.

547A

High-Throughput Phenotyping Systems for Resistance Screening Against Bipolaris Brown Spot and Rhizoctonia Sheath Blight in Long-Grain Rice

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Rice production is increasingly threatened by fungal diseases whose severity has intensified under climate change. Rice brown spot, caused by *Bipolaris oryzae*, progresses rapidly, producing visible lesions within three days under high temperature and humidity. Although this rapid onset is advantageous for pathogenicity assays, the small, scattered lesions hinder precise manual quantification. To address this limitation, we developed an automated image-based system to measure lesion area percentage with 90–100% precision. Using this platform, approximately 150 long-grain rice accessions were screened, enabling reliable identification of resistant germplasm with substantially greater throughput than manual scoring. Rice sheath blight, caused by *Rhizoctonia oryzae*, represents another major constraint, particularly during the critical August growth period when disease pressure increases under climate-driven high humidity. To establish a robust resistance evaluation method, 150 long-grain accessions were inoculated at the basal sheath with 14-day-old cultures and incubated at 31 °C and 100% RH. Lesions developed by 4 days after inoculation and expanded upward to 15–20 cm by day 10, providing clear and reproducible criteria for rating disease severity. Together, these methodological advances provide a unified, high-throughput framework for evaluating resistance to two major fungal diseases of rice and support the accelerated discovery of resilient germplasm for breeding and disease management programs. (This work was supported by the Rural Development Administration of Korea (RS-2025-02214096))

548B

Save the Ketchup! Population genetics and phylogenomics reveal clones from two obscure taxa in the *Fusarium solani* Species Complex that cause a devastating disease on California processing tomato

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California grows a large majority of the US commercial processing tomatoes used to produce many familiar tomato products, including ketchup, paste and sauce. Starting in the late-2010s, a stem rot and canopy decline disease outbreak emerged, causing up to 40% yield loss and > 90% plant mortality. We found that three members of the *Fusarium solani* species complex, *F. noneumartii, F. martii* and *F. falciforme*, are associated with this disease. Experiments showed *F. falciforme* not contributing to outbreak symptoms, with isolates either non-pathogenic or minor foot rot pathogens. In contrast, severe outbreak symptoms are caused by *F. martii* and *F. noneumartii* (a.k.a. *F. solani* f.sp. *eumartii*). Using amplicon- and genomics-based approaches combined with somatic compatibility grouping (SCGs), we demonstrate that single clonal genotypes/SCGs in both species (*F. martii* FM-1 and *F. noneumartii* FN-1) are primarily responsible for the current outbreak. *F. martii* is a previously unknown tomato pathogen, but has been present in California tomato at least since 2000. *F. noneumartii* clone FN-1 is more virulent than *F. martii*, and has been present as a weaker (foot and root rot) tomato pathogen in California since at least 1996. California isolates associated with weak disease in the 1990s are also members of the FN-1 clone; these older isolates showed virulence similar to that of FN-1 isolates from the current severe outbreak, indicating that the recent severe disease is not due to a genetic change in FN-1. Phylogenomic analyses of *F. martii* and *F. noneumartii* based on k-mer and mini-BUSCO approaches resolved both species together with a third tomato pathogen within the FSSC, *F. solani-melongenae*, distinct from *F. falciforme* as well as other related species including *F. keratoplasticum* and *F. solani* s.str. Based on this we propose the name Fusarium stem rot and vine decline for the newly discovered outbreak-associated disease caused by *F. noneumartii* and *F. martii*, distinguishable from Fusarium foot rot caused by *F. falciforme*. Our ongoing work focuses on improving diagnostic assays for *F. martii* and *F. noneumartii* pathogens, and using population genomic analyses to make inferences about the evolution of pathogenicity and host associations. Studies are also underway with *F. falciforme* to better understand epidemiological connections between endophytic / poor hosts such as tomato, and more severely affected symptomatic hosts such as melon and legume crops.

549B

Inhibitor of Apoptosis Proteins are highly conserved across fungi, with notable expansions in Lecanoromycetes

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Inhibitor of Apoptosis Proteins, or IAPs, are one of the only regulatory elements of animal apoptosis with homologs in fungi; as such, they provide a unique window into the regulation of fungal programmed cell death. Fungal genomes have previously been assumed to contain exactly one IAP gene, which is essential in some species and not in others, but a broad assessment of IAP homologs across the fungal kingdom has not been published to date. Remarkably, our bioinformatic analysis revealed that many fungal genomes encode multiple IAPs, including examples with up to eleven unique genes, and showed that IAPs are nearly universal in fungi. We have previously interrogated the function of AnBIR1, the IAP of the model fungus *A. nidulans*, and found that it is essential for survival, inhibits caspase-like activity, and its precise regulation is required for functional sporulation, secondary metabolite composition, and cell wall integrity. We are now working to characterize the IAPs of two species of *Letharia,* a lichen- forming fungus, whose genomes encode ten and eleven distinct IAP homologs. We have also identified a third lichen-forming fungus, *Physcia stellaris*, which has as many as twelve of these genes, and are examining other Lecanoromycete genomes for similar expansions. We are interested in exploring the evolutionary history of this gene expansion and its impact on IAP functions in this unique organism with both computational and wet lab approaches. Overall, this work assesses the functional conservation and diversification of IAPs across the fungal kingdom and aims to refine our understanding of fungal programmed cell death.

550B

Mycodnaviridae: a new group of giant viruses discovered as past and present infections of zoosporic fungi

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Giant viruses of the phylum Nucleocytoviricota have emerged as particularly notable due to their increasingly recognized impacts on eukaryotic genome evolution. Their origins are hypothesized to predate or coincide with the diversification of eukaryotes, and they have been detected in hosts that span the eukaryotic tree of life. But surprisingly, such viruses have not been definitively found in Kingdom Fungi, though genomic and metagenomic work suggests a putative association. Here we report both “viral fossils” and active infection by giant viruses in fungi, particularly in the zoosporic phyla Blastocladiomycota and Chytridiomycota. The recovered viral genomes span up to 350 kb, encode over 300 genes, and form a monophyletic family-level clade within the Nucleocytoviricota related to orders Imitervirales and Algavirales, which we name Mycodnaviridae. We observed variation in infection status among the isolates including apparent active infection and transcriptionally-suppressed states, suggesting that viral activation may be constrained to certain life stages or growth conditions of the host. Our experimental findings add to the limited natural virus-host systems available in culture for the study of giant viruses. Our findings expand the known host range of Nucleocytoviricota into a new kingdom that contains many model species. Mycodnaviridae have a global distribution, which invites inquiry into the implications of these infections for host traits, host genome evolution, and the metabolic impacts to ecosystems.

551B

Selective Media Reveal Hidden Cultivable Fungal Diversity in Soybean Microhabitats

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Culture-based approaches remain essential for linking fungal taxa to ecological function, yet the majority of plant-associated fungi remain uncultured due to strong medium-dependent biases. To expand the cultivable fraction of the soybean (*Glycine max*) fungal microbiome, we systematically evaluated seven media—½-strength R2A, R2A agar, soil extract agar (SEA), DG18 agar, Czapek-Dox agar, 10% PDA, and water agar—using soil, rhizosphere, and root endosphere samples. Across all media, over 30 fungal species were recovered based on colony characteristics and ITS rDNA sequencing. Media differed substantially in their recovery profiles: 10% PDA and water agar supportedsminan the greatest taxonomic breadth, while SEA and DG18 enabled isolation of slow-growing or rarely cultured taxa. Several isolates previously considered difficult to culture were successfully obtained, revealing fungal lineages with potential ecological and functional significance in soybean-associated microbiomes. These results demonstrate that combining selective and low-nutrient media can substantially broaden the observable diversity of soybean-associated fungi. This work highlights the importance of medium-driven community assembly in culture-based microbiome studies and provides a methodological foundation for future ecological, evolutionary, and genomic investigation of soybean-associated fungi. (This work is supported by RDA, Korea: RS-2025-02653099)

552B

Fungal diversity and their involvement in the decomposition of deep-sea submerged plants

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Fungi are the main decomposers of plants on land, and they are presumed to play an important role in the degradation of sunken plant materials in the deep sea. However, the diversity, distribution, and ecological functions of fungi associated with deep-sea sunken plant substrates remain largely unknown.

To investigate fungal diversity and their involvement in the decomposition of deep-sea sunken plant debris, we performed ITS amplicon sequencing on 20 samples collected from four sites (~700–5,800 m) in the northwestern Pacific. Amplicon analyses indicated that the marine fungal lineage Halosphaeriaceae (Sordariomycetes, Ascomycota) was dominant and widespread in deep-sea plant debris, and some samples showed distinct community patterns. To further assess functional roles, metagenomic analyses were conducted on four representative samples, including seawater- and sediment-facing branches, a heavily decayed camphor wood, and a relatively fresh pine branch. Metatranscriptomics was additionally applied to two samples (decayed camphor and fresh pine) to identify actively expressed genes.

Metagenomic analyses showed that bacteria contributed the majority of total gene abundance, whereas fungi, especially from Ascomycota, were also abundant in both the decayed camphor and fresh pine samples. Two fungal metagenome-assembled genomes (MAGs) were recovered. MAG1, a Sordariomycete lineage related to the marine wood-decomposer *Zalerion maritima,* was highly abundant in the decayed camphor, and MAG2, a Chaetothyriales-related genome, was mainly present in pine. MAG1 contained a variety of carbohydrate-active enzymes (CAZymes) involved in degrading plant biomass, including cellulose, hemicellulose, pectin, and lignin.

Metatranscriptomic data revealed that Ascomycota were likely the major active decomposers in the decayed camphor sample, expressing multiple lignocellulolytic and oxidative CAZymes (e.g., AA7, GH3, GH11, CE8, and CBM1). In contrast, fungal transcription was limited in the fresh pine sample, implying that this difference may be related to either the decomposition stage or tree species, which requires broader sampling for clarification.

Overall, our comprehensive analyses indicate that Halosphaeriaceae dominate deep-sea submerged plants and that undescribed Ascomycete lineages actively contribute to wood degradation in the deep sea. Continuing investigations into their diversity, physiology, and genomic traits will help to elucidate the adaptation, evolution, and ecological significance of deep-sea fungi.

553B

Transcriptional differences related to thermotolerance in *Cryptococcus neoformans* and related species

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Growth at high temperatures is necessary for pathogens to succeed inside of a mammalian host. *Cryptococcus neoformans* typically grows at lower ambient temperatures in the environment but must adapt to elevated temperature upon host entry. However, while all of the pathogens in the *Cryptococcus* genus can grow at high temperatures, there is a stark phenotypic divide in thermotolerance between pathogenic *Cryptococcus* species, which grow consistently at 37°C and 39°C, and non-pathogenic *Cryptococcus* species and closely related *Kwoniella* species which show impaired or no growth at these temperatures. We hypothesize that differences in transcriptional responses to heat stress may underlie the phenotypic difference in heat tolerance between pathogenic and nonpathogenic *Cryptococcus* species. We will use RNA-sequencing to measure transcriptional differences across temperature conditions and identify differentially expressed genes. In addition to shifts in expression magnitude, we expect distinct gene switches from inactive to active, and vice versa, reflecting regulatory responses related to thermotolerance. This will clarify how thermotolerant fungi rewire transcriptional networks to withstand heat stress, improving our understanding of the molecular basis of fungal survival in host-like environments.

554B

Fungi from Plants and the Soil They Were Growing In

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Preharvest corn is colonized by multiple fungi. These fungi could originate in the soil the plant is growing in, the seed that was planted, airborne contamination, or inoculum carried by insects. We evaluated the mycobiomes in corn ears and the soil the plant was growing in to determine the degree that the soil mycobiome could be contributing to the plant mycobiome. Our hypothesis was that corn and soil harbor overlapping mycobiomes and that these mycobiomes also could differ by geographic location.

We used metabarcoding, to evaluate ITS sequences of 12 paired soil and corn samples from two locations in Nepal, Dadeldhura and Kailali. Total DNA was extracted from corn and soil and was sequenced. The fastq sequences were processed through the mothur pipeline and OTUs assigned to fungal taxa at the genus level. R Studio was used to analyze and compare the mycobiomes.

Most of the OTUs were for Ascomycetes (60%), with 22% for Basidiomycetes and 18% for other fungi. *Fusarium* was ubiquitous in all samples, and *Sarocladium, Ceratobasidium,* and *Lasiodiplodia* were found in all corn ears. Nine genera were associated with 90% of the sequences in Dadeldhura and six genera were associated with 90% of the sequences from corn in Kailali. All genera contained species known to be plant pathogens, except for *Wallemia,* which was recovered in Kailali. Mycobiome diversity was higher in corn from Dadeldhura but in soil it was higher in Kailali. In soil, 102 fungal genera composed 90% of the sequences from Dadeldhura and 131 genera composed 90% of the sequences from Kailali.

Corn and soil mycobiomes were clearly differentiated in a PCoA plot. *Aspergillus* and *Penicillium* are known mycotoxin producers that were more abundant in corn from Kailali than from Dadeldhura, but were rare in soil from both locations. Corn and soil mycobiomes overlapped. *Fusarium* was in all samples, otherwise genera abundant in corn were not common in the soil and vice-versa. Clear differences also were observed based on location, but these differences were less than the differences observed between corn and soil. The strong differences between plant and soil mycobiomes suggest that soil fungi colonizing the plant must grow rapidly there and that most fungi do not get past the plant's barriers to colonization. The existence of numerous genera in the corn that were not found in the soil suggests that means other than soil reserve were serving as the principal inoculum for the mycobiome found in the corn ears.

555C

A fungal volatile organic compound shifts fungal communities to enhance drought resilience in pasture systems

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Volatile organic compounds that are naturally biosynthesised by beneficial fungi (fVOC) have emerged as potential tools for enhancing plant stress resilience. However, the impacts of these novel biologically active compounds on non-target fungal communities remain under studied. This study examined effects of a fVOC on fungal abundance and drought resilience across perennial ryegrass, white clover, and mixed pasture systems. A drought-stress experiment was conducted under controlled conditions using perennial ryegrass and white clover plants. The rhizosphere and root microbiomes were profiled using ITS2 amplicon sequencing. Plants grown from seeds treated with fVOC had altered fungal communities. In the rhizosphere, fVOC treatment reduced dominant families including Nectriaceae (-14%), Chaetomiaceae (-13%), Mortierellaceae (-6%), Filobasidiaceae (-10%), and Cladosporiaceae (-10%). In the root, fVOC reduced Nectriaceae (-21%) and Hoehnelomycetaceae (-37%), and greatly increased Lentitheciaceae (+121%). These fungal microbiome shifts were associated with enhanced plant performance (15-48% greater biomass under drought) for both clover and the mixed pasture. This study demonstrated that fVOC, by altering fungal root and rhizosphere associations, increased plant drought stress resilience. This may provide a new, nature-based opportunity to instill climate-resilience into these pasture species.

556C

Integrated Genomic and Metabolomic Evidence for Divergent Fumonisins and Beauvericin Production in *Fusarium proliferatum*

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Fusarium proliferatum is a major fungal pathogen implicated in fumonisin contamination of cereals, posing significant risks to food safety and animal health. Current research emphasizes the interplay between genetic determinants and metabolic variability, particularly in the biosynthesis of fumonisins and beauvericin.

In this study, we investigated a set of nine strains cultivated on three substrates (rice, PDA, maize) to characterize their secondary metabolomic profiles, focusing on fumonisins (FBs) and beauvericin (BEA). Metabolomic analysis revealed that fumonisin B1 (FB1) production peaks on rice, whereas PDA and maize supported comparatively lower levels. Beauvericin synthesis exhibited strain-specific patterns, with notable accumulation in certain isolates across PDA and rice.

A key finding was the identification of an 86 bp deletion in the *fum2* gene, involved in fumonisins biosynthesis, in some strains, which consistently correlated with the absence of FB1 production across all media. In the case of beauvericin, beauvericin production did not correlate with nucleotide variability within the *BEA* cluster. These results align with emerging literature highlighting the role of gene cluster integrity in mycotoxin biosynthesis and suggest that when the biosynthetic potential is conserved, metabolite accumulation is likely modulated by external factors.

Furthermore, our findings support the view that *BEA* and *FUM* clusters evolutions are independent and advocate for integrated genomic-metabolomic approaches to unravel regulatory mechanisms at basis of metabolic diversity in *F. proliferatum*. Additional genomic and transcriptomic investigations are warranted to confirm these hypotheses and advance strategies for mitigating mycotoxin contamination in food chains.

557C

Quantitative Multi-Omics Reveal Functional Diversity and Carbohydrate-active Enzyme Activity of Marine Fungi in the Eastern Tropical South Pacific OMZ

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Fungi are active yet understudied members of the marine microbiome, with potentially important roles in biogeochemical cycling in the open ocean. This study combines metagenomic, metatranscriptomic, and amplicon-based approaches with quantitative internal RNA standards and quantitative PCR (qPCR) to investigate the abundance, diversity, function, and activity of marine fungi across the eastern tropical South Pacific (ETSP) oxygen minimum zone (OMZ) during the 2023 El Niño event. Sixty seawater samples were collected from the surface to 4,700 m at four stations, spanning coastal to offshore. Ribosomal RNA–depleted and polyA-enriched RNA libraries were sequenced alongside metagenomes, and internal standards were used to perform quantitative metatranscriptomics, enabling the determination of absolute transcript abundance. To complement metatranscriptomic profiles, fungal 28S LSU regions were amplified and sequenced. QPCR of the 28S rRNA gene was performed to estimate copy-normalized genome equivalents, providing depth-resolved measurements of fungal abundance. The highest absolute fungal transcript abundances were detected in surface waters (~2.0 x 10^5 fungal transcripts per mL at the surface), reflecting elevated biomass and activity, while fungal transcript abundances declined at depth and in anoxic conditions (down to ~0.07 x 10^5 fungal transcripts per mL at depth in the coastal station). Among extracellular carbohydrate-active enzymes (CAZymes), glycoside hydrolase family 7 (GH7) dominated expression (~76% of extracellular CAZyme activity), suggesting a major role in cellulose and chitosan degradation. GH16, targeting β-glucans and galactans, was most active at anoxic depths, and multicopper oxidases were expressed under oxygen-depleted conditions, indicating potential alternative oxidative or respiratory pathways. Fungal community composition varied with depth: Basidiomycota dominated surface waters, whereas Ascomycota, Chytridiomycota, Mucoromycota, and Zoopagomycota were active throughout the water column, underscoring the ecological importance of early-diverging lineages. Offshore stations exhibited lower enzyme diversity, consistent with substrate limitation. These results demonstrate that marine fungi are metabolically versatile members of OMZ ecosystems, possessing extracellular enzymatic capacities that contribute to organic matter degradation and biogeochemical cycling in nutrient-poor, oxygen-deficient ocean regions.

558C

Revision of the *Pseudopithomyces* (Pleosporales) genus supports the identification of a new toxigenic species, *Pseudopithomyces toxicarius* sp. nov., responsible for facial eczema in livestock

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Toxigenic fungi in animal forage cause significant ill health due to accidental ingestion of mycotoxins. When environmental conditions favour spore production and toxin synthesis, ingested toxins such as sporidesmin are rapidly absorbed into the bloodstream and cause liver damage leading to pithomycotoxicosis, which manifests as head shaking, peeling skin and eventual death. This syndrome has been reported worldwide but can be severe in countries with temperate climates where forages that support high spore burdens are used, such as in New Zealand, South Africa and the Azores. This syndrome was originally attributed to *Pithomyces chartarum* (transferred to *Pseudopithomyces* in 2015). In this study we re-evaluated

the *Pseudopithomyces* genus using modern species concepts and determined the range of sporidesmin synthesis. Isolates were recovered from pasture samples from across New Zealand between 2014 and 2022. International strains, type material and historical isolates from the International Collection of Microorganisms from Plants were also examined. Phylogenetic analyses resolved 15 species, including one that had not yet been identified. We described *Pseudopithomyces toxicarius* sp. nov., a newly recognised sporidesmin-producing species formerly identified as *Pse. chartarum*. Two *Pithomyces* species were transferred to *Pseudopithomyces* as *Pse. cynodontis* comb. nov. and *Pse. pavgii* comb. nov., and *Pseudopithomyces pandanicola* was placed in synonymy with *Pse. palmicola*. Sporidesmin was detected in 80% of sporulating *Pse. toxicarius* isolates and in only one *Pse. chartarum* isolate at low concentrations. Whole genome sequencing revealed that all sporidesmin-producing isolates contained a putative sporidesmin gene cluster. Toxin production in other *Pseudopithomyces* species remains unresolved due to limited availability of cultures. Four species were detected in New Zealand, *Pse. toxicarius* and *Pse. chartarum* were isolated in both main islands, *Pse. palmicola* was recovered twice from pasture in the North Island, and an undescribed taxon, *Pse.* sp. 'gladiolus NZ' was also identified. No species appear unique to New Zealand indicating a global distribution. Pithomycotoxicosis in New Zealand appears to be predominantly caused by *Pse. toxicarius,* and not *Pse. chartarum* as originally thought.

559C

Fusarium Strikes Back: Expanding the Diversity of Fumonisin Producers in South African Maize

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Fumonisins (FUMs) were first discovered in the Eastern Cape (EC) province (former Transkei region) in South Africa, where *Fusarium verticillioides* was identified as the producer. Despite this historical importance, the diversity of FUMs-producing *Fusarium* in the region remains poorly studied.

Across a three-year survey of the mycotoxins and fungi occurring in maize from small-holder farms in the EC, more than 600 *Fusarium*isolates were identified based on *tef1* and *rpb2* sequences. Fumonisins were detected only in the OR Tambo district, whereas deoxynivalenol (DON) and zearalenone (ZEA) were most common in Alfred Nzo district, including two DON-positive samples exceeding the 2 000 µg/kg regulatory limit. Beauvericin (BEA), fusaric acid (FA) and moniliformin were consistently detected across seasons and districts, forming a "*Fusarium* mycotoxin cocktail" in both symptomatic and asymptomatic cobs and highlighting the frequent co-occurrence of these cocktails.

Across all years, the community was dominated by *Fusarium fujikuroi* species complex (FFSC) species, including two new species. These taxa occurred at FUMs-positive sites, including samples that did not contain *F. verticillioides*, indicating additional contributors to FUMs risk in the region. Liquid chromatography coupled to tandem mass spectrometry (LC–MS/MS) profiling of representative isolates confirmed that both novel species produced FA, one species produced BEA, and one species produced FUMs, consistent with field toxin profiles.

The next phase will involve whole-genome sequencing of both species, followed by comparative analyses of the FUMs biosynthetic clusters. This study expands the known diversity of toxigenic FFSC members in EC maize systems and provides a genomic foundation for understanding FUMs biosynthesis in the region where it was first discovered.

560C

Genetic and ecological diversity of *Fusarium falciforme* as a circumglobal plant pathogen of crop and landscape plants

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Long considered a soil saprobe and opportunistic human pathogen, *Fusarium falciforme* has more recently gained recognition as an important crown and root rot pathogen of agricultural crops and landscape plants worldwide, driving plant mortality and yield losses. Since 2017, there have been up to 11 new pathogen reports published each year, cumulatively documenting impact on more than 50 plant species. It is currently unknown whether this represents a single pathogen strain with a broad host range or whether differentiation has driven the existence of host specific strains, as documented in other *Fusarium* species. In California alone there are over two dozen plant species impacted by *F. falciforme* as well as several endophytic hosts, making this an ideal model system to study fungal diversity at a genetic and ecological level. The genetic diversity of pathogenic *F. falciforme* isolates was characterized based on *tef1a* cladogram analysis of over 50 isolates and crops and 17 countries. Complimenting multi-gene and somatic compatibility group (SCG) analyses were also undertaken for pathogenic and endophytic isolates within the California model system. Based on initial studies there are at least 10 haplotypes of *F. falciforme* and 37 SCGs, seven of which contain isolates from multiple crops. Haplotype and SCG groupings did not align with host relations (eg. plant family). Isolates representing six clonal groups, recovered from five plant families, and nine plant species were all able to cause stem rot lesions in cowpea (13 to 19.4 mm) comparable the isolate from cowpea (16.7 ± 2.2 mm) ($P > 0.05$), pointing to shared ecological traits despite high levels of genetic diversity. In addition, isolates recovered as endophytes were also able to cause disease in cowpea (15.1 to 19.4 mm lesions), suggesting conserved pathogenicity traits across isolates recovered as pathogens and endophytes. This research indicates that while *F. falciforme* is an incredibly genetically diverse organism, diversity is seemingly uninfluenced by host of origin, with all isolates possessing shared virulence traits. Our study also indicates a role of endophytic hosts as cryptic pathogen reservoirs which may be important for disease management. Additional studies are underway to further test the hypothesis that highly diverse *F. falciforme* isolates may represent a single broad-host range pathogen, providing critical information for disease diagnosis and management.

561A

Assessment of filamentous fungi with dye-degrading potential isolated from Basque estuaries

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Contamination of aquatic environments has become a big issue over the last years. Coloured wastewater effluents released by several industries, especially the textile sector, may not only be mutagenic, carcinogenic, or toxic, but also block light penetration into the water, restricting photosynthetic processes, inhibiting the growth of aquatic biota, and interfering with gas solubility in water. Filamentous fungi derived from marine environments are well known as a potential genetic resource for various biotechnological applications, including bioremediation. In this context, this work focused on the isolation of filamentous fungi from sediment samples collected from estuaries of the Basque Country. Their phenotypic characterization led to the identification of strains potentially capable of degrading industrial dyes. Three isolates belonging, based on ITS sequencing, to the genera *Acremonium* (M38), *Monodictys* (M63), and *Cladosporium* (M104) were selected for further study due to their potential to degrade and/or adsorb industrial dyes such as Remazol Brilliant Blue R (Reactive Blue 19), Congo Red (Direct Red 28) or Indigo Carmine (Acid Blue 74). Genomics, transcriptomics and proteomics analyses of those strains will provide key information on the genome features of those species and the mechanisms and activities induced for the degradation/adsorption of these dyes. In summary, this study shows that marine fungi from the Basque coast could be a valuable source for the development of new biotechnological tools for reducing dye pollution and protecting aquatic environments.

562A

Enhanced Competitiveness of Neck-Derived Magnaporthe oryzae Isolates Across Rice Tissues

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Rice blast, caused by *Magnaporthe oryzae*, affects multiple rice tissues, yet the extent to which tissue origin influences pathogenic behavior remains unclear. To investigate tissue-associated variation, we examined three representative isolates: f1-7 (leaf origin), fcn12-25 (neck origin), and fcn14-1 (panicle origin). Co-inoculation on the cultivar Shindongjin revealed striking competitiveness of the neck-derived isolate fcn12-25, which accounted for 79.1% of recovered lesions, compared with 13.9% for the leaf-derived isolate and 7.0% for the panicle-derived isolate. Pathogenicity assays on two additional cultivars, Hopum and Saechungmoo, showed that the neck-derived isolate consistently produced disease, whereas the other isolates caused little or no infection. In panicle inoculations, however, both neck- and panicle-derived isolates induced severe symptoms, suggesting tissue-adaptive pathogenic potential. These results indicate that *M. oryzae* isolates exhibit tissue-associated specialization and that neck-derived isolates may possess enhanced aggressiveness and competitive fitness during rice infection. Ongoing in vivo studies aim to identify the molecular determinants underlying this tissue-specific variation and its role in shaping blast disease dynamics. (This work was supported by the Rural Development Administration of Korea, RS-2025-02214096.)

563A

Cellular responses of *Aspergillus sydowii* to growth at extreme chaotropic concentrations of $MgCl_2$

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Studies on microbial adaptations to salt stress, particularly in fungi, have focused mainly on high concentrations of NaCl. The effects of other inorganic salts on biological systems are much less well known. This study investigates the effects of $MgCl_2$ (0–2.0 M), a chaotropic salt, on the halophilic fungus *Aspergillus sydowii* (EXF-12860) and compares these results with those obtained with the kosmotropic NaCl. While *A. sydowii* thrived at $MgCl_2$ concentrations previously considered lethal (up to 2.0 M), differences in morphological, physiological, metabolic, and molecular responses were observed. At 0.5–1.0 M, growth rates were similar under both salts, but higher $MgCl_2$ concentrations (1.5–2.0 M) significantly inhibited growth, reduced hyphal elongation, and decreased cell density. During growth in the presence of NaCl, a wide range of carbon sources was utilized, but high $MgCl_2$ interfered with substrate metabolism and uncoupled growth from metabolic activity. Both salts induced the production of compatible solutes (glycerol, erythritol), with higher accumulation under $MgCl_2$. Oxidative stress responses were also stronger under $MgCl_2$, including increased catalase and glutathione peroxidase activity. Transcriptomic analyses revealed significant changes in gene expression under $MgCl_2$stress, with upregulation of ion transport, cell wall remodelling, glycerol biosynthesis, and oxidative defense pathways. In contrast, NaCl triggered responses focused on osmotic balance and maintenance of cell integrity. These findings emphasize the need for a deeper investigation of microbial tolerance mechanisms in chaotropic environments.

564A

Fungal responses under stress: Contrasting trophic strategies over time

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Fungi have crucial roles in our ecosystems that range from carbon cycle mediation to disease contribution. This is due to their diverse trophic strategies, including pathotrophic fungi, saprotrophic fungi, and those with facultative strategies that span both. These strategies differ in how fungi obtain resources: pathotrophs draw nutrients from living hosts, whereas saprotrophs decompose organic matter in the environment. Some species are obligate specialists in one mode, while facultative fungi can shift between pathogenic and saprotrophic lifestyles. What drives changes in these fungi under stress imposed by global change drivers, such as drought and heat, is currently an open question that must be addressed to better understand fungal benefits and threats to our ecosystems. Therefore, our objective was to compare responses to global change drivers, heat, drought, and their interaction, of different fungal species categorized as either facultative pathotrophs or obligate saprotrophs. We hypothesized that facultative pathotrophs will demonstrate more variable

responses to global change drivers. For this, we measured mass specific respiration (MSR), a trait that reflects fungal catabolic activity, of 28 facultative pathotrophs and 16 obligate saprotrophs under heat, drought, and heat plus drought for 48, 96, and 192 hours. We observed variation in MSR within and between each trophic mode at each time interval. Heat and heat plus drought resulted in suppressed MSR in facultative pathotrophs, while MSR in obligate saprotrophs remained consistent between control and stress treatments. Both trophic groups converged in variance from the 48-hour time cycle to the 192-hour time cycle. Together, our results support the hypothesis that facultative pathotrophs display greater variability in their metabolic responses to climate stress, likely reflecting the plasticity inherent to their dual lifestyles. Our findings highlight the need to directly link environmental stress, metabolic state, and lifestyle switching in facultative fungi to assess their potential benefits and threats under global change.

565A

Genome-scale model, *in vitro* experimentation, and stable cultures of algae, cyanobacteria, and fungi support a *s*ymbiotic exchange of amino acids for melanin

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Melanin is a complex biomolecule that confers broad resistance to abiotic and biotic stressors in fungi. Polyextremotolerant fungi (PEF) are the only group that constitutively produce melanin, incorporating it into their cell walls, allowing them to colonize rock surfaces, icebergs, and desert soils. However, these extreme oligotrophic ecosystems lack organic matter for consumption. Instead, it is hypothesized that PEF engage in lichen-like symbioses with photoautotrophs to obtain essential carbon and nitrogen for growth. We previously characterized a novel PEF named *Exophiala viscosa,* which we isolated from a biological soil crust (BSC) in the arid tundra of the Canadian Rockies. BSCs are microbial communities that form on barren soils and are rich in algae and cyanobacteria. *E. viscosa* exhibits a unique phenomenon of releasing melanin into its environment, which is documented in only two other organisms. In this study, we used *in silico* genome-scale modeling (GSM) and *in vitro* experimentation to identify metabolic and genetic triggers that induce melanin excretion.

We hypothesized that symbiotic algae and cyanobacteria induce melanin excretion in *E. viscosa* by providing organic carbon and nitrogen. Our previous work established the first GSM of the PEF *E. dermatitidis*, which was used as the foundation for *E. viscosa*'s GSM. With these models, we identified key metabolites, reactions, and regulatory mechanisms involved in melanin biosynthesis. Corroborating experimental results supported our findings, indicating specific amino acids significantly influence melanin excretion in *E. viscosa.* We believe this is due to an increase in the pool of acetyl-CoA, the precursor molecule for DHN melanin. We propose that cyanobacteria specifically provide amino acids via the GOGAT pathway to *E. viscosa*, supplying carbon and nitrogen sources while also inducing melanin excretion for abiotic protection. In our previous work, we successfully established stable cultures of WT *E. viscosa, Coccomyxa simplex, and Anabaena inaequalis* in oligotrophic media lacking carbon and nitrogen for at least 49 days. These tri-culture results, amino acid drop-in tests, and our model's *in silico* predictions support our hypothesis that amino acids are exchanged for melanin. Moving forward, we aim to perform additional genetic modifications targeting predicted and logic-based melanin regulatory pathways, as well as mimicking melanin from amino acids using media drop-ins in place of algae and cyanobacteria.

566A

The impact of geospatial variables and local mycobiota on the landscape of drug-resistant *Aspergillus fumigatus* in the United Kingdom

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Aspergillus fumigatus is a major aetiological agent of the disease aspergillosis, which is responsible for the annual deaths of an estimated 2 million people, globally. *A. fumigatus* occupies a soil niche environment and is exposed to agricultural fungicides. Agricultural fungicides share similar mechanisms of action as clinical antifungals. Therefore, resistance towards fungicides leads to inefficacy of clinical therapeutics. The widespread application of fungicides has led to a growing incidence of fungal antimicrobial resistance (fAMR). Drug-resistant *A. fumigatus* has been identified in up to 49% of clinical and environmental samples. Environmental reservoirs, termed "hotspots", of drug-resistant *A. fumigatus* have been identified in sources such as retail plant products, fruit and compost. However, associations between local environmental variables, ecological associations with other fungi and the development of hotspots remain unexplored in the United Kingdom. Here, we performed fAMR surveillance of *A. fumigatus* across 94 sites including farms, industrial composters and private residences in the UK. A combination of terrestrial and aerial samples were collected to identify hotspot locations and airborne exposure sites. We were able to calculate the resistance fraction from each site and associate this with geospatial variables including temperature, fungicide application and rainfall. Using a Bayesian statistical framework, we have been able to identify the impact of land usage on the prediction of fAMR generation. Furthermore, through the incorporation of 163 whole-genome sequenced isolates from aerial sampling into phylogenetic generalised linear mixed models (PGLMM), to uncover whether occupancy in the air is not only driven by environmental gradients and resistance profiles but also evolutionary constraints. Additionally, we have performed mycobiome analysis of terrestrial samples to uncover the ecological interactions that may drive the development of resistance. These results highlight the impact of anthropogenic behaviours, microbial communities and environmental conditions on the emergence of fAMR in *A. fumigatus*. Understanding variables driving the generation of fAMR hotspots will offer novel avenues for targeted intervention through evidence-based-policies to help mitigate the development of fAMR and ultimately improve therapeutic outcomes.

567A

Fungal trait responses under global change drivers

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Fungi play crucial roles in ecosystems by mediating nutrient cycling, forming symbiotic relationships with plants and animals, and acting as causal agents of disease. Yet, as global change accelerates, our understanding of how fungal physiology, genetics, and life-history strategies to determine ecosystem resilience remains limited. Identifying genetic and functional traits that enable fungi to tolerate or adapt to stress is essential to understanding their evolutionary pathways and the stability of the ecosystems they support. In this study, we examined how different fungal species, and strains within species, responded to three global change drivers: warming, drought, and nitrogen pollution. We quantified multiple functional traits linked to ecological performance and potential genetic trade-offs among trophic modes, including traits reflecting competitive advantage (colony area and biomass production), stress tolerance (pigment production), metabolic investment (mass-specific respiration), and catabolic capability (decomposition). We conducted one-week incubations in potato dextrose (PD) media, except for decomposition experiments, which lasted six weeks. Experimental conditions included warming (37 °C), drought (20% sugar alcohol for dehydration), and nitrogen pollution (0.05M ammonium nitrate and urea), with control conditions consisting of PD media at 20 °C without nutrient or sugar additions. Each species and strain were tested in triplicate, resulting in 720 total observations per trait. We analyzed responses using mixed-effects models and effect sizes. Our results show strong interspecific variation in response magnitude and direction without strong phylogenetic signal for any of these responses. While drought tolerance was widespread, responses to warming and nitrogen were more variable and, in some cases, inhibitory. Interestingly, trends emerged based on functional categories. Specifically, pathogenic fungi tended to tolerate stressors differently than saprotrophic species, suggesting that life-history strategies influence stress responses. This work provides a functional foundation for ongoing genomic analyses aimed at identifying the genetic determinants of stress tolerance and ecological function. Altogether, this work advances our understanding on how evolutionary and ecological processes shape fungal adaptation and resilience under global change.

568A

Influential fungi: arbuscular mycorrhizal fungal genomic identity drives shifts in soil bacterial community structure in two agricultural crop species

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Arbuscular mycorrhizal fungi (AMF) are obligate symbionts of most plants that improve plant growth by transferring nutrients into plant roots through networks of soil hyphae. The use of these fungi as bio-stimulants presents a desirable and sustainable alternative to excessive fertilizer application in the agricultural industry. Some strains are homokaryotic (their nuclei consist of one single haplotype), while other strains have been identified as heterokaryotic (they possess nuclei from two distinct parental haplotypes); these two forms of nuclear organization have been associated with variation in functional traits. Studies have found that the presence of AMF can produce shifts in soil microbial communities, and that different AMF species possess distinct hyphae-associated bacterial communities. However, no studies have investigated AMF genotype-based microbial selection on a known soil community. We hypothesize that distinct AMF genotypes and nuclear organizations can exert varying selection on the soil bacteria via enrichment or competition with certain bacterial taxa. This study sought to test this by comparing the effects of AMF strain identity/organization and plant host species (*Sorghum x drummondii* and *Medicago trunculata*) inoculated with eight strains of *Rhizophagus irregularis* and a known microbial wash in a randomized greenhouse experiment. DNA was extracted from soil samples for full-length 16S rRNA amplicon sequencing to generate bacterial community profiles of each AMF strain treatment. Subsequent PERMANOVA results indicate significant and interactive effects between plant host species and AMF strain, as well as with AMF nuclear organization. Our results show for the first time that even at the level of fungal strain, AMF can significantly alter the surrounding soil bacterial community, presumably due to specific genotypes or molecular interactions leading to positive or negative selection on distinct microbial taxa. Further analyses will identify bacterial indicator species associated with each AMF strain and will investigate functional variation in each treatment (e.g. colonization, spore production) linked to the observed shifts in bacterial community structure.

569A

Disrupting Microbial Communities: Investigating Patulin as a Driving Force of Microbiome Community Dynamics

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Microbial communities are shaped by a continuous exchange of chemical signals between fungi and bacteria, orchestrating interactions that range from cooperation to competition. This work investigates how fungal metabolites, specifically patulin, influence these processes. Patulin, a lactone mycotoxin produced by *Penicillium expansum*, exhibits important functions at low, subinhibitory concentrations that do not impact microbial growth. Rather than acting solely as a toxin, patulin functions as a communication-modifying molecule that disrupts bacterial quorum sensing (QS) and alters microbial community dynamics. At subinhibitory concentrations, patulin broadly interferes with QS-associated behaviors in Gram-negative bacteria, significantly reducing biofilm formation in *Pseudomonas aeruginosa*, *Agrobacterium tumefaciens*, and *Chromobacterium subtsugae*, disrupting swarming motility in *P. aeruginosa* and *Pseudomonas koreensis*, and suppressing violacein production in the QS biosensor strain *C. subtsugae*. A LasR-GFP reporter assay in *Escherichia coli* further demonstrates that patulin disrupts AHL binding to the LasR receptor, directly interfering with a key QS regulatory pathway. The ecological consequences of this QS disruption were evaluated using the THOR (The Hitchhikers Of the Rhizosphere) synthetic microbiome, composed of *P. koreensis*, *Flavobacterium johnsoniae*, and *Bacillus cereus*. In this system, *P. koreensis* produces koreenceine antibiotics that inhibit *F. johnsoniae*, while *B. cereus* protects and stabilizes *F. johnsoniae* populations when present in the community. Fungal extracts from wild-type, but not patulin-deficient, *P. expansum* strains enhanced *F. johnsoniae* fitness and significantly reduced *P. koreensis* production of koreenceine A and C, the most bioactive forms against *F. johnsoniae*. These findings suggest that patulin disrupts competitive dynamics within the THOR community. Collectively, this work identifies patulin as a QS-interfering metabolite, highlighting communication disruption as a mechanism shaping microbiome community composition.

570B

Born wild, acting sus: Cheating *re-shapes* fungal virulence and antifungal resistance

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Filamentous fungi are not solitary microbes but complex social organisms that form multinucleate networks. In the plant pathogen *Botrytis cinerea*, infection depends on the cooperative secretion of “public goods” such as virulence factors and detoxifying enzymes that are shared within the colony. However, this cooperative secretion of public goods creates a vulnerability: the emergence of “cheaters” that exploit these resources without contributing, gaining a reproductive advantage at the expense of the group’s collective fitness. We investigated these social dynamics by using a WT (M3a) and its derived transformant (*Bctom1*) engineered to over-express both tomatinase and hph cassette. This setup allowed us to contrast tomatinase secretion as a cooperative "public good" (detoxifying α-tomatine for the group) vs hygromycin resistance as a "private good" (protecting only the carrier). *In-vitro*, pairwise competition assays revealed that WT exhibited severely restricted growth on α-tomatine alone, it behaved as a "cheater" in mixed cultures. By exploiting the tomatinase provided by *Bctom1*, the WT gained a significant relative fitness advantage despite not paying the full metabolic cost of enzyme production. For hygromycin resistance we observed negative frequency-dependent selection: the wild-type gained a competitive advantage specifically at lower starting frequencies. To test whether these interactions carry over to host-pathogen interaction, we infected tomato leaves with single and mixed inoculum. Mixed infections confirmed that *Bctom1* rescues the WT in planta, driving competitive success, the WT gained advantage at lower starting frequencies. To explore these dynamics further, we employed ordinary differential equation modelling to simulate cheating under varying selection pressures. Our models identify scenarios where cheaters persist despite group fitness costs, allowing us to investigate which environmental and physiological parameters are key to the competitive success of cheaters. Additionally, we model how the multinucleate nature of *B. cinerea* spores may enhance the capacity of multiple nuclei to coexist in the population after generational bottlenecks. Overall, the findings reveal how social conflicts constrain virulence, antifungal resistance and mechanistic understanding for the persistence of low-virulence strains in nature.

571B

Interactions between arbuscular mycorrhizal and ergot alkaloid-producing endophytic symbionts in morning glories.

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Many plants associate with symbiotic fungi that affect their growth and stress responses. We studied the effects of inoculating an arbuscular mycorrhizal (AM) fungus, Funneliformis mosseae, onto roots of the plant Ipomoea tricolor which already has an above-ground fungal endophyte, Periglandula clandestina. Specifically we were interested in the effects of the AM fungus on ergot alkaloid production by P. clandestina, in addition to effects on plant growth. Ergot alkaloids are compounds that protect plants and also have psychoactive effects and pharmaceutical applications. Currently there is little research for the relationship of AM fungi and fungal endophytes and none on the effects of AM fungi on Periglandula species. Two groups of I. tricolor were planted in a soil and sand mix containing or lacking the AM fungus F. mosseae. The plants were cultivated for 6 weeks in a growth room and then harvested, divided into tissues, and processed for growth metrics and ergot alkaloid accumulation. Plants inoculated with the AM fungus grew significantly larger than the non-inoculated plants ($p = 0.0041$), having an average total dry weight of 281 mg compared to 215 mg. Surprisingly, plants not inoculated with the AM fungus had greater alkaloid concentration in above ground tissues than AM fungus-inoculated plants ($p= 0.038$): 451 µg/g tissue compared to 289 µg/g tissue. Below ground alkaloid concentration was similar ($p=0.25$) between the two groups, as was below ground weight ($p=0.57$). Although plants not inoculated with AM fungus had lower overall biomass, their higher ergot alkaloid concentrations resulted in an approximately equal overall ergot alkaloid quantity as in inoculated plants. Thus, with respect to ergot alkaloids, benefits provided to the plant by the AM fungus were not passed on to the endophytic symbiont, P. clandestina.

572B

Opposing Roles of Nitrogen and Potassium in Modulating Rice Brown Spot Severity

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Nutrient availability is a key factor shaping host physiology and fungal infection dynamics, yet its role in the Bipolaris oryzae–rice interaction remains underexplored. We investigated how nitrogen (N) and potassium (K) levels modulate brown spot severity using three rice cultivars (Chucheong, Hopum, Sindongjin) grown under controlled nutrient regimes. Seedlings received 0–100% of the recommended N or K fertilization rate prior to inoculation with B. oryzae strain F1371 (5×10^3 conidia mL^{-1}). Disease progression was quantified through lesion area measurements and temporal assessments from 24 to 144 hours after inoculation. High N availability consistently intensified disease development, enhancing lesion expansion and accelerating symptom progression across all cultivars. In contrast, elevated K significantly reduced lesion formation, indicating a protective effect that may reflect improved host resilience or altered physiological responses. These opposing nutrient effects were highly reproducible and align with the concept that host nutrient status can fundamentally shift the trajectory of fungal pathogenesis. Our results highlight nutrient balance as a critical and underappreciated determinant of disease outcomes in the rice–Bipolaris system. This work provides a physiological framework for future mechanistic studies investigating how nutrient signaling, fungal infection biology, and host defense responses intersect to shape nutrient-dependent pathogenesis. (This work was supported by the Rural Development Administration of Korea (RS-2024-00400211))

573B

Emergence of Alternaria-Induced Leaf Spot on Rice Under High-Temperature, High-Humidity Conditions in Korea

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Rice disease surveys conducted from late May to mid-October 2025 aimed to monitor the occurrence of blast and brown spot in long-grain rice cultivars. Unexpectedly, leaf spot symptoms appearing from late July through early September were found not to be caused by *Magnaporthe oryzae* or *Bipolaris oryzae*. Instead, cultures isolated from symptomatic tissues were consistently identified as *Alternaria* spp., based on morphological characteristics and ITS sequence analysis. Most isolates represented saprophytic or weakly pathogenic *Alternaria* species, yet they were associated with distinct necrotic lesions on living rice tissue. These *Alternaria*-associated lesions appeared during periods of sustained high temperature and humidity, suggesting that environmental stress may facilitate opportunistic infection or colonization of weakened host tissue. By early October, typical brown spot lesions caused by *B. oryzae* were also observed, contrasting sharply with the earlier *Alternaria* lesions. This work documents the first detection of *Alternaria*-induced spot-type disease symptoms on rice in Korea and highlights the potential for climate-driven shifts in fungal community dynamics and opportunistic pathogenicity in rice agroecosystems. (This work was supported by the Rural Development Administration of Korea, RS-2025-02214096.)

574B

Many sites, many stories: context-dependent fungal responses to global change drivers

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Fungi play crucial roles in ecosystem-scale processes; they mediate biogeochemical cycles, they form symbioses and are beneficial to some organisms, and cause disease and are detrimental to others. These roles are being affected by global change drivers, such as drought, warming, and nitrogen pollution. Despite extensive site-level studies, large-scale syntheses that test whether fungal responses converge across ecosystems remain rare. That is, it is unclear whether certain fungal groups consistently respond to specific global change drivers regardless of site-level environmental context. This information is crucial for simplifying predictions of fungal responses under stress which could consequently improveEarth system models and could even help develop climate mitigation strategies. To address this gap, we assessed fungalcommunity responses to global change drivers across twelve experimental sites across the USA and Puerto Rico manipulating drought, warming and nitrogen addition. We hypothesized that site-level differences in community structure, shaped by ecosystem type, experimental design, and treatment duration, would outweigh treatment effects. We extracted DNA from soil samples, performed Illumina amplicon sequencing targeting the ITS regions, and analyzed taxonomic, diversity, and functional patterns. Our results showed that indeed, fungal responses were highly site-specific. Particularly, at several sites, the fungal community composition was significantly similar in treatments compared to controls, but in others, there were no significant similarities in response to treatment. We saw similar trends for diversity and functional analyses. However, indicator species revealed a unifying signal: facultative trophic modes were present in higher abundance under treatments compared to controls. Together, these findings highlight the context dependency of fungalresponses to global change, driven by local environmental heterogeneity, while also identifying cross-site consistencies that may help pinpoint fungal groups with predictable stress responses. These insights provide foundational data for incorporating fungal functional dynamics into ecosystem and climate models, and they open the door to investigating life-history traits that may help predict the future of fungal communities and the ecosystem-scale processes they mediate.

575B

Re-parameterising wheat stem rust (*Puccinia graminis* f. sp. *tritici*) epidemiological models to explore disease dynamics in a changing climate

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Climate change is driving fluctuations in environmental conditions that are reshaping the distribution, biology, and aggressiveness of many plant pathogens, increasing the threat of disease-related yield losses in major crops. One concerning consequence is the potential return of historically devastating diseases as climate change alters their distribution [1].

In the United Kingdom (UK), reports of wheat stem rust caused by *Puccinia graminis* f. sp. *tritici* (*Pgt*), a basidiomycete rust fungus, have risen in recent years, suggesting a potential resurgence of this disease that was once vanquished in the region [2, 3]. Because *Pgt* exhibits relatively high thermal requirements for infection and development, ongoing climatic warming is expected to modulate epidemic risk. Yet, most epidemiological models for wheat stem rust are still parameterised using data from North American *Pgt* isolates collected in the mid-20th century, limiting their relevance under current climatic and pathogen population conditions [3].

To generate updated, regionally relevant epidemiological parameters, we performed controlled-environment experiments using contemporary UK *Pgt* isolates to quantify the effects of temperature and leaf wetness duration on key stages of the pathogen life cycle. We measured epiphytic fungal development leading to infection, the *in planta* latent period, and sporulation dynamics across a range of environmental regimes. These experiments yielded revised estimates of cardinal temperatures and leaf wetness thresholds for UK *Pgt* isolates fungal development.

These new parameters are being integrated into climate-driven epidemiological models of infection and disease development to refine projections of future wheat stem rust risk in the UK. Our findings advance understanding of infection biology in modern *Pgt* populations and strengthen preparedness of the UK for the potential re-establishment of this high-impact wheat disease.

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576B

Resistance of *B. cinerea* to Different Methods of Specialized Metabolites

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Botrytis cinerea is a host-generalist necrotrophic pathogen that infects 80-90% of crops, resulting in billions of dollars in economic losses globally each year. B. cinerea is an especially precarious pathogen, as it can infect crops at all stages, from growth in the field to storage in our fridges. Plants can defend themselves against B. cinerea by releasing plant-specific metabolites (SMs) that B. cinerea can experience in volatile and non-volatile forms. Recent studies have focused on using SMs as alternative control strategies because they readily break down in the environment. Yet, most studies have focused on single routes of exposure, limiting our understanding of how B. cinerea responds to SMs. The route of exposure to SMs may alter B. cinerea's tolerance, detoxification, or evasion of SMs, so determining the importance of the route of exposure is integral to developing future biocontrol methods. In the first-pass effect, compounds can be partially detoxified before entering a fungal cell via the infection cushion or haustoria. In contrast, the proposed second-pass effect bypasses the detoxifying step and immediately enters the fungi via passive diffusion across the fungal body. To identify variation in resistance, we exposed 4 genetically distinct isolates of B. cinerea to SMs via three routes of exposure: direct (liquid), semi-direct (agar), and indirect (fumigation). Resistance was estimated based on changes in growth and morphological responses. Preliminary data suggest that indirect (fumigation) exposure is more effective at reducing hyphal growth than direct exposure. Our work advances the development of targeted, plant-derived pesticides for use across eudicots to control a widespread pathogen.

577B

A Novel Fungal Association Characterizes Crohn's Disease in a South Korean Cohort

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Alterations in the gastrointestinal (GI) microbiota have been linked to a multitude of human health outcomes, including Inflammatory Bowel Disease (IBD), and improper recognition of fungal antigens is associated with exacerbated GI tract inflammation in models of IBD. However, bacteria remain the predominant focus for studies of the IBD-associated microbiota. Additionally, while recognition of the fungal microbiota has increased in recent years, inconsistencies in the direction of fungal diversity changes and in which specific fungal taxa may be implicated in IBD remain. In this study, we characterized the bacterial and fungal GI microbiota of healthy South Koreans (n=52) and South Korean patients with Crohn's disease (CD, n=34) to determine if any specific fungal species are associated with CD in this population. Stool samples were collected from this cohort, and 16S rRNA V4 and ITS1 sequencing were performed using the extracted DNA to profile the bacterial and fungal compositions of these groups. QIIME1 and DADA2 were utilized to process the resulting data and profile bacterial and fungal community composition. Differential taxa and correlations between bacterial and fungal species were determined using a variant sparse partial least squares discriminant analysis (sPLS-DA) framework using mixOmics. We observed that the fungal species *Candida albicans* and *Aspergillus ruber* were overrepresented in patients with CD compared to healthy controls. We found that *A. ruber* was the most well-connected species in a cross-kingdom correlational analysis and was often correlated with bacterial species that are associated with GI health. Importantly, we also found that *A. ruber* can inhibit permeabilization of GI epithelial monolayers by *C. albicans*. Overall, this study highlights the fungus *A. ruber*, thus far undescribed in the GI microbiota, as being associated with CD in a human cohort with naturally limited genetic and dietary variability. We demonstrate here the need for a greater focus on cross-kingdom approaches in future studies of the microbiota in human health. Additionally, this study implicates *A. ruber* as a potential modulator of disease in CD.

578B

Gene expression analysis of *Neurospora discreta* strains isolated from burnt Joshua trees after the Cima Dome fire in California

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A lightning strike sparked the Cima Dome fire that burned more than 43,000 acres of the Mojave National Monument east of Barstow, California, in August 2020. The charred area included a large Joshua tree woodland. Patches of orange fungal growth were spotted on many Joshua trees within a few weeks after the fire. A total of 24 fungal growth samples from three sites were collected and geographic coordinates recorded. ITS sequencing of genomic DNA isolated from the streak-purified purified samples demonstrated a close phylogenetic relationship to *Neurospora discreta*. A pairwise mating test between all 24 strains was conducted using synthetic crossing medium, and production of fruiting bodies and ascospores recorded. Based on production of ascospores, the findings revealed evidence for two large groups of opposite mating type strains, a potential homothallic strain and a small group that did not mate with any other strain in the study. Colony radii and conidiation amount were measured at different temperatures, with an optimum of 30-35°C observed for most strains and traits. RNA was isolated from the original samples, subjected to Illumina sequencing, and the reads mapped to the genome sequence for *N. discreta* strain FGSC8579. The results from DESeq analysis demonstrated that many isolates share a common pattern of gene expression, while others are more divergent. Results will be presented for the patterns of gene expression and the morphological phenotypes of the strains after hierarchical clustering analysis.

579C

Shedding light on the growth pattern of the fairy ring fungus *Marasmius oreades*

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What it means to be a fungus varies within the lifetime of the individual and over generations. As the life cycle proceeds, ploidy as well as morphology changes, which alter the evolutionary context, ecological functions and growth pattern of the fungus. However, much of this diversity in growth forms, and what factors determine them, remain to be discovered. In particular, there is a great need for studies addressing growth characteristics in of fungi their natural environment. The development of metagenomic methods, however, have made the cryptic parts of the life cycle accessible for analysis. The study presented here is an effort to uncover the ecological implications of the vegetative stage of the fairy ring-forming basidiomycete *Marasmius oreades* in nature by utilizing metagenomic tools. Specifically, we want to investigate potential underlying mechanisms behind the radial growth progression of the ring. To do this, a total of 44 soil samples were collected along transects drawn across two individual *M. oreades* fairy rings and sequenced with metagenomic shotgun sequencing. We first confirmed that the mycelium of the *M. oreades* fairy ring truly forms a ring, as we detected elevated levels of *M. oreades* reads within the mushroom-producing zones, but not outside or inside of this zone. Second, we used a transplant experiment in which parts of the rings were moved to different places and in different directions across the ring, and again used metagenomics to screen for fungal growth after one season. With this approach, we found that the mycelium continues to grows forward in its original direction, regardless of how the placement and orientation of the mycelium is altered. This is the first attempt to describe fairy ring growth at a molecular level, and can help us understand what the ecological strategy of being a fairy ring is.

580C

Global patterns of diversity and distribution in *Aspergillus* fungi are driven by human and environmental influences

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Aspergillus fungi are key producers of pharmaceuticals, enzymes, and food products and exhibit diverse lifestyles, from saprophytes to opportunistic pathogens. The genus is divided into 28 taxonomic sections sharing traits (e.g., natural products) and lifestyles (e.g., xerophily, pathogenicity). Understanding global patterns of *Aspergillus* occurrence and diversity would deepen our ecological understanding and help monitor pathogens and producers of mycotoxins and diverse natural products. To identify key environmental factors influencing their geographic distributions and estimate the impact of future climate change, we trained a random forest machine learning classifier on 19,105 terrestrial occurrence records for 27 taxonomic sections and 96 environmental variables to predict distributions of each section. We found that regions with high section diversity are concentrated in temperate forests, suggesting that areas with mild seasonal variation serve as diversity hotspots. Section range estimates revealed extensive variability, and low range overlap suggests distinct niches. The top predictors of average section richness were the index of cumulative human impact, ecosystem productivity, and forested vs non-forested ecoregions. Our future climate analyses revealed considerable variation in section range estimates in response to changing climates; suitable habitats for some sections are predicted to expand (e.g., section *Restricti*), and others to contract (e.g., section *Nigri*) or remain stable (e.g., section *Fumigati*) in the next few decades. Our findings reveal that both natural and human factors influence the macroecology of *Aspergillus* fungi and highlight their ecological diversity, including the diversity of their responses to changing climates, which is of relevance to pathogen and mycotoxin risk assessment.

581C

Roadkill Wildlife Reveals Potential Reservoirs of Pathogenic *Sporothrix* Species from Paraná, Brazil (2017–2023)

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Anthropogenic pressures on natural ecosystems intensify contact between wildlife and synanthropic environments, creating conditions that favor the emergence of infectious diseases. The rapid expansion of zoonotic sporotrichosis in South America highlights the need for innovative surveillance strategies capable of identifying sylvatic reservoirs. In this context, roadkill represents an underutilized resource for monitoring *Sporothrix* circulation within human-modified landscapes. In Brazil alone, approximately 15 wild animals are killed on roads every second—an estimated 1.3 million per day and over 475 million per year—posing a threat to biodiversity. We conducted a molecular survey of 81 roadkilled vertebrates (mammals, birds, and reptiles) collected along the BR-376 and PR-445 highways, which traverse the Atlantic Forest in Paraná, Brazil (2017–2023). Genomic DNA extracted from visceral organs (heart, liver, lung, and spleen) was screened via a triplex real-time PCR (qPCR) assay targeting β-tubulin gene polymorphisms with TaqMan hydrolysis probes for *S. brasiliensis* (Sbra-FAM), *S. schenckii* (Ssch-NED), and *S. globosa* (Sglo-VIC). *Sporothrix* DNA was detected in 13.6% (11/81) of the samples. *Sporothrix schenckii* was the most frequently identified species detected in the cardiac and hepatic tissues of *Leopardus guttulus* (southern tiger cat), *Didelphis albiventris* (white-eared opossum), two *Lepus europaeus* (European hares), *Colaptes melanochloros* (green-barred woodpecker), *Piaya cayana* (squirrel cuckoo), *Selenidera maculirostris* (spot-billed toucanet), and *Oxyrhopus* spp. (false coral snake). *Sporothrix brasiliensis* was detected in *Columbina picui* and *Crypturellus tataupa*, challenging the thermal-exclusion hypothesis and suggesting that birds may serve as potential vectors. Additionally, *S. globosa* was identified in *Dasyprocta* spp., *Columbina picui*, and *Oxyrhopus* spp., further expanding the host spectrum to include caviomorph rodents and reptiles. These findings indicate that wildlife in fragmented corridors may function as relevant reservoirs, biological amplifiers, and potential mechanical vectors, rather than incidental hosts. The detection of *Sporothrix* in visceral organs across diverse taxa underscores the erosion of boundaries between sylvatic and synanthropic transmission cycles. Therefore, incorporating roadkill biosurveillance into a One Health framework is essential for monitoring environmental pathogen loads and anticipating zoonotic spillover.

582C

Accumulation and localization of europium in the filamentous fungus *Podospora anserina*

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Due to their wide geographical distribution, abundance, capacity to accumulate heavy metals, and variety, fungi are considered as good bioindicators of environmental pollution. Fungi are also able to accumulate natural and anthropogenic actinides, and can affect their migration in the environment. The study focuses on the accumulation of europium (Eu), an analogue of trivalent actinides in the filamentous fungus *Podospora anserina*, used as a model system. Using speciation modelling, biochemical and cytological approaches, we studied the influence of europium concentration during different fungal developmental stages in order to understand the mechanisms of transfer and accumulation. We present some promising results on Eu accumulation, and localization in *P. anserina*. At europium concentration higher than 10-5 M, europium affects both mycelium growth and sexual reproduction without altering the morphology of *P. anserina*. In particular, by a fluorescence microscopy approach, we followed the accumulation of Eu in different cellular compartments and at all developmental stages, and showed an accumulation in asci and ascospores during their maturation.

583C

Ergot alkaloids in the fungus *Aspergillus leporis* affect the foraging behavior of the slime mold *Physarum polycephalum*

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Ergot alkaloids produced by fungi affect mammals and insects. We hypothesized they also can affect the interactions of fungi with protozoans. We investigated the interaction of the acellular slime mold *Physarum polycephalum* with the soil-inhabiting, ergot alkaloid-producing fungus *Aspergillus leporis.* Ergot alkaloid profiles were manipulated by using cultures of different ages as well as a CRISPR/Cas9-based gene knockout in the ergot alkaloid pathway. Wild-type *A. leporis* and a previously characterized *easD* knockout strain were grown on sucrose yeast extract agar (SYE) for 6 or 13 days. Plugs (8-mm diameter; 400 µL) of fungus colonized SYE were removed and inserted into wells cut into water agar plates. An explant of *P. polycephalum* was placed equidistant between the two fungus sources. Observations of preference (or lack thereof) of *P. polycephalum* for either fungus plug were recorded 20 hours after inoculation. Quantities of ergot alkaloids were measured from replicate plugs from the same fungus-colonized plates by high performance liquid chromatography. Total ergot alkaloids did not vary by strain and day, but 6-day wild type samples were dominated by lysergic acid α-hydroxyethylamide (LAH) which was converted mainly to lysergic acid by 13 days. The early pathway intermediate chanoclavine-I was the most abundant ergot alkaloid in both 6-day and 13-day samples of the *easD* knockout strain. When presented with 6-day-old samples of SYE colonized with wild-type *A. leporis* (containing mainly LAH) or the *easD* knockout of *A. leporis* (containing mainly chanoclavine-I), *P. polycephalum* preferred the *easD* knockout strain over the wild type ($P < 0.0001$). In contrast, when given the same options at 13 days (at which time LAH concentrations were significantly reduced in the wild type), the most common outcome was for *P. polycephalum* to not make a clear choice ($P < 0.0001$). With these older samples, there was no preference for the *easD* knockout over the wild type ($P = 0.57$), which by 13 days had lost 83.5% of its LAH. Our data indicate that ergot alkaloids affect the interaction of *P. polycephalum* with *A. leporis* and suggest that the slime mold is especially sensitive to LAH.

584C

Tissue specificity, ecological function, and salinity tolerance of fungi associated with the pneumatophores and leaves of black mangrove (*Avicennia germinans*)

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Mangroves are woody-plants that have been identified as keystone species in their ecosystems due to their providing invaluable ecosystem services including habitat, structure, and nutrients. Not phylogenetically related, mangrove species have each adapted to saline conditions through convergent evolution of one of two major mechanisms: removal of salt from water at the root vs. at the leaf. Whereas microbes associated with mangroves adapted to remove salt at the root may tolerate salinity due to cohabitation of saline environments with mangroves, we predict that microbes living within stem and leaf tissue are most likely to have novel adaptations due to having to tolerate salt water taken up by the plant for photosynthesis. We also predict that such microbes may mitigate salinity stress of their hosts. To begin to understand the functional ecology of mangrove-associated microbes in this context, we profiled small metabolites and fungi from the pneumatophores and leaves of *Avicennia germinans* (black mangrove; removes salt at the leaves). We surveyed three adult trees from each of three distinct sites at Elmer's Island Wildlife Refuge in Grand Isle, Louisiana, generating metabolome (i.e., volatile small molecule) and mycobiome (i.e., fungal) profiles. For the mycobiome, we generated a collection of cultivable endophytes isolated from pneumatophores and apparently healthy leaves, as well as a database of full-length sequences from the fungal internal transcribed spacer generated directly from plant DNA extracts from the same tissues. We cut pneumatophores into sections to additionally examine differences in fungi based on degree of submersion in tides. We hypothesize the alpha-diversity of halotolerant fungi to be greatest in lower sections of pneumatophores more often submerged in tides, as well as leaves from which this species excretes salt. Here, we present results from a community analysis of small molecules and fungal communities from these samples, including analyses of tissue specificity, co-occurrence between metabolites and fungi, and salinity tolerance of fungal endophyte cultures. Our results provide important baseline information regarding fungi associated with a keystone species supporting ecosystems threatened by global climate change, and pave the way for much needed follow-up studies including additional mangrove species and a broader geographic range.

585C

The floral mycobiome of the foundational dryland shrub *Larrea tridentata* (creosote): how pollinator exclusion and climate variability shape fungal community assembly

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Flowers host diverse fungal communities that affect nectar chemistry, floral signaling, and plant–pollinator interactions. Yet the composition and assembly of floral mycobiomes remains poorly understood, especially in dryland ecosystems. Yeasts and other fungi occur naturally in floral nectar and pollen and influence the nutritional value of pollinator resources. Floral visitors contribute to the transmission of fungi and other microbes to subsequent visitors and plants. *Larrea tridentata* (creosote bush), a dominant desert shrub supporting more than 100 native bee species at the Sevilleta National Wildlife Refuge (SNWR) provides an ideal system for examining plant–pollinator–microbe interactions and their consequences for plant and pollinator ecology. Despite extensive knowledge of bee diversity at SNWR, little is known about the fungi inhabiting flowers or how pollinators contribute to dispersal. Nor is it known the extent to which the floral mycobiome affects pollinator and plant fitness. The current study examined the fungal community of flowers from *L. tridentata* along three 500 m transects spanning a shrubland-grassland ecotone. In addition, it examined the effects of inter-annual variability in rainfall in experimental plots at the Sevilleta Long Term Ecological Research (LTER) Mean-Variance Experiment (MVE), where the magnitude and variability of rainfall are experimentally manipulated. The floral fungal community was assessed primarily by sequencing the ribosomal RNA ITS2 region (Illumina platform). Across transects, we examined the effects of floral tissue type, flower age, and pollinator exclusion. In MVE plots, we examined fungal community responses to precipitation variability. Culture methods were employed to help determine the overlap in fungi between flowers and insect visitors. Results indicate that of the variables examined the exclusion of pollinators and other insects had the most dramatic effect on the fungal community associated with *L. tridentata* flowers, with a different community composition and lower Shannon diversity when pollinators were excluded. Although anther and nectary tissues along with floral age differed modestly in community composition within both bagged and unbagged treatments, these effects were small, highlighting pollinator visitation as an important driver of floral mycobiome assembly. These results indicate a dynamic floral mycobiome influenced by both intrinsic floral tissue characteristics and pollinators.

586C

Metatranscriptomics of natural communities reveals functional differentiation and seasonal nutrient transport in the Populus soil microbiome

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The plant–soil microbiome is a critical interface where roots, fungi, and other microbes exchange carbon (C) and nitrogen (N), yet we lack a community-level view of how these exchanges are coordinated over time in natural systems. Using a seasonally resolved metatranscriptomic dataset of Populus tremuloides fine roots collected across an elevational gradient, we characterized the functional landscape of the aspen soil microbiome through time. By annotating fungal and host transcripts with CAZyme families, transporters, and core C–N metabolism pathways, we resolve clear functional differentiation among fungal guilds. Specifically, we find pronounced functional differences between ectomycorrhizal (ECM) basidiomycetes and ascomycetes, and a distinct behavioral profile of cryptic rhizosphere taxa such as dark septate endophytes. Integrating plant and fungal transporter expression, we infer seasonal trajectories of C and N movement between Populus and its fungal partners. C and ammonium transport appear to be linked and both peak late in the growing season, whereas nitrate transport exhibits inverse patterns. Notably, inferred N transfer to the host is decoupled from seasonal patterns of soil N availability, which we hypothesize reflects elevated fungal energetic demands and intensified C competition during fall mushroom production. Our work demonstrates how genome-resolved metatranscriptomics can link guild-specific functional traits to seasonal C and N flows in a natural forest rhizosphere, providing a mechanistic framework for predicting when cooperative nutrient exchange gives way to belowground carbon competition.

587C

Assessing fungal growth and health risks in post-flooded facilities following Hurricane Helene

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In September of 2024, western North Carolina was devastated by Hurricane Helene which resulted in historic flooding and environmental disruption. Infrastructure damage of flooded homes and buildings, in addition to damp and humid conditions, led to a bloom of microbial (fungal and bacterial) growth. Four months post-Hurricane Helene, we collected environmental samples in and around three flooded facilities in the town of Black Mountain, NC. Indoor and outdoor environmental samples (~100) were collected from non-remediated and partially remediated facilities and including swabs of built surfaces (sinks, toilets, walls) and materials (plastic folders, air filters, documents), soil samples, water samples, and outdoor vegetation We sought to determine (1) the relative abundance and diversity of microbial species present at flooded sites, and (2) whether any species (particularly fungi) were known to cause infections or produce harmful allergens, mycotoxins, or volatile organic compounds (VOCs).

To do this, we performed bulk sequencing utilizing PacBio SMRT Sequencing targeting the Internal Transcribed Spacer (ITS) region for fungi or 18S region for bacteria. In tandem, we cultured fungal isolation from the environmental samples to create a Black Mountain Fungal BioBank. This resource includes fungal isolates with species level identification achieved by sequencing three diverse genomic regions (ITS, β-tubulin, and Large Subunit of rRNA). Thus far, we have identified several distinct fungal species previously linked to opportunistic fungal infections, and others known to produce harmful allergens and mycotoxins. In ongoing work, we are assessing the susceptibility of these opportunistic pathogens to existing antifungal drugs and will additionally test novel antifungal compounds for therapeutic value.

588C

Incidence of Fungal Infections in the United States over the Last 10 Years using Electronic Health Records

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Electronic Health Records (EHR) are now used in medical facilities ubiquitously worldwide. ICDcodes (International Classification of Diseases) are standardized codes used to record diseases anddiagnoses within EHR. We have used the ICD9 and ICD10 codes for pathogenic fungi to analyze the distribution of these fungi in the United States over time using the Oracle/Cerner Real World database. We have studied fungal distribution by U.S. state over time from 2013 to 2023, observing state-specific foci of fungal infections. We observed an overall increased fungal incidence for *Histoplasma, Blastomyces*, *Coccidioides*, *Paracoccidioides*, and *Aspergillus,* while the incidence of *Cryptococcus* is decreasing over time. The incidence of *Candida, Pneumocystis*, *Talaromyces,* and the dermatophytes is currently being determined.

While analyzing at the ICD codes for pathogenic fungi, several issues have been identified. There is no ICD code for specific fungal diseases such as systemic candidiasis, or chronic mucocutaneous candidiasis. There is also no ICD code specific for a particular fungal species. This means the ICD codes do not allow physicians to distinguish*Candida auris* from *Candida albicans.* These issues with ICD codes for fungal infections will be presented. Issues with ICD codes can lead to problems with diagnosis, treatment, research, and public health monitoring.

589C

Seasonal Mating-Type Imbalance Suggests Restricted Sexual Reproduction in Field Populations of *Bipolaris oryzae* in Korea

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Sexual reproduction can profoundly influence the adaptive potential of fungal plant pathogens. In 2025, Korea experienced an unusually severe outbreak of rice brown spot caused by Bipolaris oryzae, raising new questions about the population structure and evolutionary dynamics of the pathogen during epidemic expansion. To assess whether the observed genetic diversity may be linked to sexual recombination, we conducted a weekly field survey over a three-month rice growing period and collected a total of 679 isolates. Mating type idiomorphs (MAT1-1 and MAT1-2) were identified. Across the entire outbreak-season dataset, isolates were distributed as MAT1-1:MAT1-2 = 288:391 (42.4% vs 57.6%), a significant deviation from the expected 1:1 ratio ($\chi^2 = 15.62$, $p \approx 7.7 \times 10^{-5}$). Although both mating types were present throughout the season—indicating potential for sexual reproduction—the imbalance and the estimated probability of encountering an opposite mating type (~48.9%) suggest that opportunities for random mating were limited. These data are consistent with predominantly clonal proliferation during the outbreak, possibly shaped by unequal mating-type fitness, spatial structuring, or recent demographic expansion. This study provides a high-resolution population snapshot of B. oryzae during a major field outbreak and establishes a foundation for further investigation into whether sexual recombination contributes to the pathogen's evolutionary trajectory in rice agroecosystems. (This work was supported by the Rural Development Administration of Korea (RS-2024-00400211))

590A

MERCK complex and downstream regulatory genes operate development and secondary metabolism in *Aspergillus nidulans*

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Aspergillus spp. contribute significantly to annual food and feed losses through the production of mycotoxins such as aflatoxin B1 (AFB1) and Sterigmatocystin (ST). These mycotoxins, part of a broader group of secondary metabolites (SMs) produced by *Aspergillus spp.,* are regulated by molecular mechanisms driven by post-translational modifications. A five-protein complex known as the 'MERCK' complex was identified and associated with secondary metabolism in both *Aspergillus nidulans* and *Aspergillus flavus*. The absence of this complex results in defects in sexual fruit body formation, sporulation and ST production. To uncover the MERCK complex's role in the regulation of SM biosynthesis in *A. nidulans*, RNA-Seq and ChIP-Seq were used to identify key direct targets of the MERCK complex at the primary metabolism (PM) stage (20 h of growth) and the SM stage (48 h of growth) in deletion mutants and wild type (WT). Comparison of deletion mutants with the wild type identified 214 genes commonly downregulated at the SM stage across all mutants. The top five most differentially regulated genes with regulatory functions (e.g., transcription factors, chromatin-binding proteins, and kinases) were selected for further functional characterization. The bZIP transcription factor mdrC, which is consistently downregulated in all MERCK mutants and is directly bound by RstB (R from MERCK), is required for normal growth, epigenetic regulation, transcriptional control of regulatory genes, stress response, sporulation and spore germination, sexual fruiting-body formation and maturation, as well as secondary-metabolite production. Protein pull-down experiments using MdrC-GFP and MdrC-3xHA tagged strains revealed that it binds with proteins involved in the synthesis of SMs antibiotic 2,4-dihydroxy-3-methyl-6-(2-oxopropyl)benzaldehyde (DHMBA) and Aspercryptins along with key proteins involved in amino acid metabolism. Collectively, the data indicate that the MERCK complex and its downstream regulatory network integrate developmental pathways with the regulation of secondary-metabolite production in *A. nidulans*.

591A

The Unfolded Protein Response is involved in antibacterial defense in fungi

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Saprophytic mushroom *Coprinopsis cinerea* responds to antagonistic bacteria with the significant overexpression of genes encoding antibacterial effectors. One of the most highly induced molecules upon bacterial confrontation is the secreted lysozyme LYS1, which cleaves peptidoglycans in the bacterial cell wall (Kombrink et al., 2019).

We recently discovered that the *lys1* gene is also strongly induced by dithiothreitol (DTT), a reducing agent and inhibitor of protein folding in the endoplasmic reticulum (ER). DTT is a well-established elicitor of the unfolded protein response (UPR), a central signalling network required for ER homeostasis and conserved across eukaryotes. In fungi, the UPR is known to overlap with regulatory pathways controlling thermotolerance, antifungal drug resistance, virulence, and pathogenic development (Hartig and Heimel, 2020).

Comparative transcriptomic analysis further revealed that DTT does not only induce *lys1* but the entire set of genes previously identified as the core antibacterial defense response in *C. cinerea* (Kombrink et al., 2019). Our findings indicate a functional connection between antibacterial defense and the UPR, thus suggesting an additional role of the UPR in fungi besides those already characterized.

To elucidate this connection, we first monitored the induction of both the UPR and antibacterial defense upon DTT or bacterial treatment at different time points. All treatments induce both responses 2 hours post inoculation. However, the UPR subsides in bacterial-treated *C. cinerea* following overnight incubation, pointing towards stimulus-specific temporal dynamics. We then inoculated *C. cinerea* with bacteria or DTT and the known UPR inhibitor 4μ8C to investigate the effect of the latter on *lys1* expression. The significant downregulation of *lys1* in the presence of 4μ8C suggests that the UPR might not only overlap with but also be required for antibacterial defense. We are now confirming this hypothesis by monitoring the induction of antibacterial defense genes in UPR-null mutants of *C. cinerea*.

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592A

Decoding Stress-Specific Roles of Ribosome-Associated Quality Control Initiation in *Cryptococcus neoformans*

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Cryptococcus neoformans (*Cn*) is at the top of the WHO's fungal priority pathogens list (FPPL) due to its ability to cause invasive fungal diseases in immunocompromised populations, resulting in 19% of AIDS-related deaths annually. Our lab focuses on translatome reprogramming, which is the intricate process by which *Cn* prioritizes the production of stress-responsive effectors that allow it to adapt to host stressors including thermal and oxidative stress.

Disome profiling demonstrates that the translational responses to thermal and oxidative stress differ in the accumulation of collided ribosomes in *Cn*. This led us to investigate the ribosome associated quality control (RQC) pathway which has conserved roles in recognizing and resolving aberrant translation resulting in ribosome collisions. We hypothesize that RQC recognition of collisions is a signal for initiation of translatome reprogramming, allowing efficient adaptation to the host environment. The role of RQC in *C. neoformans* translatome reprogramming has never been investigated, however, many of the RQC protein components characterized in other systems are conserved in *Cn*.

The recognition steps of the RQC pathway in *S. cerevisiae* is mediated by Hel2, an E3 ubiquitin ligase that targets the 40S ribosomal subunit, and Asc1/RACK1 (Gib2 in *Cn*), a 40S ribosome-associated protein. To begin investigating the initiation steps of RQC, we have generated *hel2*Δ and *gib2*Δ knockout strains and found that these strains are sensitive to oxidative stress in minimal media. Hel2 is also sensitive to the translational inhibitors anisomycin and cycloheximide, as well as RNA damaging agents, indicating that Hel2 is involved in the response to translational stress. In addition, both mutants exhibit altered patterns of Gcn2 activation in response to stress. Further investigation into the roles of these initiating RQC protein components could provide insights into fungal specific recognition of cell stress at the ribosome, potentially illuminating mechanisms that initiate translatome reprogramming in *Cn*.

593A

Transcription factors encoded by non-clustered genes directly activate biosynthetic gene cluster expression during *Epichloë*-grass symbiosis

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Fungi produce diverse bioactive small molecules known as secondary metabolites (SMs). The genes that specify production of each SM are typically organised into discrete biosynthetic gene clusters (BGCs). These BGCs often contain a gene encoding a transcription factor (TF) that is responsible for directly activating the expression of the other genes from that cluster. These integrated TF genes are then themselves regulated by global TFs in response to environmental cues. However, approx. half of all fungal BGCs do not contain integrated TF genes and it is usually not known how expression of these BGCs is directly activated.

Fungi from the genus *Epichloë* form persistent symbiotic associations with grass hosts. During asymptomatic growth "*in planta*" (within their grass hosts) these fungi simultaneously express many BGCs that specify SMs which protect the host plant against herbivory and disease. Interestingly, BGCs from *Epichloë* spp. almost universally lack integrated TF genes, and it is not known how these BGCs are activated in response to symbiosis. These BGCs are transcriptionally silent in axenic culture, suggesting they may be directly activated by global TF(s) in response to host signals, though such TFs have not been identified. We investigated whether and how *Epichloë* fungi employ TFs to activate BGC expression *in planta*.

By comparing transcriptomes across three different *Epichloë*-host associations, we identified five candidate genes encoding TFs that may activate BGC expression during symbiosis. These genes were not located near any BGCs, and deletion analysis established that four of these genes were not essential for establishing an asymptomatic symbiosis with the grass host. However, transcriptomic and metabolomic analyses revealed that these TFs are responsible for the global activation of BGCs *in planta*, with each TF regulating a subset of the total clusters present. These included the characterised BGCs that specify the indole diterpene, ergot alkaloid, epichloëcyclin, and Ile/Leu-glycoside SM classes, along with uncharacterised BGCs and small secreted protein genes. Our findings demonstrate that *Epichloë* spp. use the combined action of multiple conserved TFs to achieve global activation of BGCs in response to symbiosis.

594A

Identification of potential DNA N6-methyladenine readers uncovers a broad regulatory role of adenine methylation in non-dikarya fungi

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DNA methylation is a key epigenetic modification, principally through 5-methylcytosine (5mC) and N6-methyladenine (6mA). Recent studies have revealed high levels of 6mA in the genomes of green algae, ciliates, and non-dikarya fungi, in sharp contrast to the extremely low levels reported in the rest of eukaryotes. In those eukaryotes with high levels of 6mA, the symmetric form predominates and is associated with high gene expression, raising fundamental questions about its function and the identity of its molecular readers.

Here, we characterize the only nuclear YTH-domain containing protein in *Rhizopus* microsporus, YDC. YTH proteins are well-established readers of RNA m6A; therefore, their presence in organisms with abundant genomic 6mA is particularly intriguing. Phylogenetic analyses show that *R. microsporus* YDC clusters with counterparts from ciliates, green algae and other non-dikarya fungi that also display high genomic 6mA levels. This phylogenetic clustering is lost when only the isolated YTH domain is considered, suggesting that additional conserved regions outside the canonical fold contribute to 6mA recognition in DNA.

Electrophoretic mobility shift assays showed that full-length fungal YDC preferentially binds fully methylated dsDNA, with reduced affinity for unmethylated dsDNA, supporting a mechanism of DNA 6mA recognition. To identify additional proteins involved in DNA 6mA binding, methylated DNA probes were used in pull-down assays, which recovered candidate 6mA interacting proteins involved in DNA repair, RNA/DNA-binding functions, protein–interaction modules, and chromatin-chaperone activities, reinforcing a mechanistic link between adenine methylation, genome integrity pathways, and chromatin dynamics.

Together, these findings suggest a lineage-specific evolutionary function for YDC, provide evidence for 6mA-dependent DNA recognition, and suggest that adenine methylation plays a broader regulatory role in transcription, chromatin organization, and genome maintenance in basal fungi.

595A

Deciphering of the non-canonical unfolded protein response (UPR) pathway governs the pathogenicity of *Candidozyma auris* (*Candida auris*)

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Candidozyma auris (formerly *Candida auris*), first identified in 2009, is an emerging pan-drug-resistant fungal pathogen. Despite the high mortality associated with candidiasis due to its resistance to multiple antifungal drugs, the signaling pathways that regulate stress responses and virulence in *C. auris* remain poorly understood. To address this gap, we investigated the pathobiological roles of the unfolded protein response (UPR) pathway—a highly conserved cellular mechanism that alleviates endoplasmic reticulum (ER) stress by restoring protein-folding homeostasis. In fungi, the canonical UPR pathway involves the ER-resident sensor Ire1 and the transcription factor Hac1, which is activated via Ire1-mediated splicing of its mRNA. This mechanism has been well-characterized in model fungi such as *Saccharomyces cerevisiae*, *Candida albicans*, and *Cryptococcus neoformans*. In contrast, other fungi like *Schizosaccharomyces pombe* and *Candida glabrata* utilize a non-canonical UPR pathway known as RIDD (regulated Ire1-dependent decay), in which Ire1 directly degrades ER-associated mRNAs to reduce the protein-folding burden. To explore how the UPR functions in *C. auris*, we first confirmed that its Ire1 ortholog contains conserved kinase and RNase domains. Surprisingly, the *HAC1* ortholog in *C. auris* lacks the intron commonly found in other fungi, which is typically required for Ire1-mediated splicing and activation of Hac1. RT-PCR analysis following tunicamycin-induced ER stress showed no evidence of alternative splicing, indicating that *C. auris* does not employ the canonical Ire1-Hac1 splicing mechanism. Nonetheless, deletion of either *IRE1* or *HAC1* resulted in hypersensitivity to ER stress, suggesting that both genes play critical roles in the stress response. These findings reveal a non-canonical UPR pathway in *C. auris*, highlighting the need to understand species-specific adaptations in emerging fungal pathogens.

596A

Comparative Transcriptome and Mutational Analysis Reveal Nitrogen-Responsive Regulators of Filamentation in *Ustilago maydis*

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Nitrogen availability greatly influences the developmental process of *Ustilago maydis*, a fungal pathogen of maize. The ammonium transceptor gene *ump2* plays a crucial role in sensing low nitrogen conditions and promoting filamentation. Deletion of *ump2* disrupts this process, resulting in a non-

filamentous phenotype. However, the key regulators underlying this characteristic remain unclear. This study aims to investigate the role of *ump2* in regulating this morphological change and identify secondary suppressor mutations that restore filamentous growth in *ump2* knockout strains. Transcriptome profiling was performed on haploid cell type FB1 and solo-pathogenic SG200 strains grown under nitrogen starvation. Among genes differentially regulated in both the FB1 and SG200 knockout backgrounds, a proportion that were upregulated in one background were down-regulated in the other, suggesting a strain-specific role of *ump2*. Likewise, suppressor mutants of *ump2* knockout derivatives were evaluated by whole genome sequencing to identify UV-induced variants associated with phenotype restoration. In total, 348 and 239 mutation sites were distributed in intergenic, intron, exon, and upstream regions of FB1 and SG200 UV-induced mutants, respectively. These findings reveal the existence of multiple regulatory genes that interact or act downstream of *ump2* and can rescue the filamentous phenotype.

597A

Tissue-specific activation of genes regulated by Polycomb Repressive Complex 2 in *N. crassa* fruiting bodies

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Fungal sexual development encompasses major transcriptional and morphological reprogramming events. Fruiting bodies typically comprise multiple layers of unique tissue types and are metabolically expensive to generate. Therefore, it is essential to tightly regulate and coordinate developmental signals to prevent unnecessary energy investment into building these structures. In other systems, Polycomb Repressive Complex 2 (PRC2) is a highly conserved regulator of multicellular development that represses genes by establishing domains of histone H3 lysine 27 tri-methylation (H3K27me3) across conditionally activated genes. In *Neurospora crassa*, PRC2-methylated genes show exquisite cell-type specificity and are specifically activated during sexual development. Loss of PRC2 results in the precocious formation of perithecia-like structures (dubbed false perithecia), even in the absence of a compatible mating-type partner. However, bulk ChIP-seq has revealed that only subtle changes to the distribution of H3K27me3 occur in perithecia. One potential regulator of PRC2-methylated genes is the predicted pioneer transcription factor FSD-1, which binds to the promoters of strongly expressed PRC2-methylated genes that show subtle reduction in H3K27me3 levels. Together, we propose that PRC2 maintains cell-type specificity of developmental genes. Furthermore, upregulation of sexual development genes occurs via subtle, tissue-specific modifications to the epigenome and coordinated activity of specific transcription factors.

598A

A long non-coding RNA mediates crosstalk between fungal thermal adaptation and azole drug response in *Aspergillus fumigatus*

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Fungal pathogens are remarkable in their ability to adapt to mammalian (37°C) and avian physiological temperatures (38-42°C) to cause infection. Saprophytic mold *Aspergillus fumigatus* (AF) is unique in its ability to adapt to temperature and can grow above 50°C. Here, we show that AF adaptation to increased temperature results in morphological changes and, more importantly, increased fungal growth in presence of azoles. Importantly, this is a reversible phenotype and is mediated by a long non-coding RNA whose RNA levels negatively correlate with temperature. Our data show that lncRNA levels differ during saprophytic phase (25°C), mammalian infection phase (37°C) and increased temperature exposure (42°C), thus acting as a link between temperature adaptation and fungal azole response. Furthermore, the overexpression of lncRNA in azole-resistant isolates increases fungal azole susceptibility and, importantly, improves the azole treatment outcomes in a murine model of infection, clearly showing the role of lncRNA in infection outcomes for azole drug-resistant isolates. Through our adaptation experiment, we also show that fungal adaptation to sub-MIC voriconazole concentration is a precursor to the development of a non-reversible novel drug resistance mechanism that is lncRNA dependent. Taken together, these data show that AF adapts to increased temperature by downregulating lncRNA levels that impacts fungal azole response and the subsequent exposure to azoles results in evolution of non-CYP51-dependent azole resistance.

599A

Dynamics of Alternative Polyadenylation (APA) at 3' UTR regions in wild-type Neurospora crassa and CFIm mutants to investigate a short circadian clock period length defect

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The circadian clock is an entrained process in many organisms that oscillates approximately every 24 hours, and it is maintained at the cellular level to synchronize processes according to the time of day. These rhythms are regulated by a transcription-translation feedback loop (TTFL) consisting of positive arm transcription factors and a negative arm complex that closes the daily feedback loop by inhibiting the positive arm. Compensation is a regulatory property of circadian clocks that maintains oscillation period length despite changes in environmental conditions such as temperature and nutrient availability. Intact circadian compensation is important to buffer clock period and phase from regular changes in hormones and other biotic and abiotic factors in the environment.

Previous work has identified a polyadenylation cleavage factor complex (CFIm) as being important in circadian period maintenance. CFIm knockout mutants Δcpsf5 & Δcpsf6 show circadian period lengths of around 19 hours, approximately 2 hours shorter than wild type controls under most nutrient conditions. Interestingly, the circadian clock of Δcpsf5 & Δcpsf6 mutants is shortened about 4 hours under nitrogen starvation in "old" fungal growth regions (i.e. the inoculation area, 2.5+ days after incubation). The CFIm complex has been shown to regulate alternative polyadenylation (APA) of Neurospora and other fungal species, and mammalian studies have shown that dysregulation of APA can lead to the development of cancer. Through the use of Neurospora crassa, a well-studied model for circadian biology, this project aims to elucidate the how changes in APA under different nutrient contexts lead to changes in circadian period length to better understand the function of clocks. To compare 3' UTR regions between the wild type and CFIm mutant, samples grown in starvation conditions, where the shortened period phenotype is revealed, will be analyzed using long-read Nanopore RNA sequencing. To see the dynamic differences not having a functioning CFIm complex has on period length, an 11-point timecourse will be performed using a Δcpsf5 Δcpsf6 double mutant strain and the different timepoints will be subjected to RNA sequencing to analyze gene expression levels and APA patterns at the

3' UTR. This work also requires new computational methods to quantify the link between poly(A) tail location, alternative splicing in the coding region, and circadian rhythms in transcription and post-transcriptional regulation. Proper maintenance of circadian rhythms is critical for human health as its dysregulation has been linked to diabetes, cardiovascular disease, and cancer, highlighting the importance of first understanding the circadian mechanism of APA in a more genetically tractable eukaryote.

600A

Can the presence of a fungal volatile organic compound improve plant resilience to abiotic stress?

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Climate change is a matter of increasing concern, as the severity, duration, and area affected by drought have steadily risen since the 1970s. These changes have severely affected the agricultural industry, as drought stress is a major contributor to crop loss. Therefore, it is crucial to develop new strategies to address this problem.

This research aims to evaluate the protective effect of a specific fungal volatile organic compound (FVOC1) when applied as a seed coating. The FVOC1 seed coating treatment's effects were assessed regarding plant emergence and resilience to drought stress adaptations. By conducting a comprehensive transcriptomic analysis, it was possible to identify the transcriptional changes induced by the presence of the FVOC1 molecule. The application of FVOC1 led to significant changes in gene expression in plants under drought-stress conditions, enabling them to cope more effectively with stress. Moreover, when plants grown from FVOC1-treated seeds were exposed to a second round of drought stress, they exhibited a more specific and refined response to this stress.

This research highlights the importance of fungal volatile organic compounds as promising molecules for developing novel strategies and technologies to enhance crop resilience to the environmental challenges that global warming, water scarcity, and a growing population will impose on global food security.

601A

Functional dissection of the RNAi pathway in the oomycete *Phytophthora sojae*

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The oomycete *Phytophthora sojae* is a devastating pathogen of soybean, causing multi-billion US dollar losses every year across the world. Due to its economic importance and well-established toolkit, *P. sojae* has been developed as a model system to study oomycete biology, genetics, and pathology. Epigenetic regulation enables organisms to rapidly adjust gene activity without altering their underlying DNA sequence, and gene silencing is one of the most important epigenetic mechanisms for protecting genome integrity and fine-tuning gene expression. Although multiple pathways contribute to gene silencing, they are generally grouped into two major categories: Transcriptional Gene Silencing (TGS), which involves DNA methylation and histone modifications, and Post-Transcriptional Gene Silencing (PTGS), which is mainly mediated by RNA interference (RNAi) via small interfering RNA (siRNA). In previous studies, we identified the core RNAi components across *Phytophthora* species and uncovered their diverse evolutionary trajectories. We also observed that TGS-associated histone marks, including H3K9me3 and H3K27me3, strongly coincide with siRNA hotspots across the *P. sojae* genome, especially at effector loci encoding small secreted virulence proteins. These findings suggested a potential link between RNAi, heterochromatin, and effector regulation, yet the RNAi pathway itself has not been functionally characterized in *P. sojae*. In this study, we generated deletion mutants of three core RNAi components, namely RDP1, *DCL1*, and *DCL2*, and systematically investigated their biological functions. Deletion of *rdp1* did not noticeably affect siRNA production or virulence, suggesting that additional RNA-dependent RNA polymerases participate in RNAi. In contrast, deletion of either *dcl1* or *dcl2* disrupted siRNA profiles and led to impaired growth and reduced virulence. Together, these results demonstrate that the Dicer-mediated RNAi pathway is critical for *P. sojae* development and infection. In addition, comparative analysis of siRNA profiles revealed pronounced differences in siRNA enrichment between *P. sojae* and *P. infestans*, suggesting that RNAi functions have diverged considerably during *Phytophthora* evolution. We are now investigating how RNAi interacts with heterochromatin and how this relationship shapes effector gene regulation.

602A

Post-transcriptional regulation of the *C. neoformans* stress response by the RNA-binding protein Gis2

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The pathogenic yeast *C. neoformans* can proliferate in the lungs of immunocompromised human hosts causing cryptococcal pneumonia. Without treatment this can progress to cryptococcal meningitis, which is responsible for over 100,000 deaths annually. Adaptation to host-associated stressors is required for *C. neoformans* virulence and consists of a rapid and extensive shift of the mRNAs being actively translated. During this "translatome reprogramming", homeostatic, ribosomal protein transcripts are repressed to reallocate translational resources for production of stress response proteins. The details of this complex process are not fully understood. Two proteins that were identified to interact with the 3' UTR of mRNAs that are repressed during this stress response are homologs Gis2 and Znf9. Both of these proteins contain the same number of SS nucleic acid binding motifs but differ in the presence of an RG-rich methylation site. Mutants lacking these two proteins have different phenotypes, yet the mRNAs encoding each are repressed as part of stress response. Phenotypic analysis and RNA-sequencing data indicate a disruption in mitochondrial function of the *gis2*Δ mutant, which lacks the RG-rich methylation site found in Znf9. Additionally, the *gis2*Δmutant had increased activation of the Gcn2 pathway which regulates translational stress response. Unexpectedly, despite this increased activation of Gcn2 in *gis2*Δ, there is reduced downstream activity, resulting in a reduction in expression of integrated stress response transcripts compared to the WT strain. This is consistent with a model in which hyperactivation of Gcn2 signaling can be detrimental to an effective downstream stress response. Determining the mechanism by which Gis2 and Znf9 contribute to *C. neoformans* adaptation to host stress can provide a novel avenue of exploration for targets of antifungal drugs.

603A

Evolution of short amino-acid insertions in Gcn4 rewires metabolic gene regulation in the *Candida* lineage

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This study seeks to understand how expression patterns change over evolutionary time, specifically how new genes come under the control of a transcription factor (TF). Using the well-characterized regulator of amino acid starvation Gcn4 as a test case, we found that Gcn4 brought genes involved in central and fatty-acid metabolism under its control in the *Candida albicans* clade. We hypothesized that this occurred through protein-coding changes within Gcn4's unstructured region as such regions are not under strong purifying selection, tend to evolve rapidly, and can become fixed over short evolutionary timescales. To test this hypothesis, we replaced native *C. albicans* Gcn4 with Gcn4 from the distantly related yeast *Saccharomyces cerevisiae*, whose unstructured region has diverged substantially over ~200 million years of evolution. This experiment revealed that the expression of recently added Gcn4-activated genes is attenuated through protein-coding changes that arose during this evolutionary window. Using a novel computational tool that scans fungal genomes from the Y1000+ project, we identified multiple 5–10 amino-acid stretches that recently emerged in the *Candida* clade. We determined that one of these stretches is necessary and sufficient to attenuate the expression of the recently gained gene orf19.2607 (a paralog of a glycolysis-pathway gene). We further found that attenuation of this gene is driven by the DNA-binding cofactor CaPho2 (Grf10), which interacts with Gcn4 by binding a site 11 bp upstream of the Gcn4 site. Together, these results suggest that (1) new genes can come under a TF's control through protein-coding changes in its unstructured region, and (2) such changes, that appear as fixed peptide sequences over short evolutionary timescales, can encode interactions with other transcription factors. Overall, this study provides new insights into transcription-circuit rewiring and how gene-expression patterns evolve.

604A

Chromatin dynamics of ergosterol biosynthetic gene regulation in wild-type and azole-resistant *Aspergillus fumigatus*

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Aspergillus fumigatus is a ubiquitous saprophyte responsible for chronic pulmonary infections and life-threatening invasive aspergillosis. Recently, *A. fumigatus* was classified as a critical priority pathogen by the World Health Organization in part due to rapid emergence of antifungal resistance. The first-line therapy for *A. fumigatus* infections is the azole drug voriconazole, which targets Cyp51A to inhibit ergosterol biosynthesis. Transcription of *cyp51A* and other ergosterol biosynthesis genes is controlled by multiple transcription factors, including SrbA, AtrR, and others, yet how these factors modulate chromatin structure to regulate gene expression is unknown. To determine how chromatin changes in response to ergosterol limitation, cells were grown in the presence or absence of voriconazole and examined by ATAC-seq. Following voriconazole exposure, a subset of ergosterol biosynthetic genes displayed enhanced promoter accessibility. We will present findings from our ongoing work to: (1) quantify differential accessibility in voriconazole-exposed cells, (2) identify enriched motifs within these regions, and (3) integrate chromatin accessibility data with histone modification profiles. We also examined chromatin structure in two azole-resistant *A. fumigatus* strains. The most prevalent azole-resistant alleles of *cyp51A* contain short promoter duplications of 34 and 46 base pairs. Both duplications include binding sites for the global regulators of ergosterol biosynthesis: SrbA and AtrR. In both alleles, the *cyp51A* promoter was hyperaccessible, even in the absence of voriconazole. Future studies will determine if DNA sequence, SrbA, or AtrR is responsible for increased promoter accessibility in the tandem repeat mutants.

605A

sit1 regulates morphological development and aflatoxin biosynthesis in *Aspergillus flavus*

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Aspergillus flavus is a mycotoxigenic plant pathogen that contaminates important crops such as oilseeds, spices, and nuts, posing serious risks to food safety and public health due to its production of aflatoxins, potent carcinogenic compounds linked to liver cancer. Iron is an essential micronutrient for fungal growth and metabolism, supporting processes such as energy generation, DNA synthesis, and enzymatic function. The *sit1* gene encodes a siderophore transporter involved in iron acquisition, suggesting a potential role in regulating fungal development and secondary metabolism. In this study, we generated a *sit1* deletion strain and its complementation strain and compared them with the wild type to investigate the functional role of *sit1* in fungal morphology and secondary metabolism. Deletion of *sit1* reduced colony diameter. In addition, the *sit1* mutant showed a decrease in sclerotial formation. Notably, aflatoxin production was significantly decreased in the *sit1* deletion strain compared to the wild type and complementation strain. Together, these results show that *sit1* is required for normal development and contributes to the regulation of aflatoxin production, suggesting that *sit1* could be a potential target against *A. flavus* plant colonization and mycotoxin contamination.

606A

Gene Regulatory Network Inference Reveals Novel Regulators of Cellulose Degradation in Neurospora crassa

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Lignocellulose is the most abundant renewable biomass on Earth and holds great potential as a sustainable energy source. Unlocking this potential requires efficient access to plant cell wall components with minimal environmental impact. In this context, the study of plant cell wall degrading enzymes (PCWDEs) produced by cellulolytic fungi such as Aspergillus, Trichoderma and Neurospora is key. Indeed, in recent years, Neurospora has emerged as a powerful model system for dissecting the transcriptional control of cellulose degradation.

PCWDE production is a tightly regulated process, where transcription factors (TFs) such as CLR1, CLR2, XLR1 and CRE1 has already been characterized. However, additional TFs likely remain to be discovered to fully understand, and modulate, cellulose deconstrucion. In this work, we aimed to uncover novel TFs involved in this process. To achieve this, we applied a systems biology approach by integrating published transcriptomic datasets and employing machine learning algorithms to infer gene regulatory networks and uncover potential transcriptional regulators. Through this analyses, we found 14 TFs, including the 5 previously described (CLR1, CLR2, XLR1, PDR2 and ARA1), as key regulators in lignocellulose metabolism. The remaining 9 TFs had not been, to date, functionally characterized. To validate their role in plant biomass degradation, we analyzed deletion mutants grown on different carbon sources. Notably, 5 of the 7 tested candidates showed obvious growth phenotypes on complex substrates, supporting their involvement in plant cell wall deconstruction. These findings, validated by complementation analyses, expand our understanding of the transcriptional network controlling lignocellulose degradation in Neurospora and provide novel targets for the development of fungal strains with improved biotechnological potential.

607B

Retrotransposon-mediated transition from sexual to asexual development in the katsuobushi fungus, *Aspergillus chevalieri*

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Katsuobushi, a dried bonito, is a traditional Japanese fermented food. A particular type of katsuobushi, known as karebushi, is produced through repeated cycles of cultivating xerophilic fungi on the surface of bonito fillets. These processes reduce the moisture content and refine the flavor of karebushi. *Aspergillus chevalieri* is one of the predominant fungi on the surface of karebushi. This species includes two types that differ in their life cycles: a teleomorphic type, which produces cleistothecia (sexual fruiting bodies), and an anamorphic type, which produces conidia (asexual spores) under identical environmental conditions, such as on minimal agar medium. This contrasts with the model fungus *Aspergillus nidulans*, which switches between sexual and asexual development depending on environmental factors such as oxygen availability and light. When cultivated on minimal agar medium, the teleomorphic strain of *A. chevalieri* initially produces cleistothecia. However, upon prolonged incubation, it partially begins to form conidia. Conidia isolated from these cultures, when subcultured on minimal agar medium, consistently exhibited an anamorphic phenotype, indicating a loss of sexual fertility. Comparative genomic analysis between the teleomorphic strain and its anamorphic mutant using PacBio long-read sequencing revealed the insertion of an approximately 5.8-kb retrotransposon upstream or inflame of a gene encoding an HMG-box transcription factor in the anamorphic mutant. Disruption of this transcription factor gene in the teleomorphic strain resulted in the acquisition of an anamorphic phenotype, whereas complementation restored the teleomorphic phenotype. These findings indicate that this transcription factor is essential for sexual reproduction in *A. chevalieri*. Furthermore, expression levels of the mating-type genes *MAT1-1* and *MAT1-2* were reduced in the anamorphic mutant, suggesting that this transcription factor regulates *MAT* genes expression.

608B

Role of the *osaA* transcription factor gene in development, secondary metabolism and virulence in the mycotoxigenic fungus *Aspergillus flavus*

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Aspergillus flavus is an opportunistic fungal pathogen that affects numerous economically relevant oil-seed crops, including corn, cotton, peanuts, and various tree nuts. While colonizing the plant tissues, *A. flavus* generates several powerful mycotoxins, most notably aflatoxin B_1, considered the most carcinogenic natural compound known. The infection of crops by *A. flavus* and the resulting aflatoxin contamination pose significant global challenges to both public health and the economy. Current control measures are inadequate to mitigate the harmful effects of *A. flavus*. Molecular genetic research may reveal new targets for the development of innovative strategies to combat the negative effects of this pathogen. Here, we characterized a putative WOPR transcription factor gene, *osaA*, in this agriculturally relevant fungus. Our study revealed that *osaA* is a repressor of conidiation in *A. flavus*. In addition, *osaA* is required for normal production of sclerotia. Importantly, removal of the *osaA* gene results in a decrease in aflatoxin B_1 production. Unexpectedly, transcriptome analysis revealed that while several aflatoxin biosynthetic genes were upregulated in the mutant, although the actual toxin levels were reduced, suggesting that *osaA* may influence aflatoxin production through post-transcriptional or cofactor-regulated mechanisms. Other *osaA*-dependent changes in secondary metabolism were observed, including a significant decrease in cyclopiazonic acid production. In addition, deletion of *osaA* resulted in upregulation of genes involved in the production of aspirochlorine, a potent anti-fungal compound. Importantly, *osaA* is indispensable for normal *A. flavus* seed colonization; deletion of *osaA* significantly reduced fungal burden in corn kernels. Furthermore, aflatoxin B_1 production in seeds also decreased in the absence of *osaA*. The *osaA* mutant presented alterations in oxidative stress and temperature sensitivity, as well as a decrease in cell wall chitin content, which could contribute to the decrease in pathogenicity in this strain. Understanding the regulatory scope of *osaA* provides new insight into fungal biology and emphasizes its potential as a molecular target for controlling fungal seed colonization and mycotoxin contamination.

609B

RNAi restriction of a novel mycovirus prevents viral induced alterations in transcription and virulence in a critical human fungal pathogen

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RNA interference (RNAi) is a widely conserved genome-defense mechanism that protects eukaryotes against viruses and transposon genome parasites. We previously showed that RNAi loss leads to hypermutation and anti-fungal drug resistance in the human fungal pathogen *Cryptococcus neoformans*, illuminating the potential clinical relevance of this occurrence. In this study, we identify mycovirus restriction as another clinically relevant RNAi function in *C. neoformans*. Through investigation of two RNAi-deficient clinical isolates of *C. neoformans*, we identify a novel dsRNA mycovirus of the Totiviridae family we have named CnTV1. Interestingly, RNAi deficiency in these isolates results from the insertion of a nuclear mitochondrial DNA segment (NUMT) into the key RNAi component Ago1. We find that restoration of RNAi in sexually produced zygotes eliminates CnTV1. To further test the role of RNAi in CnTV1 persistence, we performed CRISPR-mediated allele exchange at the mutated RNAi loci which similarly led to CnTV1 elimination in vegetative cells. Through genetic mutations and chemical treatments, we identified several RNAi-independent factors involved in CnTV1 regulation, including a core RNAi component that unexpectedly promotes viral accumulation in the absence of functional RNAi. To assess the functional impact of CnTV1, we developed a cytoplasmic-mixing approach to generate isogenic strain pairs in an RNAi-deficient background that differ only in viral infection status. With these strains, we show that CnTV1 causes altered gene expression and moderately increased virulence in a mouse infection model. Our findings reveal that RNAi opposes infection of *C. neoformans* with a virulence-influencing mycovirus, highlighting another clinically relevant consequence of RNAi loss in this WHO priority fungal pathogen.

610B

Mapping nuclear cooperation in fungal syncytia

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Many fungi are syncytia, a form of cellular organization where many nuclei co-exist in a common cytoplasm. The prevalence of syncytia across fungal lineages, and broadly across eukaryotic kingdoms suggests that multinucleation, as an alternative to uninucleate organization, might itself be beneficial and adaptive in diverse contexts. How multiple, coexisting nuclei contribute to fungal growth, environmental sensing and resilience is poorly understood. Intriguingly, across many multinucleate systems including filamentous fungi, mammalian skeletal muscle, algal cells and slime molds, syncytia have been observed to show inter-nuclear differences such that the same cytoplasm hosts multiple gene expression states. How and why do syncytial nuclei show transcriptional variation? One hypothesis is that such transcriptional variability may permit global control of gene expression while compensating for noisy expression at the level of individual nuclei. This can lead to bet-hedging, or even nuclear “cooperation”. Previously, we showed that in the syncytial ascomycete *Ashbya,* a minimal cell cycle circuit can promote spatial variation in protein translation and potentially promote cell cycle asynchrony. This implies that transcriptional variation associated with the cell-cycle may be programmed within *Ashbya* cells. To understand how such a program may impact transcriptome-wide variation and further enables functional differentiation across syncytial nuclei, we performed single-nucleus RNA sequencing on two highly diverged species: *Ashbya* and *Mucor,* sampling different growth conditions and developmental states. This yielded high-quality single-nucleus transcriptomes for thousands of nuclei. Unsupervised clustering of the transcriptomes revealed a striking diversity of transcriptional “signatures” within and across growth conditions in both organisms. These signatures show enrichment for specific biochemical functions and stress response genes. However, it is unclear if these nuclear signatures represent coexisting states within a single cell, and if they arise as a result of cell-cycle control. To investigate the possibility of nuclear cooperation, we are currently in the process of mapping the spatial variability of these signatures using single molecule FISH, along with cell cycle state estimation. The outcome will reveal how fungal syncytia leverage multinucleation to orchestrate distinct gene expression programs in a shared cytoplasm across hundreds of nuclei.

611B

Facultative heterochromatin mediated by core and accessory chromosome-encoded H3K27-specific methyltransferases controls virulence in a fungal phytopathogen

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In many fungal phytopathogens, infection is regulated by accessory genomic regions enriched in facultative heterochromatin, but the precise role of this chromatin state in virulence is poorly understood. Here we examined the two facultative heterochromatic marks H3K27me and H3K36me in the tomato-pathogenic *Fusarium oxysporum* isolate *Fol* 4287, by genetically analyzing the corresponding histone methyltransferases Kmt6 and Ash1. *Fol* has three *KMT6* homologs, two of which, *KMTB/C*, are encoded on an accessory chromosome. Loss of the core gene *KMT6A* resulted in strongly reduced but not abolished H3K27me, revealing that methylation of subtelomeres can be taken over by the accessory copy Kmt6b. To the best of our knowledge, this demonstrates for the first time that accessory fungal histone modifiers can contribute to the overall epigenetic profile. Deletion of *KMT6A* or *ASH1* resulted in derepression of *in planta*-induced effector genes under axenic conditions. In the case of Ash1, H3K36-mediated H4 deacetylation is likely involved in gene silencing under non-inducing conditions. *KMT6A* deletion mutants were blocked at early stages of root infection and non-pathogenic on tomato plants, but were still able to kill the animal host *Galleria mellonella*. Constitutive overexpression of the pathogenicity-related transcription factor *FTF1* in the Δ*kmt6a* background restored effector gene activation *in planta* but not pathogenicity, suggesting that additional facultative heterochromatin-regulated virulence factors are essential for plant infection.

612B

RNA isoforms and structure rapidly respond to temperature changes in *Histoplasma*

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Temperature is a key signal sensed by thermally dimorphic fungi, including *Histoplasma ohiense* (*Ho*), to elicit developmental programs that facilitate pathogenesis. Since RNA is a classic thermoresponsive molecule, we hypothesized that fungal transcriptomes harbor temperature-responsive RNA structures that could regulate fungal biology. Since *Ho* displays dramatic temperature regulated biology, growing as hyphae at ambient temperature and as yeast at host temperature, it is an ideal system to identify putative RNA thermometers.

To interrogate the role of RNA molecules in thermosensation, we generated the first isoform-level transcriptomic atlas of *Ho* yeast (37˚C) or hyphae (22˚C) using Nanopore direct RNA-seq and short-read RNA-seq, with validation of transcription start-site annotations by 5'-seq. We annotated 14,014 genes and 50,791 isoforms, including those that displayed differential splicing or alternative 5' leaders and 3' trailers that were differentially enriched in yeast or hyphae. Initial annotation of differentially expressed isoforms revealed that one of the splice events increased the length of the transcript region encoding a phase separation domain in a key regulatory transcription factor that is required for yeast-phase growth, suggesting that differential RNA isoforms could play important regulatory roles in the response of *Ho* to temperature. Differential leader and trailer isoforms were equally provocative. We identified 2,140 and 3,516 protein coding genes with longer leader and longer trailer isoforms, respectively. A time course following a shift from 37˚C to 22˚C revealed that some longer leaders and trailers arise or disappear as early as 2 hours post shift. Rapid regulation of leader and trailer isoform levels post temperature shift and at various developmental stages suggest isoform-driven regulation of temperature-dependent development. We are currently exploring the effect of these differential isoforms on translation.

To extend these studies to identify thermoresponsive RNA structures, we adopted a transcriptome-wide RNA structure probing approach, DMS-MaPseq. Preliminary analysis revealed 5' leader and 3' trailer regions that have different structures at either 37˚C or 22˚C, including temperature-responsive structural elements in the mRNA leader structures of ubiquitin and BiP. Molecular characterization and structure-function assays of these and other putative thermosensory structural elements are currently underway.

613B

Evolution of RNA binding in the fungal regulatory protein Ssd1

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Proteins gain and lose functions over time, as evolutionary selective pressures change. Ssd1 is a fungal protein that regulates growth and cell wall integrity. Fungal Ssd1 is also a "pseudoenzyme", related to the Dis3L2 family of exonuclease enzymes. Ssd1 has gained a novel regulatory RNA-binding function, prior to losing an ancestral nuclease enzymatic function, over its evolutionary history. Interestingly, some extant Ssd1 homologs have sequence signatures of both nuclease activity and RNA-binding, suggesting descent from ancestral bifunctional homologs undergoing this transition. The activity of modern Ssd1 is regulated by Cbk1, a serine/threonine protein kinase that phosphorylates the unstructured Ssd1 N-terminus. We have characterized the *Saccharomyces cerevisiae* Ssd1 RNA-binding surface, and shown how a key molecular interaction between these residues and a conserved motif in the 5' UTRs of target cell wall protein-encoding mRNAs leads to translational repression and promotes cell wall integrity. We have shown that the genetic interaction of Cbk1 regulation of Ssd1 is due in large part to these RNA-binding residues, consistent with a model where Cbk1 regulates RNA-binding by Ssd1. To study the functional evolution of Ssd1, we developed a plasmid-based system to express Ssd1 fungal homologs in *Saccharomyces cerevisiae ssd1Δ* strains. We observed conservation of the fungal-specific RNA-binding function in fungal clade Dikarya and a subset of mycorrhizal fungal homologs, suggesting evolution of this function in the common ancestors of these 2 groups, >700 mya. We confirmed that the fungal-specific RNA binding function is not conserved in *Schizosaccharomyces pombe* Dis3L2, but is conserved in some potentially bifunctional homologs. This extant data forms the foundation for a broader effort to reconstruct the ancient molecular events that led to functional changes in the Ssd1/Dis3L2 family. The project serves as an exemplary dissection of the molecular evolutionary processes that underly an ancient transition in fungal lifestyle, from unicellularity to hyphal growth and saprotrophy.

614B

Mechanisms behind nitrous oxide production and lignocellulose degradation in anaerobic salt marsh sediment fungi deduced through transcriptomics

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The role facultative denitrifying fungi may play in nitrogen and carbon cycling processes in dynamic salt marsh sediment environments is poorly understood. To deduce mechanisms that contribute to these processes, transcriptomic profiling was performed using salt marsh sediment isolates capable of N_2O production, *Purpureocillium lilacinum BL2022* and *Trichoderma virens XP2022*. Isolates were incubated with differing redox states (sulfidic and oxic), nitrite (NO_2^-) concentrations (0, 10, 100 µM), and carbon sources (glucose and *Spartina alterniflora* stems). One target of our analysis was nitric oxide reductase (*P450nor*), the diagnostic gene for fungal denitrification. In *P. lilacinum*, *P450nor* was positively correlated with nitrite reductase (*NirK*), another gene in the denitrification pathway. *NirK* was not identified in *T. virens*. Nitric oxide synthase (*NOS*) expression, however, was positively correlated with *P450nor* in both species, and is potentially an unrecognized nitric oxide-providing mechanism for *P450nor*. Differential expression analysis revealed concurrent upregulation of *P450nor*, *NirK*, and *NOS*, under sulfidic conditions (*NirK* limited to *P. lilacinum*). In addition to positive N_2O and CO_2 headspace gas correlations, measured from gas-tight vials, results strongly support the role of *P450nor* as part of the denitrifying respiratory chain, and suggests a role for fungal *NOS* in sulfidic systems. Although p450nor was previously hypothesized to be associated secondary metabolisms, the expression of *p450nor* in our strains did not show any significant correlation with biosynthetic gene clusters.

Analysis on extracellular CAZymes revealed that a large suite of CAZymes were upregulated under sulfidic conditions in *P. lilacinum*. These CAZymes, however, were primarily glycoside hydrolases, while auxiliary activities, carbohydrate esterases, and polysaccharide lyases were primarily upregulated under oxic conditions. Conversely, the majority of CAZymes in *T. virens* were upregulated under oxic conditions, irrespective of CAZyme family.

Regardless of fungal strain, substrate analysis via dbCAN-sub revealed that highly refractory materials, such as lignin and polyphenols, in addition to other cell wall components, are potential substrates for CAZymes upregulated under sulfidic conditions. In summation, results confirm the role of *P450nor* in anaerobic fungal metabolism and highlight the capability of fungi to break down lignocellulose under sulfidic conditions.

615B

Developing transposable element reporter systems in *Cryptococcus*

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Cryptococcus neoformans (*Cn*), the most common cause of fungal meningitis, is a significant healthcare burden with a high mortality rate and high degree of patient suffering. For individuals with HIV/AIDS, *Cn* infection is a severe co-morbidity and kills an estimated 180,000 people annually. Despite the concerning mortality rate and disease burden of *Cn*, our understanding of how *Cryptococcus* species adapt to cause infection, develop antifungal resistance, and persist in the human host is limited. Since *Cryptococcus spp.* are ubiquitous environmental fungi, they must successfully overcome the environment-to-host transition to cause infection. One major stressor is high body temperature since *Cryptococcus* grows poorly at 37°C. To identify stress-induced genetic changes that occur in *Cryptococcus*, our lab employs a forward-genetic assay to screen for mutations that result in antifungal drug resistance following growth under stress conditions, including growth at 37°C. Using this approach, we previously demonstrated that mutations by transposable elements (TEs) contribute to drug resistance in *Cn* and *Cryptococcus deneoformans* (*Cd*), a closely related disease-causing species, when grown at human body temperature. Specifically, we have identified two **l**ong **t**erminal **r**epeat (LTR) retrotransposons, Tcn3 and Tcn12, that mobilize under heat stress conditions (37°C). To systematically study the movement of these LTR retrotransposons in response to different environmental and host stressors, we introduced each element separately into a naïve environmental strain of *Cd* at a common locus. We confirmed heat stress-induced TE mobility for both elements in this background and determined the mutation rates into reporter genes (*URA3* and *URA5*). To better quantify stress-induced TE mobilization across the cryptococcal genome, we are pursuing a dual approach that applies our established forward-genetic screen with the concurrent development of a fluorescent TE reporter tool. Experiments are underway to validate fluorescent TE reporter constructs integrated into the *Cd* genome. Both methods will allow us to characterize additional mutations and TE mobilization events in response to environmental and host stressors critical for survival during infection (e.g. high temperature, pH changes, reactive oxygen species). Fundamentally, development of these reporter systems will enable us to quantify and assess how TE mobilization impacts *Cn* adaptation to the hostile host environment.

616B

Genome-wide CRISPRi Exploration of Plant-derived Compounds for Antifungal Potential

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Fungal pathogens pose a major threat to human health and agriculture, compounded by rising resistance to existing antifungal agents. To identify new sustainable antifungal leads, we performed a high-throughput screen of 794 diverse plant extracts against *Candida albicans*. Dozens of extracts significantly inhibited fungal growth. Notably, extracts of *Tea seens* and *Gentiana lutea* reduced *C. albicans* proliferation by over 40%, indicating potent antifungal activity.

In addition, investigated the mechanism of action of a plant-derived phenolic, ferulic acid (FA). Using a genome-wide CRISPR interference (CRISPRi) screen, we identified 194 gene knockdowns that heightened FA sensitivity and 12 that conferred resistance. Notably, repression of the squalene synthase gene ERG9 conferred marked resistance to FA, implicating the ergosterol biosynthesis pathway in FA's activity. ERG9 knockdown upregulated the HMG-CoA reductase paralogs HMG1 and HMG2, consistent with FA targeting HMG1/2 and depleting ergosterol. Deep proteomic profiling of FA-resistant *Cochliobolus heterostrophus* strains further revealed conserved upregulation of ergosterol biosynthetic enzymes.

Functionally, FA synergized with fluconazole, reducing its MIC by ~8-fold in *C. albicans* and restoring susceptibility in azole-resistant strains. In planta assays demonstrated dose-dependent inhibition of *C. heterostrophus*, significantly reducing lesion formation on maize leaves.

Together, these findings establish FA as a plant-derived antifungal that disrupts conserved sterol biosynthesis, enhances azole efficacy, and suppresses fungal disease in planta. This integrated approach, combining plant extract discovery with genome-wide CRISPRi functional genomics and deep proteomics, provides a framework for identifying eco-friendly antifungals that could expand both clinical and agricultural toolkits.

617B

Light controls gene functions through alternative splicing in fungi

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Light controls important biological processes in fungi by regulating transcriptional gene-activation. Beyond the regulation of mRNA transcript abundance, light regulates alternative splicing (AS) in the filamentous fungi *Aspergillus nidulans*, *Trichoderma guizhouense* and *Neurospora crassa*. Blue light-regulated AS was involved in ergothioneine biosynthesis and conidiation in *T. guizhouense*, which required the blue light receptor BLR1. Blue light activated the MAPK HOG (Sak) pathway which then transmitted the signal via the serine/threonine kinase SRK1 to the AS key regulator SRP1. SRK1 and SRP1 are important for light-induced conidiation. The light-activated HOG pathway led to an increase of the SRK1 protein level and its phosphorylation status. Phosphorylated SRK1 translocated from the cytoplasm to the nucleus to interact with SRP1, thereby regulating AS efficiency. This study unravels another level of complexity of fungal environmental sensing and responses and also first describes the entire cascade from an environmental signal to the splicing machinery.

618B

Response of *Candida albicans* white and opaque cells to phagocytosis by macrophages suggests that opaque cells are "pre-adapted"

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Candida albicans is a normal resident of the human gut and mucosal microbiomes but is also an opportunistic fungal pathogen. It undergoes several morphological transitions, one of which is white-opaque switching, where *C. albicans* reversibly alternates between two distinct cell types, named "white" and "opaque." Each state, which is maintained by a complex transcriptional feedback loop, is heritable through many cell divisions. To date, most research on interactions between *C. albicans* and the innate immune system has utilized white cells. Here, we examine the response of opaque cells following phagocytosis by murine macrophage cell lines and compare it to the response of white cells. White cells are known to rapidly form hyphae that can rupture macrophages, but we show that opaque cells continue to proliferate as yeast-form opaque cells within the macrophage. Before phagocytosis, white and opaque cells differ markedly in the mRNAs they express and therefore enter macrophages as two distinct types of cells. We were surprised to observe that, within macrophages, the transcriptional profiles of white and opaque cells became much more similar to each other. This convergence was driven, in part, by the up-regulation, in white cells, of a set of genes that were already expressed in opaque cells prior to macrophage exposure. These observations indicate that opaque cells, compared to white cells, are "pre-adapted" for life within host macrophages.

619B

Pmk1-dependent regulation of a Mst12–Bip1 transcriptional network governing pathogenesis by the rice blast fungus, *Magnaporthe oryzae*

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Rice blast disease, caused by the filamentous fungus *Magnaporthe oryzae*, poses a major threat to global rice production. To invade its host, *M. oryzae* undergoes tightly regulated morphogenetic transitions that enable penetration and secretes effector proteins to suppress host immunity and promote colonisation. Effective infection, therefore, depends on mechanisms that translate external cues into transcriptional programmes controlling both morphogenesis and effector deployment. The pathogenicity of the blast fungus has been linked to several interconnected signalling pathways, including the cyclic AMP–dependent protein kinase A pathway, the Pathogenicity Mitogen-Activated Protein Kinase (Pmk1) cascade, the cell wall integrity pathway, and epigenetic regulators, but the transcriptional networks that act downstream of these pathways remain poorly defined.

Our results show that Pmk1-dependent phosphorylation of the transcription factor Bip1 (B-ZIP Involved in Pathogenesis-1) is required for rice blast infection, placing Bip1 as a key effector of this conserved MAPK pathway during pathogenesis. We also identify a *BIP1* promoter element required for infection and potentially regulated by the transcription factor Mst12. Global transcriptomic analyses reveal that Bip1 and Mst12 co-regulate a common subset of genes, including multiple effector genes, and other Pmk1-dependent transcription factors during plant infection. Together, these findings support a model in which Mst12 functions as a central regulator of pathogenesis-associated gene expression, operating within a hierarchy of transcription factors that couple upstream signalling to the transcriptional reprogramming required for successful host colonisation by *M. oryzae*.

620B

Acute temperature shifts drive rapid remodeling of the morphogenetic program in *Histoplasma*

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Temperature plays a critical role in altering the developmental program and virulence of thermally dimorphic fungal pathogens, including *Histoplasma*. In the environment, *Histoplasma* grows as multicellular hyphae that produce vegetative spores. Upon inhalation by a mammalian host, hyphal fragments and spores undergo a developmental change to give rise to parasitic yeasts that secrete virulence factors and cause disease. This morphological transition can be recapitulated in the laboratory, where temperature is a sufficient signal to shift cultures of *Histoplasma* between hyphal and yeast forms, which grow at 22°C and 37°C, respectively. How *Histoplasma* links temperature to changes in its morphology and gene expression is not well understood. Here, we shift yeast cells from 37°C to temperatures ranging from 33°C to 22°C, revealing that the normal yeast-phase transcriptome is exquisitely sensitive to temperature and requires growth at 37°C for its maintenance. Expression of the majority of these transcripts is dependent upon the Ryp (Required for Yeast Phase) transcription factors, which were previously identified in our laboratory as key regulators of the morphogenetic program in *Histoplasma* and do not change in abundance over the course of these experiments. To understand if the DNA-binding activities of Ryp1, Ryp2, and Ryp3 are temperature-dependent, we performed Chromatin Immunoprecipitation followed by sequencing (ChIP-Seq) in parallel with our transcriptomic studies. We observed a global loss of Ryp association from promoters within two hours of a shift away from 37°C and hypothesize that this striking change in DNA-binding activity accounts for rapid downstream transcriptomic changes. This study provides the first mechanistic insight into how *Histoplasma* senses temperature via regulation of the activity of highly conserved transcription factors, which relay this signal to downstream pathways involved in morphogenesis and virulence.

621B

Comparative transcriptome analysis among *roc1* mutants in the white-rot fungus *Pleurotus ostreatus*

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White-rot fungi degrade all three major wood components—cellulose, hemicellulose, and lignin—by secreting numerous enzymes. To investigate the regulatory mechanisms controlling the expression of the degrading enzyme-encoding genes, our previous study isolated the mutant strain AZp1 from the monokaryotic *Pleurotus ostreatus* strain PC9 by UV mutagenesis. AZp1 exhibited greatly elevated cellulose- and xylan-degrading enzyme activities. Through linkage analysis and complementation test using genetic transformation, it was also shown that a single-nucleotide mutation in the 3′

downstream region of *roc1*, which encodes a putative Zn_2Cys_6-type transcription factor, was identified as responsible for this phenotype. However, transcriptional upregulation was previously shown for only a limited number of putative cellulose- and xylan-degrading enzyme genes using quantitative RT-PCR. Here, to comprehensively characterize transcriptional changes in AZp1, we performed RNA-seq. AZp1, the Δ*ku80* strain generated from PC9 (20b), and the Δ*roc1* strain generated from 20b were cultured on toluene/ethanol-extracted beech sawdust medium supplemented with 1.3% (v/v) wheat bran for 13 days, followed by total RNA extraction and comparative RNA-seq analysis. Among 53 carbohydrate-active enzyme gene families predicted in the genome of *P. ostreatus*, the total TPM value for each of seven AA- and GH-encoding gene families was more than two-fold higher in AZp1 than in 20b, whereas it was reduced to less than half in Δ*roc1* compared with 20b. Based on their reported catalytic functions, these seven families are predicted to encode cellulose-, xylan-, xyloglucan-, and glucomannan-degrading enzymes. These results suggest that Roc1 plays a crucial role in regulating the transcription of cellulose- and specific hemicellulose-degrading enzyme-encoding genes in *P. ostreatus*.

622C

Cys-bZIP transcription factors function as redox sensors regulating development and stress responses in the oomycete *Phytophthora infestans*

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Unlike basic leucine zipper (bZIP) transcription factors found in animals, plants, and yeast, which contain an invariant asparagine residue essential for DNA binding, nearly half of the bZIPs in the oomycete *Phytophthora infestans* have replaced this asparagine with cysteine (Cys-bZIP). While our lab identified DNA targets for Asn-bZIPs using *in vitro* binding studies, 14 out of 15 Cys-bZIP types failed to bind DNA under standard assay conditions. We hypothesize that the novel cysteine residue functions as a redox sensor, modulating DNA binding or dimerization in response to the cellular oxidation state. To understand the function of these novel Cys-bZIPs, we focused on Cys-bZIP3, Cys-bZIP5, and Cys-bZIP7, which were selected to represent the structural diversity of the family. Functional characterization of Cys-bZIP5 via hairpin RNA-mediated gene silencing highlighted its critical role in stress management and development. The Cys-bZIP5-silenced mutants exhibited a 10-fold reduction in dry weight under standard growth conditions and hypersensitivity to 1 mM H2O2 compared to the wild type. Intracellular reactive oxygen species (ROS) quantification using dichlorodihydrofluorescein diacetate (DCFH-DA) suggested that significantly elevated ROS levels existed in the mutant. A tomato leaf infection assay demonstrated that the mutant strains displayed delayed infection progression compared to wild type. To investigate how ROS might affect bZIP behavior, we used electrophoretic mobility shift assays (EMSA) to test the binding of Cys-bZIP7 to a DNA motif identified from a protein binding microarray (PBM) study. The results showed that Cys-bZIP7 binds strongly in the reduced state but weakly in the oxidized state. To elucidate the gene networks controlled by Cys-bZIP3 and Cys-bZIP5, we are currently employing chromatin immunoprecipitation sequencing (ChIP-seq) with FLAG-tagged proteins expressed in *P. infestans*. To investigate further, we are using the Systematic Evolution of Ligands by Exponential Enrichment method (SELEX) to identify the motifs bound by additional bZIPs and study the effects of oxidation. The findings will help show how Cys-bZIPs serve to regulate growth, development, and stress responses in oomycetes.

623C

Deletion of Elongator Protein 1 (Elp1) relieves heterochromatin defects in a Pol II mutant of *Schizosaccharomyces pombe*

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Heterochromatin is a repressive epigenetic state that controls developmental and environmental responses. However, the full mechanism of its regulation remains elusive. Here, we focus on a previously described RNA polymerase II (Pol II) variant called *m203* in *Schizosaccharomyces pombe,* which has a single substitution mutation within the Rpb2 subunit of Pol II (*rpb2-N44Y*) that antagonizes heterochromatin formation at a pericentromeric reporter locus. By determining how Pol II controls heterochromatin, we sought to uncover new factors and mechanisms of epigenetic regulation. We find that *rpb2-N44Y* is a gain-of-function mutation. Furthermore, the heterochromatin defects of the *m203* variant require a subunit of the Elongator complex called Elongator Protein 1 (Elp1), a protein that canonically promotes $mcm^5s^2U_{34}$ tRNA modifications and is associated with diverse stress responses. While the single knockout of Elp1 in the *m203* strain majorly restored heterochromatin formation, single knockouts of the Elp3 or the Elp5 subunits of Elongator showed only modest effects. Furthermore, mcm^5s^2 U_{34} tRNA modifications are dispensable for Elp1-dependent heterochromatin. In contrast, the heterochromatin required core factors that are critical for heterochromatin formation, including protein mediators of the RNA interference (RNAi) pathway and H3K9 methylation. Overall, our study reveals two distinct Rpb2-centric pathways, via RNAi or Elp1, that can positively or negatively regulate heterochromatin, respectively. Furthermore, our findings reveal a novel chromatin function for Elp1 that does not rely on Elongator-dependent $mcm^5s^2U_{34}$ tRNA modifications. This work expands our understanding of how Elp1 can influence chromatin biology. Indeed, we are beginning to identify differential modes of environmental response regulation by Elp1 and other distinct Elongator subunits, findings which are unexpected given the current paradigm in the field that Elongator subunits primarily regulate cell development and environmental responses as a single functional complex.

624C

The regulatory network of 186 transcription factors of a model mushroom during development and cellulose degradation

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Mushroom-forming fungi play an important role in lignocellulose degradation and their fruiting bodies are a nutritious source of food. Several transcription factors have previously been identified that regulate aspects of their lifestyle, especially mushroom development. However, little is known about their target genes or their regulatory network. Here, we used the high-throughput technique DNA Affinity Purification followed by Sequencing (DAP-Seq) to identify the binding sites and putative target genes of 186 transcription factors in the model mushroom *Schizophyllum commune*. There is considerable variation between the transcription factor families, but notably bZIP and fungal-specific Zn2Cys6 transcription factors have large numbers of binding sites in the genome. Transcription factors preferentially bind around 500 bp upstream of the translation start site of their target genes, underscoring that the promoters are compact. Consensus promoter binding motifs were identified for the majority of transcription factors. We demonstrated the strength of the DAP-Seq dataset by identifying the regulatory networks that underlie development and cellulose degradation, resulting in novel candidate regulators of these processes. Transcription factors Ftr1 and Bzt1 were functionally characterized and were indeed regulators of mushroom development. This DAP-Seq dataset is the largest of its kind for any fungus, and will be a valuable resource to study the regulation of the lifestyle of mushroom-forming fungi.

625C

AtrR Interaction Network and Its Role in Azole Resistance in *Aspergillus fumigatus*

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Aspergillus fumigatus is an ubiquitous environmental mold and a major opportunistic pathogen responsible for invasive aspergillosis, particularly in immunocompromised patients. Development of antifungal drug resistance, especially to azoles, poses a serious challenge to treatment and limits therapeutic options. AtrR, a fungal-specific Zn_2-Cys_6 transcription factor, plays a critical role in azole resistance by directly regulating the expression of *cyp51A* (encoding the azole drug target) and *abcG1*/*cdr1B* (an ABC transporter involved in drug efflux). To better understand how AtrR modulates azole resistance, protein extracts from an AtrR-TAP tagged strain were purified under native conditions followed by mass spectrometry. Several proteins co-purified with AtrR and were confirmed as interactors by co-immunoprecipitation, including chromatin assembly factors (Ngg1, Arp4), a proteasomal degradation component (FbdA), and a nuclear-localized protein of unknown function (NcaA). Single, double, and triple deletions of *ngg1*, *fbdA*, and *ncaA* resulted in progressively increased azole sensitivity. Interestingly, these mutants exhibited azole susceptibility without altering expression of known AtrR targets such as *cyp51A* or *abcG1*. To identify additional AtrR-dependent genes contributing to azole resistance, RNA-seq analysis was performed comparing wild-type and the triple deletion strain in the presence and absence of azole drug. Transcriptomic analysis revealed novel AtrR-regulated genes, including one involved in sphingolipid biosynthesis, that may play a role in azole resistance. We have also developed an inducible protein degradation system that can proteolyze fusion proteins between *A. fumigatus* genes-of-interest and green fluorescent protein (GFP). We will discuss progress in using this system to degrade GFP fusion proteins, including AtrR-GFP, in vitro and in vivo. These findings highlight AtrR as a central regulator of azole resistance and suggest that its interaction network extends beyond canonical drug resistance genes, implicating chromatin remodeling, protein turnover, and lipid metabolism in antifungal resistance mechanisms.

626C

Upregulation of a mobile carotenoid gene cluster during fungal plant infection

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Starships are a newly discovered group of transposable elements in filamentous fungi. *Starships* are unique because of their massive size (~100kb on average) and ability to mobilize dozens of cargo genes with putatively adaptive functions. Because of the ability to horizontally transfer genes between fungi, *Starships* are agents of rapid adaptation. *Starships* may carry genes that increase fungal fitness, play a role in plant host specificity, and are predicted to play a role in the virulence of plant pathogenic fungi. *Macrophomina phaseolina* is a destructive plant pathogen capable of infecting at least 97 economically important crops, with yield losses reaching 100% and damages amounting to hundreds of thousands of dollars each year. Analysis of *Starship* elements across 12 *M. phaseolina* strains revealed a correlation between *Starship* copy number and pathogenicity on soybean. However, an expanded analysis is needed to understand the role of *Starships* in plant pathogenicity across all plant hosts and geographic locations. Our research aims to map out the *Starship* landscape in *M. phaseolina* across many plant hosts and countries of origin to understand the distribution of *Starships* and their potential role in disease outbreaks. We used starfish, a *Starship* annotation pipeline, to annotate *Starships* in 465 *M. phaseolina* genomes from 91 host plants and 23 countries. We then conducted a transcriptomics analysis of a reference strain to compare *Starship* cargo expression *in planta* vs *in vitro*. This work led to the discovery of two homologous carotenoid gene clusters that are upregulated during infection of soybean, one carried on a *Starship* and one present in the core genome. Next, we will analyze the distribution of this cluster across our entire *Macrophomina* dataset and across all fungi, then we will validate the role of these genes in plant infection by reverse genetics and infection assays. Ultimately, this research seeks to predict fungal population diversity and adaptation by exploring the role of *Starship* cargo in fungal plant pathogenesis. These predictions will then inform and improve strategies for managing agricultural fungal diseases more effectively.

627C

Epigenetic control of cellulase gene expression via HDA-2/SIF-2–mediated regulation of MAK-1 phosphorylation and CLR-2 activity in *Neurospora crassa*

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Histone deacetylases (HDACs) are evolutionarily conserved epigenetic regulators that modulate chromatin architecture and gene expression. In filamentous fungi, however, their biological roles remain only partially understood beyond circadian regulation and oxidative-stress responses. In this study, we dissected the molecular function of the class-I HDAC HDA-2 (NCU02795) in the model cellulolytic fungus *Neurospora crassa* (*N. crassa*). Deletion of *hda-2* or its binding partner *sif-2*, as well as introduction of a catalytically inactive HDA-2 allele, equally impaired radial growth, biomass accumulation and lignocellulase production, demonstrating that HDA-2 required both its deacetylase activity and complex formation with SIF-2 to support normal development and cellulase induction. Transcriptomic profiling showed a pronounced decrease in the expression of the principal cellulase genes and their master activator *clr-2*. Consistently, ChIP–qPCR revealed that loss of HDA-2 markedly reduces CLR-2 occupancy at the *cbh-1* promoter. Constitutive expression of *clr-2* gene could not restore the defect of cellulases production. Cellulases gene expression could only be restored when CLR-2 overexpression occurred in conjunction with the introduction of phosphorylated model MAK-1. These data indicated that HDA-2 not only affected the transcript level of CLR-2 but also, via modulation of the phosphorylation status of MAK-1 mitogen-activated protein kinase, influenced the binding of CLR-2 to the promoters of its downstream cellulase-genes. Our findings establish an HDAC-mediated regulatory node that synchronises transcriptional priming with MAPK-dependent chromatin targeting of the regulation of cellulases expression, offering a conceptual framework for engineering hyper-cellulolytic filamentous fungi.

628C

Telomere anchoring to the nuclear envelope through Lem2 is required for *Ustilago maydis* pathogenicity

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The transcriptionally silent heterochromatin is primarily situated at the nuclear periphery, predominantly tethered by lamina-associated proteins (LAPs). This peripheral location is crucial for complete gene silencing and has important roles in cell development and fate. However, its impact on fungal pathogenesis remains largely unexplored. Our research focused on Lem2, the LAP homolog in yeast known to anchor and silence centromeres and telomeres at the nuclear periphery, to investigate its role in the pathogenicity of *Ustilago maydis*. The findings clearly establish that Lem2 is essential for virulence, particularly for penetrating host tissues. Most of the *Δlem2* mutant filaments become arrested at the appressorium-mediated plant penetration stage. We determined that the *Δlem2* strain exhibits impaired nuclear migration and detachment of telomeres from the nuclear periphery. Intriguingly, when we artificially re-anchor telomeres to the nuclear envelope in the *Δlem2* mutant, nuclear migration defects are partially rescued. This suggests that telomere anchoring to the nuclear envelope is crucial for the nucleus to migrate from the appressorium into the invading filament. To investigate why telomere release impedes nuclear migration, we conducted a transcriptomic analysis during the filamentation process. The *Δlem2* mutant displayed elevated expression of G1–S specific genes and several kinases associated with cell cycle checkpoints, which could explain the nuclear migration defects. Overall, this study not only significantly advances our comprehension of fungal pathogenicity but also highlights new opportunities for exploring how the spatial organization of chromatin can dictate pathogen behavior.

629C

Transcriptome analysis in response to beech wood sawdust to identify a new regulator for lignocellulose-degrading enzyme-encoding genes in the white-rot fungus *Pleurotus ostreatus*

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The regulatory mechanisms controlling the expression of lignocellulose-degrading enzymes in white-rot fungi remain largely unknown. To investigate these mechanisms, we performed RNA-seq to characterize transcriptional response to lignocellulose in the dikaryotic *Pleurotus ostreatus* 20b×PC15, followed by functional analyses of differentially expressed putative DNA-binding transcription factor (TF)-encoding genes. Comparative RNA-seq was performed on samples collected 0.5 and 48 hours after transfer from BIII agar plates (Kirk *et al*. 1986 *Enzyme Microbial. Technol.*) onto toluene/ethanol-extracted beech wood sawdust (BWS). Among 55 analyzed AA- and GH-encoding gene families, the total TPM value for each of the 15 families increased more than 10-fold at 48 hours. Based on reported catalytic functions, ten of these upregulated families are predicted to participate in cellulose and hemicellulose degradation. RNA-seq was also performed on the ligninolysis-deficient Δ*gat1*×PC15 strain: *gat1* encodes a GATA-type DNA-binding TF. It was shown that the total TPM values for *vp*/*mnp*s were markedly lower in Δ*gat1*×PC15 than in 20b×PC15. From these transcriptome datasets, we identified two putative TF genes as candidates for regulators of lignocellulose-degrading enzyme-encoding genes, as both were upregulated approximately five-fold at 48 hours in 20b×PC15, but not in Δ*gat1*×PC15. For functional analysis, dikaryotic single-gene disruptants of these TF genes were generated in the PC9×PC15 background using plasmid-based CRISPR/Cas9. Each gene disruptant and the parental PC9×PC15 were grown on BWS, and extracellular enzyme activities were compared at 10 and 20 days. Disruptants of one TF gene exhibited significantly reduced cellulose-degrading activities at 10 days and xylan-degrading activities at 20 days when AZCL-HE-Cellulose and Xylazyme AX were used as substrates, respectively. In addition, Mn^{2+}-dependent peroxidase activity toward 2-methoxyphenol in the disruptants was significantly lower at 10 days but increased at 20 days compared with PC9×PC15. These results identify a previously uncharacterized TF that may contribute to the transcriptional regulation of lignocellulose-degrading enzyme-encoding genes.

630C

Investigating Uniparental Inheritance of Mitochondrial DNA During Sexual Reproduction in *Cryptococcus neoformans*

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Mitochondrial DNA uniparental inheritance (mtUPI) is prevalent in eukaryotic species, but the mechanism remains unclear. In anisogamic species like mammals where the size of the two gametes - egg and sperm - differ drastically, uniparental inheritance is thought to be largely achieved by passive dilution of mitochondria DNA. However, mtUPI is also widespread in the realm of fungi despite the fact that both gametes are of a similar cell size, which is likely the ancestral state of eukaryotic species. This indicates additional mechanisms are necessary to ensure strict mtUPI. Unfortunately, the meiotic progeny of the two model yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* can inherit mtDNA from either parent, rendering them ineffective for studying mtUPI. Here we propose to study mtUPI in *Cryptococcus neoformans*. Previous studies showed that mtUPI is established early in the process of bisexual mating, likely soon after zygote formation. Several hypotheses may explain the mechanism underlying this phenomenon: (1) α mitochondria are excluded from entering the zygote or the resulting dikaryotic hyphae due to an uneven mix of organelles; (2) α mitochondria are selectively degraded; (3) α mtDNA is selectively degraded; or (4) a combination of the above mechanisms contributes to strict UPI. **My goal is to test these hypotheses to better understand the mechanism underlying mitochondria uniparental inheritance during sexual reproduction in *C. neoformans***. My preliminary data using both genetics and cell biological approaches indicate that α mitochondria can enter the zygote. Thus, selective degradation of either α mitochondria or α mtDNA is likely the major factor controlling mtUPI.

631C

Ssb1 chaperone orchestrates stress-induced translatome reprogramming and virulence regulation in *Cryptococcus neoformans*

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Cryptococcus neoformans, an omnipresent opportunistic basidiomycete ranked first on the WHO fungal priority pathogens list with ability to adapt to host conditions, such as elevated temperatures, oxidative stress nutrient limitations and the immune response. Ssb1, a cytosolic, ribosome-associated Hsp70

family chaperone, facilitates co-translational protein folding and prevents nascent chain misfolding, predominantly under stress conditions. Ssb1 has been functionally characterized in model fungal species, however, its role in translatome reprogramming in *C. neoformans* remains unexplored. In this study, we investigated the role of Ssb1 in translational reprogramming under thermal and oxidative stress in *C. neoformans*. Analysis of translational output in the *ssb1*Δ mutant revealed a decrease in overall protein synthesis under all the conditions. Polysome profiling supports overall reduced translational capacity in the absence of Ssb1, with half-mer shoulders detected on polysome peaks, consistent with a ribosome biogenesis defect. Dissociation of all ribosomes by EDTA treatment revealed a decrease in the abundance of 60 S subunits relative to 40 S in the *ssb1*Δ mutant relative to the wild type. The *ssb1*Δ mutant exhibited a significant growth defect and heightened sensitivity to thermal and oxidative stress compared to the wild type. In the absence of Ssb1, the activation of Gcn2 and downstream induction of the ISR in response to oxidative stress was defective. Complementation of the Ssb1 reestablished the stress tolerance phenotype, confirmed the specificity of the observed defects. Our findings also demonstrate that Ssb1 deletion significantly reduces urease activity and capsule induction, while melanin synthesis remains unaffected in the *ssb1Δ* strain under normal growth conditions, and the mutant also exhibits avirulence in *Galleria mellonella* larvae. This study collectively establish that Ssb1 is important for overall translational capacity in *C. neoformans*¸ and loss of Ssb1 results in exacerbated stress sensitivity. Future work will investigate the specific mRNAs that exhibit altered translation in the absence of Ssb1.

632C

tRNA-m1A methylation supports hyphal growth of Neurospora crassa through controlling cellular energy metabolism

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Fungi hyphal growth requires a sustained energy supply to support its high biosynthetic demands. However, the role of tRNA modification in regulating hyphal growth remains unclear. Here, we show that the Trm6/Trm61 complex catalyzes dynamic N1-methyladenosine at position 58 (m1A58) in 42 transfer RNAs (tRNAs) of Neurospora crassa, significantly influencing tRNA stability and protein translation. The absence of this modification significantly reduces the hyphal growth rate. Notably, deletion of Trm61 specifically reduced the stability of iMet-tRNA, while overexpression of this tRNA partially rescued the hyphal growth defect. Multi-omics analysis revealed that m¹A58 deficiency significantly reduced the translation efficiency of multiple genes involved in glycolysis and oxidative phosphorylation, leading to dysregulation of overall energy metabolism and consequently inhibiting hyphal growth. Furthermore, overexpression of iMet-tRNA in the trm61 deletion mutant effectively enhanced the expression of energy metabolism-related genes. Our findings demonstrate the tRNA m¹A58 modification governs hyphal growth by modulating cellular energy metabolism through tRNAiMet stability, coupling translational capacity to the biosynthetic demands of hyphal growth.

633C

From Energy Sensing to Epigenetic Activation: The AMPK/TrSnf1-ACE3-MST2 Pathway Orchestrates Cellulase Synthesis in *Trichoderma reesei*

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Fungal gene expression often relies on energy-sensing kinase Snf1 and histone acetyltransferase, particularly for highly expressed or rapidly modulated genes such as those that encode cellulases. *Trichoderma reesei* is the most prominent industrial cellulase producer, but most studies on Snf1 function focus on yeast. In this study, we aimed to determine the mechanisms underlying the rapid response of the Snf1 ortholog in *T. reesei* (TrSnf1) to carbon source depletion and subsequent activation of cellulase gene transcription. TrSnf1 senses glucose depletion, relieves carbon catabolite repression (CCR), and ultimately triggers cellulase gene expression. Multi-omics analysis identified the histone acetyltransferase MST2 and highlighted the regulatory role played by its interaction with TrSnf1 in cellulase expression. MST2 deletion impairs cellulase gene expression, suggesting that the interaction with MST2 is critical for its regulatory function. MST2 is involved in histone H3 acetylation and RNA polymerase binding to cellulase gene promoters. We propose that the TrSnf1-MST2-ACE3 regulatory pathway orchestrates cellulase transcriptional activation. This study targets a critical gap in the CCR regulatory network of *T. reesei*—the persistent CCR observed in CRE1-knockout strains. Our work seeks to uncover a novel CRE1-independent CCR signaling pathway and provides a theoretical foundation for developing high-yield cellulase strains through genetic engineering.

634C

QTL Mapping to identify the mechanisms of extreme CO_2 tolerance in *Cryptococcus neoformans*

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Cryptococcus neoformans is a ubiquitous free-living soil yeast and opportunistic pathogen that kills more than one hundred thousand people each year. The pathogenicity of *C. neoformans* relies on its adaptation to the host conditions. An important difference between its natural environment and the mammalian host is the concentration of CO_2. CO_2 levels in the host fluctuate around 5%, which is ~125-fold higher than in ambient air. We previously established that CO_2 tolerance is a key cryptococcal virulence trait. To identify the molecular basis for CO_2 tolerance, we selected two clinical isolates that are extremely tolerant to host levels of CO_2 for further investigation. Here, we will utilize quantitative trait loci (QTL) mapping with progeny from a cross between these two CO_2-tolerant clinical isolates and a CO_2-sensitive environmental isolate to identify genetic regions and genes regulating CO_2 tolerance. We expect to identify unique and some shared pathways to achieve CO_2 tolerance in these two clinical isolates. We plan to verify those mechanisms by genetic manipulation of these pathways in originally CO_2-sensitive strains. We hope to demonstrate some common pathways that this fungus can employ to adapt to high CO_2.

635C

Assessing impact of temperature on growth and transcriptional codon usage bias across Saccharomycotina yeasts

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Thermotolerance varies broadly across Saccharomycotina yeasts, with instances of pathogenesis being linked to the acquisition of high thermal tolerance in response to increasing environmental temperatures (e.g., Candidozyma auris). Understanding how species evolve resistance to higher temperatures is essential for combating novel fungal pathogens. Existing research methodologies are often limited to single pathogens and rely on simplistic measures such as binary growth on solid media at a single temperature in order to quantify thermal tolerance. Here, we utilize an emerging method to grow yeasts at gradient temperatures within a thermocycler and quantify cell survival to better understand thermal growth phenotypes within Saccharomycotina and identify the impact of environmental temperature on transcriptional codon usage. Preliminary data suggest strains demonstrate different growth rates according to the availability of oxygen at high temperatures, challenging existing understanding of thermal growth capabilities.

636C

Regulation of the mannanase genes by multiple transcription factors in *Aspergillus nidulans*

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Aspergillus species produce cellulolytic and mannanolytic enzymes, which are utilized in a wide range of industrial fields. Generally, orthologs of a single specific transcription factor ClrB (e.g., ManR in *A. oryzae*, ClrB in *A. niger*) regulate the expression of both enzymes. However, *A. nidulans* possesses ManS, a paralog of ClrB. While ClrB primarily regulates cellulase genes and is also involved in regulating some mannanase genes, ManS significantly affects the expression of all mannanase genes. To elucidate the regulatory mechanisms of mannanase genes by ManS and ClrB, we have been analyzing the DNA recognition by these paralogous transcription factors. In the previous conference, we reported the results of DNA binding analysis by EMSA for the transcription factors in *manE*, regulated solely by ManS, and *manB*, regulated by both ManS and ClrB. We proposed that the $CGGN_{16}CCG$ sequence within the *manE* and *manB* promoters constitutes the ManS binding site, while the CCG/CGG-rich region within the *manB* promoter enables simultaneous binding by ManS and ClrB. In this study, the activity of the identified motifs was assessed by a reporter assay. Furthermore, we investigated whether McmA, another cellulase gene regulator that acts cooperatively with ClrB, is involved in the expression of *manB*.

In the reporter assay, when galactomannan was used as an inducer, the approximately 250-bp DNA fragments containing the $CGGN_{16}CCG$ sequence derived from the *manB* and *manE* promoters exhibited high promoter activity. For *manE*, this galactomannan-responsive expression was lost in the *manS*-deficient strain. This suggests that $CGGN_{16}CCG$ is a *cis*-element involved in ManS-dependent expression. For *manB*, the 250-bp also contained the CCG/CGG-rich region alongside $CGGN_{16}CCG$. Currently, we are analyzing the activity of each motif by introducing specific mutations.

Previous EMSA studies have demonstrated that His-$ManS_{164}$ and FLAG-$ClrB_{118}$ bind as a heterocomplex to the CCG-rich region within the *manB* promoter. This region contains cellulose responsive element (CeRE, $CCGN_2CCN_6GG$), a *cis*-element in the cellulase gene promoters, where ClrB and McmA bind cooperatively. Hence, we analyzed the binding of FLAG-$ClrB_{118}$ and His-McmA to the CCG-rich region by EMSA. The results showed that the ClrB-McmA complex bound more strongly than the ManS-ClrB complex. This result suggests that, similar to the cellulase genes, binding of the ClrB-McmA complex to CeRE may regulate the *manB* expression.

637C

Pseudouridine modification regulates development, azole resistance and genome stability in *Aspergillus fumigatus*

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Aspergillus fumigatus is a major opportunistic fungal pathogen that poses a growing global health threat due to the expanding population of immunocompromised individuals and the increasing prevalence of antifungal resistance. Understanding the molecular mechanisms underlying fungal development and drug resistance is crucial for developing effective control strategies. RNA modifications have recently emerged as important epigenetic regulatory mechanisms in eukaryotes, yet their roles in pathogenic fungi remain poorly understood. Here, we identify RNA pseudouridylation as a previously unrecognized regulatory layer governing fungal growth, differentiation, and antifungal resistance. Systematic functional analyses of pseudouridine synthases revealed their essential contributions to hyphal development, sporulation, and genome stability. Perturbation of pseudouridylation altered global translation efficiency, as demonstrated by polysome profiling, and induced adaptive responses associated with enhanced antifungal resistance. These findings indicate that RNA pseudouridylation modulates translational homeostasis to fine-tune fungal stress adaptation. Collectively, our study uncovers a fundamental role of RNA modification in fungal physiology and drug resistance, providing new mechanistic insights into antifungal tolerance and potential directions for therapeutic intervention.

638A

Laboratory Evolution of a Non-Model Yeast for Citizen Science and Education

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Short generation times and large population sizes make microbial species ideal tools for providing real-time evolutionary experiences for students. The emerging potential of the yeast *Metschnikowia pulcherrima* as a sustainable source of a palm-oil substitute also presents it as an interesting candidate species for student-led investigation.

To support research into the genetic basis of improved acid tolerance in this species, an adaptive laboratory evolution protocol was adapted for use within a citizen-science model involving UK A Level Biology students (aged 16–17). The primary aim of the project was to evolve novel *M. pulcherrima* strains with increased tolerance to acetic and formic acids using non-GM approaches, and to determine the genetic basis of any resulting changes. The impact of participation on student learning, skills development and future aspirations was also assessed.

Across two years and five participating schools, students generated 147 novel strains. These were subjected to whole-genome sequencing and phenotypic characterisation. Genomic data were used to investigate the genetic basis of the observed phenotypes in this genetically under-characterised but industrially significant species. Analysis focused on SNPs and copy-number variation, with the large number of strains generated through the citizen-science model enabling identification of patterns in evolutionary responses to acidic environments. Additionally, as the protocol derived strains had evolved against either acetic acid only, formic acid only, or a combination of the two, genotypic and phenotypic data was used to consider the emergence of specialist vs generalist tolerance pathways in response to small organic acid stress. Additionally, as the protocol derived strains had evolved against either acetic acid only, formic acid only, or a combination of the two, genotypic and phenotypic data was used to consider the emergence of specialist vs generalist tolerance pathways in response to small organic acid stress.

Overall, this project demonstrates the potential of citizen science as a tool for evolutionary genetics research, offering a novel approach to improving understanding of non-model microbial species with industrial relevance, while providing clear benefits for participating students and researchers.

639A

Characterization of Colletotrichum isolates from symptomatic dry bean fields in North Dakota and western Minnesota

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Bean Anthracnose, traditionally caused by the fungus Colletotrichum lindemuthianum (Sacc. & Magnus), is an economically important disease in common bean. Anthracnose symptoms on dry bean plants include dark and slender leaf veins and petiole lesions, as well as small brown spots on stems, pods, and petioles that may coalesce and become circular and sunken. During the 2025 growing season, we surveyed dry bean fields in North Dakota and Westen Minnesota for plants that displayed Anthracnose-like symptoms. Symptomatic leaves and stems were surface sterilized and cultured on PDA media containing streptomycin (100 mg/L) and ampicillin (100 mg/L) antibiotics for 5-7 days. Fungal isolates that displayed morphological characteristics of Colletotrichum spp., were single spore purified, and maintained in culture. We are currently extracting DNA from all Colletotrichum isolates and will use primers to amplify actin and β-tubulin to characterize all Colletotrichum isolates to a species level. Furthermore, we are carrying out greenhouse inoculation trials to assess the virulence potential of all isolates. Eventually, in this study we are aiming to use comparative genomics to identify effectors of Colletotrichum spp. that are involved in pathogenicity.

640A

Finding genes essential for bacterial-fungal symbiosis using transposon insertion sequencing

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Bacterial endosymbionts are found in and impact phenotypes of diverse fungal hosts. *Luteibacter mycovicinus* is one such endosymbiont residing within foliar endophytic ascomycete *Pestalotiopsis* sp. strain 9143. This bacterium increases phytohormone production by the fungal host, increasing seedling growth and improving colonization as an endophyte. The facultative nature of this relationship allows for independent microbial culturing, bacterial genetic modulation, and in vitro reassociation, offering a tractable system. Dual transcriptomic studies on these partners have reported differential expression of bacterial secretion systems, sugar transport, and amino acid exchange, suggesting cross talk between the organisms. Further, the endosymbiont likely undergoes shifts in motility and chemotaxis when in coculture with the host. However, RNA sequencing offers limited information in fully correlating gene presence with bacterial phenotypes. Hence, we used a barcoded transposon-insertion gene knockout *L. mycovicinus* library and reassociated it with *Pestalotiopsis* sp. to identify genes contributing to symbiosis establishment. With each strain having a single gene knocked out, we profiled mutant population fitness at representative timepoints in coculture. Changes in barcode frequencies indicate the essentiality of a gene in symbiosis, uncovering specific genes and pathways enabling colonization by the bacterium. Findings from this facultative endosymbiosis can be leveraged to identify other free-living bacteria as putative endohyphal symbionts, opening up avenues for endofungal bacteria in disease prevention, bioremediation, and aspects of fungal manipulation.

641A

Unravelling the Molecular Mechanisms of Bacterial–Fungal Endosymbiosis

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Fungal-bacterial interactions (BFIs) are critically important in agriculture, biotechnology, and medicine. An important feature of BFIs is the ability of bacteria to control and exploit their eukaryotic hosts. The association of the pathogenic fungus *Rhizopus microsporus* with the endosymbiont *Mycetohabitans rhizoxinica* exemplifies a remarkable example of bacteria influencing their host's physiology. We have identified various strategies and effectors used by endosymbiotic bacteria to control their fungal host. For example, we show that a transcriptional activator-like effector (TALE) released by endofungal bacteria is an essential symbiosis factor. By combining microfluidics with fluorescence microscopy, we observed enrichment of TALE-deficient mutants in lateral hyphae. High-resolution live imaging showed formation of septa at the base of infected hyphae, leading to compartmental inclusion of endobacteria. The trapped symbionts show reduced intracellular survival, suggesting a protective host response. Beyond revealing an unprecedented function of TALE proteins, this mechanism of host control illustrates an unusual survival strategy of endosymbionts in the host and provides deeper insights into the dynamic interactions between bacteria and eukaryotes.

642A

The sealing domain of tRNA ligase (Trl1) is a potential antifungal drug target

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Some eukaryotic pre-tRNAs contain an intron that is removed by a dedicated set of enzymes. Intron-containing pre-tRNAs are cleaved by tRNA splicing endonuclease (TSEN), followed by ligation of the two exons and release of the intron. Fungi and Trypanosomes use a “heal and seal” pathway that requires three distinct catalytic domains of the tRNA ligase enzyme, Trl1. In contrast, humans use a “direct ligation” pathway carried out by RTCB, an enzyme completely unrelated to Trl1. Because of these mechanistic differences, Trl1 has been proposed as a promising drug target for fungal infections.

Here, we systematically tested whether Trl1 meets the key criteria for a viable antifungal drug target candidate. To validate Trl1 as a broad-spectrum drug target, we showed that fungi from three different phyla contain Trl1 orthologs with all three catalytic domains. This includes the major invasive human fungal pathogens, and these proteins can each functionally replace yeast Trl1. In contrast, species from the order Mucorales, including the pathogens *Rhizopus arrhizus* and *Mucor circinelloides*, have an atypical Trl1 that contains the sealing domain but lacks both healing domains. These sealing-only Trl1 orthologs can functionally complement defects in the corresponding domain of yeast Trl1 and appear to be essential for viability in *Mucor circinelloides*. Thus, Mucorales use a sealing-only enzyme together with unidentified non-orthologous healing enzymes for their heal and seal pathway. Further functional investigation of the individual domains of Trl1 in pathogenic fungal species revealed that the ligase domain is the optimal target for antifungal development. In contrast, we demonstrated that all three catalytic domains of Trl1 in the human pathogenic Trypanosome are essential and can be exploited for the development of anti-trypanosomal drugs. To enable the discovery of Trl1 inhibitors, we designed a cell-based assay system that can be used in a high-throughput setting to identify compounds that specifically inhibit fungal Trl1. This implies that drugs that target the sealing activity are more likely to be broader-spectrum antifungals than drugs that target the healing domains.

643A

Exploring biocontrol potential of a bacterial strain against Fusarium root rot in pulses through transcriptomic approaches

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Pulse crops are annual legume crops with dry edible seeds that have high protein and fiber content. One of the biggest challenges in pulse crop production across all growing regions in the United States is root rot. This disease is caused by a pathogen complex that includes *Fusarium* spp., *Rhizoctonia solani*, *Aphanomyces euteiches*, and *Pythium* spp., with Fusarium being the predominant genus. We have identified an *Achromobacter* bacterial strain that suppresses sporulation of two Fusarium spp. and Colletotrichum incanum. Our results show that *Fusarium oxysporum*, *Fusarium redolens*, and *C. incanum* produce significantly fewer spores when grown in presence of the bacterium compared to *Fusarium* spp. and *C. incanum* grown in the absence of the bacterium. Furthermore, in assays where *Achromobacter* strain and *F. oxysporum* were physically separated but gas exchange was possible, the presence of the bacterium reduced sporulation of the fungal isolates significantly, hinting towards a volatile nature of the inhibiting compound. We will conduct RNA sequencing analyses on both the bacterial and fungal interactor to identify genes involved in this specific interaction. Overall, this research aims to identify the bacterial compound that is responsible for the inhibition of sporulation in multiple fungal species and determine its underlying mode-of action. This line of research can potentially identify environmentally friendly alternatives for managing fungal diseases in pulse crops.

644A

Phytoalexins as host cues: convergent evolution of detoxification mechanisms in polyxenous pathogens *Botrytis cinerea* and *Colletotrichum tropicale*

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Throughout evolution, plants have acquired chemically diverse antimicrobial phytoalexins that limit the host range of fungal pathogens. In contrast, some fungi exhibit remarkably broad host ranges, such as *Botrytis cinerea*, which can infect more than 1,600 plant species, a trait referred to as polyxeny. However, how such polyxenous pathogens overcome diverse phytoalexins remains unclear. In this study, we investigated the mechanisms by which *B. cinerea* and the distantly related polyxenous fungus *Colletotrichum tropicale* cope with and detoxify host phytoalexins. Both fungi showed high tolerance to various phytoalexins, such as the sesquiterpenoids capsidiol (chili pepper) and rishitin (potato), the triterpenoid tormentic acid (strawberry), and the phenylpropanoid methyl trans-p-coumarate (cucumber). Each compound was metabolized into less toxic derivatives in both fungi, suggesting that their resistance is associated with active detoxification. RNA-seq analysis of both fungi after treatment with the four phytoalexins revealed that each compound induced a distinct set of genes. Notably, capsidiol induced specific ADH (alcohol dehydrogenase)-like genes in both species. Functional analyses showed that these ADHs are essential for the dehydration-mediated detoxification of capsidiol to the less toxic capsenone and for pathogenicity on capsidiol-producing hosts. Despite catalyzing the same detoxification reaction, phylogenetic analysis revealed that the ADHs in *B. cinerea* and *C. tropicale* are not orthologous but originated from completely different evolutionary lineages, supporting convergent evolution toward a common functional solution. This suggests that capsidiol can act as a strong selective pressure on fungal pathogens. Furthermore, the timing of the acquisition or lineage-specific expansion of capsidiol-detoxifying ADH in the *Colletotrichum* lineage is consistent with the emergence of capsidiol biosynthesis in Solanaceous plants. Taken together, these findings suggest that independently evolved polyxenous pathogens may have convergently established an infection strategy in which phytoalexins are perceived as host-derived chemical signals that in turn activate distinct gene sets in a host-dependent manner.

645A

Comparative phosphoproteomics identifies a novel group of Pmk1-regulated effectors deployed by the rice blast fungus *Magnaporthe oryzae* during plant infection

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The rice blast fungus *Magnaporthe oryzae* secretes a battery of effector proteins to manipulate host defence and colonise its host. The pathogenicity MAP kinase Pmk1 is a master regulator of *M. oryzae* infection-related development, controlling appressorium formation and host tissue colonisation. However, most of its downstream signalling components remain unknown. Here, we employed a comparative phosphoproteomics approach to investigate plant infection by the blast fungus. This analysis identified 62 Pmk1-dependent phosphorylated proteins associated with cytoskeleton remodelling, vesicular trafficking, protein synthesis, cell-cycle control, and a group of seven phosphorylated putative effector proteins. Transcriptional profiling of *M. oryzae* plant infection revealed that the expression of these seven putative effectors is dependent on Pmk1 activity. These Pmk1-Regulated Effectors (PREs) are specifically expressed between 24-48h post-inoculation, when the fungus rapidly colonises rice tissue. Genomic analyses revealed that *PRE* genes are highly conserved across 163 *M. oryzae* genomes, including numerous host-specialised lineages, and their identified phosphoserine residues are similarly conserved. Subcellular localisation of mCherry-tagged PREs showed that Pre1, Pre3, Pre5, and Pre7 preferentially accumulate at the Biotrophic Interfacial Complex (BIC) during invasive growth, indicating that they are cytoplasmic effectors, whereas Pre2, Pre4, and Pre6 localise to both the BIC and the host nucleus, indicating that they are nuclear effectors. Targeted gene deletion of individual *PRE* genes revealed that *PRE4*, which encodes a nuclear effector, is important for full virulence, while Δ*pre1* mutant strains exhibited a reduced virulence fitness coefficient compared to isogenic wild-type isolate. Immunoprecipitation-mass spectrometry of YFP-tagged PREs transiently expressed in *Nicotiana benthamiana* revealed that PREs are phosphorylated *in planta* at the same phosphosites identified during rice blast infection. Taken together, we demonstrate a novel approach to identify and characterise effectors deployed by *M. oryzae* during plant infection and show that PREs operate as part of a developmental programme governed by the Pmk1 MAPK. Furthermore, we provide the first evidence that fungal effectors can be phosphorylated within host cells.

646A

Splash Dispersal of *Colletotrichum* Conidia on Host and Nonhost Leaf Architectures

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For splash-dispersed *Colletotrichum* species, the outcome of a splash event depends on how conidia are redistributed over plant surfaces and which of those spores remain attached. To study these processes in a controlled way, this work combines 3D-printed splash hardware with imaging on glass and resin leaf replicas that reproduce leaf structure. Droplets containing conidia are splashed onto these surfaces, attached spores are mapped by fluorescence imaging, and changes in conidial concentration are used to estimate attachment efficiency under different conditions. The assay is applied to *Colletotrichum* species with distinct conidial morphologies and previously characterized patterns of surface adhesive, providing spatial distributions and overall attachment efficiencies that can be compared across species. Because the printed parts and resin leaves are inexpensive and easily reconfigured, the assay can be adjusted to test different surface geometries and splash regimes without major redesign. Resin leaf replicas can be cast from both host and non-host leaves with distinct vein density, trichome abundance, and surface texture, allowing attachment to be compared across defined plant surface architectures. Because identical replicas can be reused across experiments, differences in splash-driven attachment can be attributed to spore traits and leaf type rather than plant-to-plant variation. These quantitative attachment patterns offer a way to move beyond purely incidence-based descriptions of splash-dispersed epidemics, supplying experimentally grounded inputs for future models that link leaf-scale interactions to field-scale disease dynamics. The approach is readily adaptable to include reporters or stains that track adhesive material, membrane organization, or other features relevant to early surface interactions. Together, this framework allows systematic comparison of how species-specific spore traits and surface properties influence splash-driven attachment in plant-pathogenic fungi.

647A

The polyketide synthase, Ctb1, is required for cercosporin biosynthesisbut not virulence in the maize pathogen *Cercospora zeae-maydis*

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The genus *Cercospora* is one of the largest and most diverse assemblages of fungal foliar pathogens. Many *Cercospora* species are known to produce the polyketide-derived, secondary metabolite cercosporin. Cercosporin is a photoactivated perylenequinone, which absorbs light energy and generates reactive oxygen species. Recent work has elucidated the gene cluster required for cercosporin biosynthesis and demonstrated a role for cercosporin in virulence in multiple *Cercospora* species. However, it is not clear if cercosporin represents a virulence factor in all species of *Cercospora*. Considered one of the most important foliar pathogens of maize worldwide, *C. zeae-maydis* causes gray leaf spot of maize and is known to produce cercosporin. In this study, we identified an isolate of *C. zeae-maydis* that failed to produce cercosporin underfavorable culture conditions yet was fully pathogenic on maize leaves. Thus, to clarify the involvement of cercosporin in grey leaf spot of maize, an ortholog of *CTB1* (a gene encoding a polyketide synthase that catalyzes formation of a key cercosporin precursor in *C. nicotianae*) was identified and disrupted via homologous recombination in *C. zeae-maydis*. Disruption of *CTB1* eliminated cercosporin production but did not affect fungal growth and development *in vitro*. Interestingly, pre-infectious development, including germination on the leaf surface, appressorium formation, and number of appressoria per germling, in the CTB1 disruption strains was indistinguishable from the wild-type strain. Additionally, the CTB1 disruption strains were able to form lesions and sporulate on maize leaves. Taken together, these findings suggest that cercosporin may be a component of a suite of as-yet unidentified virulence factors produced by *C. zeae-maydis* during infection of maize but, alone, is dispensable for pathogenesis.

648A

Identification and characterization of *Cercospora beticola* effector candidates associated with adaptation to the sugarbeet resistance gene *BvCr4*

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Cercospora beticola, the causal organism of Cercospora leaf spot (CLS), is the most economically important disease of sugarbeet (*Beta vulgaris* subsp. *vulgaris*). Growers have recently utilized varieties that contain *BvCr4* that mediates CLS resistance. However, resistance breaking *C. beticola* populations have been found worldwide. A European study identified the gene, *AvrCr4*, associated with overcoming *BvCr4*-mediated resistance, but this association has not been examined in American populations. We conducted a population genomic analysis with *C. beticola* collected from American regions with and without *BvCr4* and identified 14 fungal genes putatively associated with breaking *BvCr4*, including *AvrCr4*. Functional validation of candidate genes indicated that *C. beticola* mutants lacking *AvrCr4* are more virulent on plants with *BvCr4* than the progenitor isolate. To explore how *C. beticola* populations are rapidly evolving to circumvent *BvCr4*-mediated resistance, we investigated how a single fungal isolate behaves under strict selective pressure. We conducted a passage experiment by inoculating a single *C. beticola* strain harboring *AvrCr4* onto three sets of 1500 sugarbeet plants. Whole genome sequencing showed that the locus containing *AvrCr4* is missing from later generations of isolates. The mechanism responsible for removing the locus is under investigation. Overall, this research aims to elucidate the interaction between *C. beticola* and the CLS resistance gene *BvCR4* to improve understanding of the *BvCr4* resistance phenotype and predict durability of similar resistance genes.

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A single laccase is essential for cell wall construction and environmental sensing in *Sclerotinia sclerotiorum*

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Laccases are a broadly conserved class of multicopper oxidases that play a key role in mediating plant-fungal interactions. In Ascomycetes, laccases are involved in pigmentation, cell wall melanin deposition, and virulence. Gene knockouts of a *Sclerotinia sclerotiorum* laccase (*Sslac2*) demonstrated a number of aberrant phenotypes that suggest altered environmental sensing capabilities. *ΔSslac2* mutants grown in culture were unable to produce sclerotia. Appressorial formation and oxalic acid production, both key virulence determinants in *S. sclerotiorum*, were markedly reduced in *ΔSslac2*. Transcriptomic analysis indicated that, relative to the wild type, *ΔSslac2* was less responsive to host plant cues. Additionally, *ΔSslac2* demonstrated increased susceptibility to antifungal compounds and cell wall stressors, decreased responsiveness to cell wall degrading enzymes, and drastically decreased hyphal hydrophobicity. Transmission electron microscopy revealed a clear difference in the extracellular matrix of *ΔSslac2* as compared to the wild type. At the time of abstract submission, we are using electron tomography to generate 3D images of the cell wall and extracellular matrix at a higher resolution. The observed phenotypes led us to suspect that variations in *ΔSslac2* cell wall architecture contributed to the altered environmental sensing. Cell wall composition analysis revealed that the *ΔSslac2* cell wall contained a lower proportion of glucose and higher proportions of mannose and N-acetyl glucosamine; it also identified variation in the sugar linkages which suggests that glucan is more highly branched in the wild type cell wall than in *ΔSslac2*. This is further supported by 1D H^1 NMR analysis, in which *ΔSslac2* is missing three peaks present in the wild type, including a key anomeric proton. In characterizing the laccase *Sslac2* from the broad host-range pathogen *S. sclerotiorum*, we have found evidence supporting a novel role for fungal laccases in environmental sensing and cell wall construction. We are currently investigating the mechanism behind this role.

650A

Host transcriptomic and metabolic pathway shifts imposed by intracellular bacteria within *Mortierellaceae*

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Mortierellaceae are ubiquitous soil fungi that are known to host *Burkholderia*-related endobacteria (BRE) and/or *Mollicutes*-related endobacteria (MRE). Whole genome assemblies of these endobacteria show significant losses in amino acid and nucleic acid biosynthesis pathways, indicative of their metabolic dependence on fungal hosts for these essential molecules. This metabolic cost is evidenced by the fact that fungal isolates hosting endobacteria have reduced growth rate compared to isogenic lines cleared of their endosymbionts. Still, it is unknown how endobacteria alter fungal genomic expression. To determine if mechanisms of host nutritional provisioning of endobacteria are conserved between endosymbiont or host species we sequenced genomes and analyzed metatranscriptomes of four isolates of *Mortierellaceae*, each containing different clades of BRE or MRE. We found that each *Mortierellaceae*-endosymbiont holobiont exhibited distinct transcriptomic responses to their respective endobacteria, but common changes induced by bacterial endosymbionts on their fungal host included energy metabolism, mitochondrial function, DNA damage repair, polyamine metabolism, and iron homeostasis. Many of the changes in host metabolism appeared to complement functional losses observed in endobacterial genomes.

651A

AI-driven protein design to understand effector function within the *Magnaporthe oryzae* rice pathosystem

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Artificial intelligence (AI)-based protein design tools have great potential to advance our understanding of molecular interactions between pathogenic fungi and their hosts. This study illustrates how AI-designed binders can be deployed to characterise enigmatic fungal effectors and reveal conserved virulence mechanisms, complementing traditional molecular approaches. The blast fungus *Magnaporthe oryzae* infects agronomically significant crops such as rice and wheat, but also subsistence crops such as fingermillet. Globally, rice blast disease has an economic impact of $66 billion each year. Like many phytopathogens, *M. oryzae* utilises an arsenal of secreted effectors to facilitate plant infection. Through a structure-based phylogenetic analysis of the *M.*

oryzae effector repertoire, we have discovered a previously uncharacterised effector, *Magnaporthe* effector protein 26 (Mep26). Structural predictions suggest that Mep26 functions as an enzymatic effector with phytate-degrading activity. Furthermore, we have identified three structural orthologues of Mep26 that exhibit low sequence similarity yet share a conserved structural fold. To probe the function of Mep26, we utilised AI-based protein design to generate *de novo* protein binders for Mep26. Designed binders were filtered *in silico* for cross-reactivity across structural orthologues to dissect the mechanism by which the Mep26 effector family contributes to fungal virulence. By targeting the binders to the putative active site, we hope to disrupt ligand binding and consequentially phytate degradation. Enzymatic assays can then to be used to test whether activity is reduced in the presence of the binders. Furthermore, by customising the binders with various tags, such as localisation signals or degradation motifs, we aim to assess whether we can phenocopy mutants lacking all members of the Mep26 family. This proof-of-concept study of the Mep26 family will allow for these techniques to be applied to other challenging research areas, such as targeting highly redundant structural families in genetically intractable organisms where multiple knockouts could prove more difficult. Finally, we provide an overview of AI-driven protein design and outline how *de novo* protein design strategies can be broadly applied in other areas of fungal protein-protein interaction research. Through this work, we aim to stimulate discussion on how emerging technologies can address fundamental questions in fungal biology.

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Pmk1-driven signal integration and developmental control in the rice blast fungus *Magnaporthe oryzae*

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During the lifecycle of the rice blast fungus *Magnaporthe oryzae*, the mitogen-activated protein kinase (MAPK) Pathogenicity MAPK 1 (Pmk1) is essential for developmental transitions, including appressorium formation, penetration peg emergence, invasive growth, and cell-to-cell movement. Various components which regulate, or are regulated by, Pmk1 are known such as the MAPK kinase Mst7 and the MAPK kinase kinase Mst11 which activate Pmk1. However, the mechanism by which Pmk1 integrates extracellular cues and produces different developmental outcomes remains largely unresolved.

Here we describe different mechanisms of Pmk1 regulation as well as dynamics of Pmk1 activity during appressorium development. First, we identify and functionally characterize a Pmk1-dependent phosphosite (S358) in the upstream kinase Mst7. This phosphosite was identified in a phosphoproteomic analysis of a Δ*pmk1* mutant (Cruz-Mireles et al.,2024 PMID: 38729111). We found and aspartate substitution of S358 had altered Pmk1 activity and was impaired in appressorium formation. This suggests Pmk1 phosphorylates the upstream kinase Mst7 to regulate its own activity thereby forming a feedback loop. Second, we show that a putative MAPK scaffold regulates stage-specific Pmk1 signalling. Ham5 has been previously shown to be a MAPK scaffold in other fungal species. We report the Ham5 orthologue is dispensable for Pmk1-mediated appressorium formation and virulence but is required for sexual development. This provides evidence that scaffolding confers different functions for Pmk1 during distinct stages of fungal development. Finally, we assessed the temporal dynamics of Pmk1 MAPK activation throughout appressorium development. Pmk1 activity shows an early peak at the germling stage, before appressorium formation, as well as a peak in activation after the incipient appressorium has formed, suggesting Pmk1 plays a role throughout infection related development. These findings provide new insight into how Pmk1 integrates extracellular cues to produce different developmental outcomes.

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Large scale proteome mapping of multidrug resistant *Candida auris* isolates

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Candida auris is a multidrug-resistant yeast that colonizes skin, spreads in hospitals, and causes bloodstream infections with 30–60% mortality. Resistance to azoles, amphotericin B, and echinocandins is common, yet the proteome signatures of drug-resistance across clinical isolates remain poorly defined. We assembled a panel of 50 clinical *C. auris* isolates spanning all major clades and quantified their proteomes in triplicate under four conditions (no drug, fluconazole, amphotericin B, and micafungin), generating ~600 LC–MS datasets. MICs (minimum inhibitory concentration) for each varied widely and were linked to distinct polymorphisms from genome-wide association analyses. Linear models relating protein abundance to MIC, with clade included as a covariate, identified sets of MIC associated proteins whose abundance separates resistant from susceptible isolates. Co-expression network analysis further revealed proteome modules and ones correlated with MIC across drugs. To dissect proteome diversity, we classified proteins across isolates into core, shell, and cloud sets based on presence/absence. Core proteins dominate the quantified proteome, while shell and especially cloud proteins show much higher variability and clade-biased presence/absence patterns, suggesting an accessory proteome layer that may contribute to strain-specific phenotypes. Ongoing work is integrating these core/shell/cloud categories, MIC-associated modules, and GWAS hits to prioritize proteins for functional testing in resistance. This study provides one of the largest proteomic surveys of *C. auris* to date and begins to map how proteome diversity and drug-responsive pathways shape antifungal resistance.

654A

Hypovirus-mediated enhancement of *Trichoderma harzianum* biocontrol activity through virus-fungus-plant interactions

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Hypovirulence induced by hypovirus infection in phytopathogenic fungi has emerged as a promising virocontrol strategy against fungal diseases, typically involving virus-fungus-plant tripartite interactions. However, the role of virus-fungus interactions in modulating the biocontrol activity of beneficial fungi against plant pathogens-representing a quadripartite (virus-fungus-fungus-plant) system-remains largely unexplored. In this study, the effects of two hypoviruses infecting *Trichoderma harzianum* were investigated. Using comparing virus-infected and -free isogenic lines, we found that hypovirus infection caused no notable changes in morphology, including mycelial growth, pigmentation, or sporulation. Nevertheless, infected *T. harzianum* exhibited significantly enhanced biocontrol activity against target pathogenic fungi. Interestingly, multiple mycovirus infections attenuated this enhanced activity, suggesting potential virus-virus interactions within the host. These findings demonstrate that hypovirus infection can improve the antagonistic performance

of *T. harzianum*, leading to enhanced plant protection. This represents a novel type of virocontrol distinct from conventional hypovirulence, highlighting the potential of mycoviruses as biological enhancers for developing superior fungal biocontrol agents.

655A

Characterization of *de novo* miniproteins targeting *Candida albicans* virulence traits

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Fungal infections affect more than a billion people annually, yet the number of licensed antifungals remains low, and drug resistance is emerging in many species, demanding new therapeutic interventions. An alternative strategy to combat mycotic disease is to target virulence traits. Such an approach can potentially expand the therapeutic target space while minimizing disruption to the host microbiota and reducing selective pressure on the pathogen to develop resistance. To explore this strategy, we designed *de novo* miniprotein inhibitors, peptides of three to four alpha helices, using a machine learning-based protein structure and binding prediction software called BindCraft. These miniproteins were designed to target virulence factors of the fungal pathogen *Candida albicans*, including the adhesin Als3 and the cytolytic peptide candidalysin. To confirm binding between the designed miniproteins and the target protein, we are employing genetic and biochemical approaches. Specifically, we tested a library of miniproteins designed against the N-terminal region of Als3 for target protein interaction through a yeast two-hybrid assay. Amplicon sequencing of the yeast two-hybrid strains isolated on medium that selected for interactions between bait (Als3) and prey (miniprotein) revealed three structurally similar miniproteins that interacted with the adhesin. Functional assays will be performed to assess the impact of these miniproteins on *C. albicans* adherence to plastic surfaces as well as mammalian cells in culture. Furthermore, a library of miniproteins designed against the candidalysin monomer were screened against synthetic candidalysin in a toxin neutralization assay. Three structurally dissimilar miniproteins with low peptide identity significantly reduced mammalian cell death in the presence of candidalysin, suggesting toxin binding and neutralization. Future work will be performed to define the mechanism by which the miniproteins interact with candidalysin directly as well as their therapeutic potential against *C. albicans* itself. Overall, this project exemplifies an innovative approach to address the need for novel therapeutics to combat serious fungal infections.

656A

Role and regulation of dispersal in Candida albicans commensalism and pathogenicity

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Candida albicans is a prevalent, generally commensal member of the human mycobiome carried by most people their entire lives. Simultaneously, *C. albicans* is a major opportunistic pathogen – in immunocompetent individuals this generally manifests in vulvovaginal (yeast infections) and cutaneous infections while in immunocompromised people, *C. albicans* can also cause superficial oral infections (thrush) or internal solid organ or bloodstream infections. *C. albicans*' pathogenesis is associated with a cycle of biofilm formation and dispersal, and its ability to invade a variety of host tissues while evading the immune system and treatment is in part due to its ability to switch between cellular states with distinct metabolic signatures, adhesins, and morphologies. *Candida* cell state transition has also been linked to host immune and metabolic regulation. Despite the importance of these transitions, dispersal– the process by which hyphal cells give rise to yeast-form cells is poorly understood. Using innovative underoil microfluidic technology, we have found that dispersal from biofilms occurs as a discrete event within biofilm development coinciding with the cessation of biofilm expansion. Both dispersal and the formation of new biofilms from dispersed cells is regulated by intercellular signaling in addition to environmental conditions. We observe that dispersed cells are morphologically and functionally heterogenous and primed for immune evasion and the formation of new biofilm. By modeling the regulatory pathways controlling dispersal, we hope to uncover potential targets for treatment to encourage commensalism over pathogenesis.

657A

Characterizing macrophage dependent spherulation in *Coccidioidies*

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Coccidioidomycosis is an endemic fungal infection that is caused by *Coccidioides* species. It occurs predominantly in the southwest region of United States and affects both healthy and immunocompromised individuals. In the environment, *Coccidioides* asexual spores, or arthroconidia, germinate to give rise to hyphae. However, in the host, these arthroconidia give rise to a multicellular pathogenic form known as the spherule, which then ruptures to give rise to individual endospores that disseminate in the body and undergo further cycles of spherule formation. To understand spherule development inside the host, we investigated the interaction between *Coccidioides* and murine bone-marrow derived macrophages in cell culture. We observed that arthroconidia without host cells formed hyphae under tissue culture conditions, but in the presence of macrophages were stimulated to develop into spherules. Blocking phagocytosis with cytochalasin D severely reduced spherule number, suggesting that phagocytosis by macrophages is required to trigger spherulation in in vitro conditions. Since interactions with macrophages promoted formation of the pathogenic phase of *Coccidioides*, we were interested in further characterization of the *Coccidioides*-containing phagosome. Ongoing studies of phagosome characteristics, including pH, host membrane markers, and phagosome-lysosome fusion will be presented.

658A

Molecular Basis of a Bacterial 'Toxin Sponge' that Protects its Fungal Partner from Antimicrobial Compounds

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Microbial community composition is determined by competition among resident microbes where community-produced antimicrobial compounds (AMCs) play a large role. Our lab studies a physically associated, bacterial-fungal pairing in which the novel bacterium *Paraburkholderia edwinii* acts as a toxin sponge to protect its fungal partner, *Aspergillus calidoustus*, from natural AMCs like phenazine-1-carboxylic acid (PCA). We have found that *P. edwinii* acts

as a ‘toxin sponge’ by sequestering and reducing the toxic molecule while forming aggregates within the fungal mycelium. Further, *A. calidoustus* lowers the local pH during stress, activating the protection program in *P. edwinii*. We have utilized this fact to create a colorimetric assay whereby the bacterial protection program can be activated in the absence of the partner fungus, enabling mutagenic screens for genes involved in protection. We have identified the regulon of a key transcription factor that promotes both association with the fungal host during stress and detoxification of PCA.

To fully establish this as a model system, our group has also rendered *A. calidoustus* genetically tractable and conducted transcriptomic studies to determine which fungal genes are implicated in the defense against PCA without, and with, the partner bacterium. Finally, we have expanded the work to show *P. edwinii* can protect *A. calidoustus* from synthetic, clinically relevant, antifungal drugs such as voriconazole, and have demonstrated that key bacterial genes implicated in PCA protection are not relevant in voriconazole protection. Taken together, our results raise the possibility that protective bacteria are a major factor in determining which fungi are able to persist in environmental and infectious microbial communities.

659A

Influence of pathogen carbon metabolism on host immunity

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Cryptococcus neoformans is an emerging fungal pathogen that remains a leading cause of fungal meningitis and is classified among the top four critical priority fungal pathogens by the World Health Organization due to its significant mortality and global health impact. *C. neoformans* first colonizes the lungs before potentially disseminating to the central nervous system, resulting in meningoencephalitis. Successful adaptation to these distinct host environments depends on efficient carbon utilization and immune evasion.

We investigated the interactions between *C. neoformans* and host immune cells during initial lung colonization, focusing on how disruption of carbon utilization pathways alters immune recognition and host response. The study employed complementary ex-vivo and in vivo approaches. Phagocytosis assays using J774A.1 macrophages were conducted to quantify yeast uptake. Primary bone marrow-derived macrophages from BALB/c mice were co-incubated with *C. neoformans* strains to assess cytokine and marker expression. In vivo, BALB/c mice were intranasally infected to evaluate fungal burden, lung cytokine responses, and tissue pathology. Total RNA harvested from infected lungs was analyzed by qPCR, and histological staining (H&E, GMS, mucicarmine) visualized fungal cells during infection.

Ex vivo analyses demonstrated that macrophages exposed to *pyk1*Δ mutant of *C. neoformans* showed unchanged phagocytic uptake compared to wild type. However, *pyk1*Δ induced a marked reduction in iNOS and an increase in arginase expression in macrophages, indicating a shift toward an anti-inflammatory activation state compared to the wild type (WT). In vivo, mice infected with the *pyk*1Δ strain exhibited significantly reduced cytokine responses overall, including lower TNF-α and IFN-γ levels, absence of titan cells, and diminished pulmonary inflammation relative to WT infection, reflecting attenuated immune recognition and reduced tissue inflammation. Despite its attenuated virulence, *pyk*1Δ persisted in lung tissue for extended periods without causing overt disease.

Collectively, these findings suggest that impaired glycolysis in *C. neoformans* dampens immunological signaling and alters host-pathogen communication at the onset of lung infection, highlighting the central role of fungal metabolic adaptation in shaping the immune response. Understanding how *C. neoformans* metabolism shapes immune recognition may reveal new therapeutic targets against this critical fungal pathogen.

660A

Characterization of Extracellular Vesicles in *Fusarium oxysporum* f. sp. *vasinfectum*

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Extracellular vesicles (EVs) are lipid bilayer-bound particles secreted by cells that mediate communication through the transport of RNA, proteins, and metabolites. In plant–pathogen interactions, EVs play key roles in cross-kingdom communication with functions in both pathogenicity and host defense. This study aims to characterize EVs produced by the phytopathogenic fungus *Fusarium oxysporum* f. sp. *vasinfectum* (FOV), a major causal agent of vascular wilt in cotton. EVs were isolated from FOV1 and FOV4 using differential centrifugation and sucrose gradients, followed by nanoparticle tracking analysis (NTA) and transmission electron microscopy (TEM)

661A

Dissecting appressorium-mediated plant infection by FIB-SEM Volumetric EM and Cryogenic Electron Tomography

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Fungal pathogens pose a significant threat to global agriculture and understanding their virulence mechanisms is essential for developing durable disease control strategies. Many of the most destructive plant infecting fungi form specialized infection cells called appressoria. Appressoria develop on the host leaf surface, undergo rapid cytoskeletal reaorganisation, and generate enormous turgor pressure that breaches the plant cell wall and enable pathogen penetration. Disruption of appressorium development either by genetic mutation or by chemical inhibition disrupts pathogenicity, making this process an attractive target for innovative crop protection approaches. However, the specialized and highly dynamic nature of appressoria have largely prevented the

resolution of their underlying mechanisms using conventional structural biology approaches. Here, we present a method to germinate fungal spores and induce appressorium formation directly on cryo-EM grids. Appressoria formed on grids display normal cytoskeletal rearrangements, septin aggregation and turgor generation, effectively recapitulating their invasion mechanism and exerting forces on the grid film. This breakthrough allows us, for the first time, to use techniques such as FIB-Milling, FIB-SEM Volume EM and Electron Tomography in cryogenic conditions to study the cellular architecture of appressoria and the establishment of fungal disease at unprecedented resolution. These findings offer a robust methodology to resolve the biological processes driving appressorium-mediated plant infection, including repolarization events that initiate host penetration. Furthermore, this approach could lead to the development of novel strategies to combat some of the most devastating plant diseases in agriculture.

662A

Interspecific interactions and virulence variation among *Fusarium* species causing root rot in pulse crops

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Root rot is one of the most destructive diseases of pulse crops worldwide. It is caused by a combination of fungi, oomycetes, and bacteria. In the Northern Great Plains, *Fusarium* spp. are the predominant members of the root-rot disease complex. Preliminary studies on *Fusarium* spp. infecting lentil indicated that instead of forming a virulent disease complex, some *Fusarium* species inhibit each other when co-inoculated. To investigate whether we are able to find similar competitive interactions for *Fusarium* spp. infecting chickpea, we collected a total of 94 *Fusarium* species, that belonged to 6 different species, namely *Fusarium oxysporum*, *Fusarium solani*, *Fusarium acuminatum*, *Fusarium equiseti*, *Fusarium redolens*, and *Fusarium avenaceum,* from field surveys in 2022 and 2023. Since F. *equiseti* has been previously reported to be weakly or non-pathogenic on chickpea, it was not included in the greenhouse pathogenicity tests. Representative isolates of the remaining five species, *F. oxysporum*, *F. solani*, *F. acuminatum*, *F. redolens*, and *F. avenaceum* were tested in single-species inoculations and 26 different multi-species combinations (10 two-species, 10 three-species, 5 four-species and 1 five-species combinations), to assess their virulence potential on chickpea. While single species inoculations and most multi-species inoculations were highly virulent, three out of ten possible two-species combinations showed a reduced level of virulence when compared to virulence potential of the corresponding single strain inoculations. We are currently introducing different fluorescence markers to the *Fusarium* strains that are members of the combinations that displayed the lowest as well as highest virulence potential and we will use quantitative PCR assays to quantify the relative biomass of each strain during co-infection. Understanding the dynamics of these interactions will provide insight into how *Fusarium* species form and act as disease complexes and may ultimately guide biocontrol strategies that exploit natural antagonism among strains to reduce root rot severity.

663A

A novel *Starship* transposon in a broad host range pathogen mediates host-specialized virulence on strawberry

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Macrophomina phaseolina (*Mp*) is a fungal pathogen of at least 97 plant species and an endophyte or opportunistic parasite with an even broader host range. Over the past 20 years, *Mp* has emerged as a destructive pathogen in strawberry-growing regions, causing crown and root rot disease in strawberry. Our previous work revealed that specific clades of *Macrophomina* were highly associated with host origin, with strawberry-derived isolates mostly clustered in clade XIII. Pathogenicity tests revealed that isolates from other hosts and in other clades were not virulent on strawberry; only strawberry-derived isolates of clade XIII were virulent to strawberry. Little was known about the genes underlying *Mp* virulence on strawberry, and to resolve this, we identified genes that differentiate strawberry-virulent isolates from non-strawberry virulent isolates using published short-read sequence data of 428 *Macrophomina* spp. isolates. We identified 569 genes enriched in the strawberry-associated clade XIII. Transcriptome data from our previous work showed 84 of these genes were disease stage-specific, and most of the genes co-upregulated during necrotrophy were localized to a ~200kbp fragment on contig_8. Genes in this fragment were present in most strawberry-derived isolates, regardless of clade, and mostly absent in isolates from other hosts. We determined that this specific region on contig_8 encompasses a novel *Starship* transposon using starfish, a tool for annotating cargo-mobilizing mobile elements. The *Starship* carries a tyrosine recombinase “captain” gene and cargo genes comprised of several cytochrome P450, HTH CenpB-type DNA-binding domains, major facilitator superfamily transporters, and metallo-dependent hydrolase. We tested the association of this *Starship* with strawberry virulence by determining strawberry pathogenicity for 69 representative isolates from 26 host plant species with varying presence/absence of the *Starship* and phylogenetic relatedness. Virulence in strawberry was highly associated with full/partial *Starship* element sequence (X^2= 47.41; $P < 0.0001$). Thus, strong evidence suggests that co-regulated expression of key virulence factors on this novel *Starship* element determines the potential for *Mp* isolates to have host-specialized virulence on strawberry. Our findings provide insights into the mechanisms of fungal pathogen host specialization and could serve as the basis of diagnostic assays to detect strawberry-virulent *Macrophomina* species.

664A

Determining the Sln1-mediated turgor sensing pathway and phospho-histidine landscape in the blast fungus *Magnaporthe oryzae*

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The *Magnaporthe oryzae* appressorium is a highly specialised infection structure capable of generating exceptionally high turgor pressure, reaching up to 8 MPa to breach the host leaf cuticle and initiate disease. The turgor-sensor kinase Sln1 acts as a core regulator of this transition, sensing when the appressorium has achieved the critical turgor threshold required for orchestrating the switch from isotropic turgor generation to anisotropic hyphal growth. This developmental transition is tightly coupled with septin-dependent cytoskeleton remodelling and actin-directed emergence of a rigid penetration peg. The Sln1 kinase localises to the appressorium pore in a turgor-dependent manner. Mutants lacking *SLN1* display hyper-melanised appressoria with excess

turgor, causing aberrant septin ring organisation, impaired appressorium repolarisation and plant infection. Sln1 is well characterised in budding yeast as a histidine-aspartate kinase, initiating a multistep phosphorelay that regulates the high osmolarity glycerol (HOG1) MAPK pathway and the Skn7 transcription factor. However, in *M. oryzae* Sln1 has been evolutionarily repurposed to integrate multiple signalling networks that underpin turgor generation including melanin biosynthesis, the protein kinase C cell-integrity pathway, and the cAMP-dependent protein kinase A signalling pathway. While the dual roles of Sln1 in turgor-sensing and osmoregulation are apparent, the mechanisms by which Sln1 coordinates both of these pathways, sensing biochemical stress and appressorium turgor remain unresolved. Tracking histidine phosphorylation of Sln1 targets has been historically challenging due to the acid and heat labile nature of phospho-histidine, and lack of suitable detection tools. The groundbreaking recent development of 1-pHis and 3-pHis antibodies has, however, provided a means to study histidine phosphorylation. Our research aims to identify and characterise downstream phosphorylation targets of Sln1, in order to determine how histidine phosphorylation regulates the transition from turgor generation to host penetration during appressorium-mediated plant infection.

665A

tRNA modification and codon usage underlie phytopathogen effector evolution and secretion

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Magnaporthe oryzae causes blast, the most devastating disease of cultivated rice, and now poses a significant threat to global wheat production. To manipulate host immunity and establish infection, *M. oryzae* deploys apoplastic effectors into the space between invasive hyphae and the plant membrane-derived extra invasive hyphal membrane (EIHM) matrix via the conventional ER-Golgi pathway, and cytoplasmic effectors into the plant membrane-rich biotrophic interfacial complex (BIC) via poorly understood unconventional protein secretion (UPS) pathway, where they are translocated into host cells by host clathrin-mediated endocytosis. Despite extensive chemical modification of transfer RNA (tRNA) anticodon across taxa, which contributes to codon decoding and translation fidelity, few physiological roles are known. Using reverse genetics and live-cell imaging, we previously showed that, in the destructive blast fungus *M. oryzae,* the translation of cytoplasmic (but not apoplastic) effector mRNAs relies on efficient decoding of AA-ending codons by the Uba4-dependent thiolation of wobble uridine-34 (s^2U_{34}), a universally conserved modification, in cognate tRNAs. Loss of *UBA4* abolished secretion of AA-ending codon enriched cytoplasmic effectors (but not AA-ending codon depleted apoplastic effectors). Synonymous AA- to AG-ending codon changes in *PWL2* mRNA restored cytoplasmic effector production and secretion in *Δuba4* strains. In the *M. oryzae* WT strain, expressing recoded *PWL2* or *AVR-Pik* caused massive protein production and super-secretion into the BIC, which destabilized the host-fungus interface and abolishing infection. We show here that Uba4-dependent decoding of AA-ending codon-enriched cytoplasmic effector mRNAs is widespread across *M. oryzae* host-infecting lineages and across taxa. Furthermore, based on the AA-ending usage rates *of M. oryzae* secretome genes, we identify two new unconventionally secreted cytoplasmic effectors. In *PWL2 and AVR-Pik*, mutational analysis coupled with FRAP (Fluorescence Recovery After Photobleaching) experiments reveals that a single conserved AA-ending codon can optimize mRNA translation speed and effector production, maintaining BIC integrity and facilitating host infection. These findings emphasize how codon usage choice on fine scales underpins the success of the *M. oryzae*-host rice plant interaction indicating it is a conserved, fundamental principle of host infection driving effector evolution.

666A

The outer membrane of a protective bacterial partner plays a key role in fungal resistance to phenazines

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In nature, microbes spanning all domains of life form communities that drive critical processes involved in host health, ecosystem stability, and more. The species-level composition of microbial communities frequently determines the distinct roles members have and the function of the community. However, the mechanisms by which microbes form relationships that influence the species-level composition are not well understood. A common mechanism of competition within microbial communities is the production of natural antimicrobial compounds (AMCs), which regulate community membership. Phenazines, a class of naturally occurring, redox-active AMCs, are toxic to many fungal species. However, sensitive fungi are repeatedly isolated from phenazine-replete environments. Our lab has discovered that some fungi are able to survive in the presence of phenazines via protection from physically associated, phenazine-tolerant bacteria. To study this phenomenon, our lab established a model system which consists of a bacterium, *Paraburkholderia edwinii*, that protects its filamentous fungal partner, *Aspergillus calidoustus*, from phenazine-1-carboxylic acid (PCA) stress. *P. edwinii* is thought to activate its protection response due to a decrease in the local pH created by *A. calidoustus* during PCA challenge. Here, *P. edwinii* forms aggregates within the *A. calidoustus* mycelium that sequester and reduce PCA, rendering the PCA less toxic to the fungus. The bacterial genes responsible for this protection response remain largely unknown. To identify which genes are vital to how *P. edwinii* sequesters and reduces PCA, I have developed an axenic transposon mutagenesis assay that synthetically mimics the presence of *A. calidoustus* during PCA stress. Preliminary results indicate key components of the bacterial outer membrane play a role in *P. edwinii's* ability to detoxify PCA. Continued investigation of these findings will help provide insight into how cooperative efforts between microbes can help assemble and maintain community structure despite AMC pressure.

667A

Brute-force or Trojan horse: How do bacteria hijack the fungal immune system?

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Fungi interact with diverse organisms in the environment, but little is known about the fungal immune system. Although nutrient-dependent fungal-bacterial interactions have been studied previously, the field lacks an understanding of a conserved fungal defense program and a tractable model system to probe the molecular mechanisms of these inter-kingdom interactions. We propose *Neurospora crassa* as a model for studying molecular mechanisms of fungal-bacterial interactions in co-culture with the generalist pathogen *Pseudomonas syringae Pst DC3000* and soil microbe *Pseudomonas putida KT2440*. We find that *N. crassa* fitness during bacterial pressure requires direct contact with bacteria, that it is dependent on the type and amount of bacterial cells, and nutrient availability.

Moving forward, we are expanding the search for bacterial genes that “hijack” the fungal immune system and enable bacterial growth across the hyphal network. We propose using bacterial RB TN-Seq libraries of *E. coli, P. putida,* and *P. syringae* inoculated onto *N. crassa* to determine the bacterial genes responsible for survival and motility along live fungal hyphae compared to abiotic surfaces. We hypothesize that after bacteria adhere to the fungal cell surface, secretion systems deliver effectors into the host cells to modulate the fungal immune response.

668A

Measuring fitness penalties of fungicide resistance mutations via *in planta* competition assays.

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Cercospora Leaf Spot, caused by *Cercospora beticola*, is the most damaging foliar pathogen of sugarbeet worldwide. Management practices involve host resistance and chemical control measures, of which, quinone outside inhibitors (QoIs) and demethylation inhibitors (DMIs) are essential management tools. In recent years, *C. beticola* resistance to QoI and DMI fungicides has increased in prevalence in the Red River Valley of Minnesota and North Dakota. Within *C. beticola* populations, the G143A amino acid substitution in cytochrome b is the primary mutation associated with QoI resistance. For DMI resistance, a silent mutation at the 170th amino acid of *CbCYP51* is the most frequently associated mutation, followed by the L144F amino acid substitution of *CbCYP51* that is coded by either a TTC or a TTT codon. To investigate the relative fitness of fungicide sensitive and resistant isolates, a direct, head-to-head, *in planta* competition assay was performed. Isolates containing the G143A mutation were co-inoculated with wildtype isolates on susceptible five-week-old sugarbeets using equal spore concentrations. To assess the relative fitness of the two most common DMI fungicide resistance mechanisms, a sensitive and a resistant isolate were each mutated to all observed haplotypes. Transformants were co-inoculated in pairs on susceptible five-week-old sugarbeets using equal spore concentrations. The relative ratio of each isolate biomass was assessed using ddPCR at 14 days post inoculation. Subsequent inoculations were performed with relative spore concentrations reflecting the recovered biomass of the isolates in competition. Preliminary results suggest there is a fitness penalty associated with the G143A mutation, and ongoing competition assays will allow us to measure a relative penalty associated with this mutation. Additionally, there is a relative fitness advantage associated with the *CbCYP51* E170 silent mutation associated with DMI resistance and experiments are ongoing to assess the fitness of isolates carrying the L144F mutations.

669A

A *Pyrenophora teres* f. *teres* effector defeats the broadly used barley net form net blotch resistance gene, *Rpt5*

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The fungal pathogen *Pyrenophora teres* f. *teres* causes net form net blotch (NFNB) on barley. A previous study used the segregating barley population Tifang × CI5791 to map a highly effective and broadly used resistance harbored by the barley line CI5791. It was concluded that this was a dominant resistance gene previously named *Rpt5,* and it mapped to barley chromosome 6H. Subsequently, *P. teres* f. *teres* isolates collected in Morocco were shown to overcome this *Rpt5* resistance in CI5791. Using a *P. teres* f. *teres* biparental mapping population of the Moroccan *P. teres* f. *teres* isolates MorSM40-3 (virulent on CI5791) and the reference isolate 0-1 (avirulent on CI5791), we identified a significant quantitative trait locus (QTL) associated with fungal virulence on CI5791. The QTL was mapped, and with the aid of genomic and transcriptomic data, we identified the candidate effector gene *v5791* in MorSM40-3. Subsequently, we assessed the contribution of *v5791* to fungal virulence using CRISPR-Cas9-based gene disruption, complementation, and allele swaps between avirulent and virulent alleles in the native *v5791* genomic position. MorSM40-3 mutants (MorSM40-3Δ*v5791*) lacking a functional *v5791* lost virulence on CI5791, resulting in an association with *Rpt5* when inoculated on the Tifang × CI5791 mapping population. Complementation of *v5791* in the MorSM40-3Δ*v5791* mutants in the gene's native position restored full virulence on CI5791 and resulted in a loss of association with *Rpt5*. An allele swap of the MorSM40-3 allele of *v5791* in the *P. teres* f. *teres* isolate 0-1 resulted in virulence on CI5791 and caused a loss of association with *Rpt5*. These findings indicated that, although *P. teres* f. *teres* is a necrotrophic pathogen, it has evolved effectors that enable it to bypass dominant host resistance by evading early recognition and defense activation, thereby facilitating colonization and disease development.

670A

tissue-dependent role for cell wall regulatory pathways on *Aspergillus fumigatus* virulence.

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Aspergillus fumigatus is a common agent of invasive lung and corneal infection, both of which are associated with poor antifungal treatment outcomes. We reason that pathways that regulate fungal adaptation or growth within the host environment may serve as novel drug targets, yet the extent to which such pathways vary across host tissues (e.g. the lung vs the cornea) is poorly understood. Here, we demonstrate that deletion of the carbon catabolite repressor gene (*creA*) in *A. fumigatus* results in a marked reduction in cell wall chitin, which is an important mediator of wall rigidity. Interestingly, whereas *ΔcreA* was able to growth through explanted lung tissue and establish infection in a murine model of invasive pulmonary aspergillosis (IPA), the mutant could not penetrate into explanted cornea and was avirulent in an *in vivo* model of fungal keratitis (FK). We reasoned that salient difference between the two host sites is matrix density, where the fungus requires high wall rigidity (increased chitin) to penetrate the dense collagen matrix of the cornea, and less rigidity to penetrate the relatively spongy lung parenchyma. We further reasoned that this may explain why others have shown that an *mpkA* mutant, which is defective in its ability to upregulate chitin in response to cell wall stress, is fully virulent in the lung. To explore if MpkA is important for virulence in the cornea, we generated an *mpkA* deletion in our background and confirmed expected phenotypes, including a marked hypersensitivity to cell wall stressing drugs, including Congo red and caspofungin, as well as severely stunted radial growth on agar. Interestingly, however, we found that growth (biomass accumulation) of *ΔmpkA* was indistinguishable from the wild-type and complemented strains in shaking broth culture, suggesting that growth of the mutant varies as function of the environmental growth matrix. Critically, and in contrast to reports from the lung, *ΔmpkA* was unable to establish infection in the murine cornea. Ongoing studies seek to determine if this loss of virulence for *ΔmpkA* can be directly tied to reduced cell wall chitin and if

chitin synthesis in the wild-type strain is different within the cornea and lung microenvironments. Nevertheless, our current results suggest that virulence pathways, and by extension novel antifungal targets, may vary as a function of host niche, with cell wall regulatory pathways being particularly attractive targets in the setting of FK.

671A

A molecular diagnostic marker for the primary causative agent of Fusarium stem rot and decline (FRD) outbreak in California processing tomatoes, *F. noneumartii* FN-1 (*F. solani* Species Complex)

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Almost all US processing tomatoes, which are used in products such as ketchup, tomato sauce and paste, are produced in the California Central Valley. *Fusarium noneumartii*, previously reported as *F. solani* f. sp. *eumartii,* causing minor (foot rot) symptoms in California and Japan, began to be observed causing severe stem rot and canopy decline in California tomatoes. This disease, named Fusarium stem rot and vine decline (FRD) is caused by a single *F. noneumartii* clonal group, FN-1. This clonal group also encompasses California isolates from tomato with minor symptoms 1990s, and isolates the cause stem rot and decline of hemp and both wilt and dry rot of potato. All FN1 isolates are able to cause FRD in tomato, regardless of host of recovery or temporal origin. Accurate diagnosis of these diseases is vital for selecting appropriate management tools such as more resistant cultivars and appropriate crop rotations. Distinct FN clones have been identified as tomato endophytes and sweet potato pathogens, which do not appear to overlap with the host range of FN-1, pointing to the need to develop clone-specific (rather than species-specific) diagnostic markers for accurate diagnosis of FN-1 driven diseases. In this study, a TaqMan qPCR-based diagnostic tool specific for FN-1 was developed targeting unique genomic loci identified through a pre-existing comparative genomics pipeline (*Phytopathology* 115:204-217). The specificity of the marker was validated through i) *in silico* screening against sequence reads and genome assemblies of 167 isolates within the *Fusarium solani* Species Complex and NCBI database and ii) wet-lab testing using a large panel of 137 isolates representing the known genetic diversity of *F. noneumartii* and several closely related species, including *F. martii* (which also includes a clonal group (FM-1) capable of generating FRD symptoms)*, F. falciforme* and *F. solani*. The TaqMan qPCR assay was shown to be highly specific to FN-1 with no false positives and false negatives when validated against an additional panel of tomato isolates identified in ongoing diagnostic efforts. This new FN-1 diagnostic tool provides a more rapid and accurate method for identification of FRD in tomato. Studies are underway to expand diagnostic utility to Fusarium dry rot of potato, by screening against an additional array of pathogenic and non-pathogenic species present in diseased tubers.

672A

Molecular architecture and dynamic remodeling of fungal cell walls revealed by Advanced Solid-State NMR Spectroscopy

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Invasive fungal infections remain a major cause of morbidity and mortality among immunocompromised patients, yet current antifungal drugs are limited by toxicity, poor efficacy and rapid emergence of resistance. The carbohydrate-rich cell wall is an attractive target as it is absent in humans; however, its structural organization, dynamics, and remodeling pathways remain poorly understood. To address this gap, we employed multidimensional solid-state NMR (ssNMR), a nondestructive atomic-resolution technique, to characterize the architecture, packing, and adaptive restructuring of fungal cell walls directly in intact cells across pathogenic and extremophilic species.

Using $^{13}C/^{15}N$ isotopic labeling and Fast magic-angle spinning, and hydration-relaxation NMR methods, we resolved the composition, linkages, and dynamic of major polysaccharides, including β-1,3-glucan, β-1,6-glucan, chitin, chitosan, galactomannan, and galactosaminogalactan in *Aspergillus fumigatus*, *Candida albicans*, and *Rhizopus delemar*. Proton-detected ssNMR on partially deuterated *Aspergillus* cell wall revealed pronounced polymorphism and conformational heterogeneity in glucan and chitin networks, identifying multiple packing states that coexist within the native wall. Second, we characterized the structural responses of *A. fumigatus* to caspofungin, demonstrating a coordinated remodeling program in which β-1,3-glucan depletion is compensated by increased chitin, chitosan, and new α-glucan and branched glucan conformers that stabilize the drug-stressed wall. Third, we examined *Rhizopus* germination and uncovered a temporally ordered remodeling trajectory: early loosening of β-glucans, progressive chitin-to-chitosan conversion, and increasing wall rigidification as spores transition from dormancy through swelling to polarized hyphal growth. Finally, to understand adaptation under extreme abiotic pressure, we investigated the halophilic fungus *Aspergillus atacamensis*, revealing that hypersaline conditions induce the incorporation of extracellular salt microcrusts, reshape hydration profiles, and reorganize glucan assemblies into densely packed, mechanically resilient architectures that support survival in salt-saturated environments. Altogether, this research provides a molecular blueprint of how fungal cell walls reorganize during drug stress, development, and extreme environments, revealing structural signatures that could guide the creation of more effective, targeted, and broadly applicable antifungal therapies.

673A

Epistasis at the cell surface: what is the role of Erg3 loss-of-function in acquired echinocandin resistance?

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Echinocandins, which target the fungal β-1,3-glucan synthase (Fks), are essential for treating invasive fungal infections, yet resistance is increasingly reported. While resistance typically arises through mutations in Fks hotspots, emerging evidence suggests a contributing role of changes in membrane sterol composition due to *ERG3* mutations. Here, we present a clinical case of *Nakaseomyces glabratus* (*Candida glabrata*) in which combined mutations in *ERG3* and *FKS2*, but not *FKS2* alone, appear to confer echinocandin resistance. Integrated analyses reveal a recurrent association between Erg3 loss-of-function and echinocandin resistance mediated by Fks variation across *Candida* species, but exclude *ERG3* loss-of-function as an independent resistance mechanism. Advances in Fks structural biology and insights into echinocandin-Fks interactions support a model of epistatic crosstalk between membrane sterols and Fks function. Understanding this interaction is crucial, as it may underlie not only acquired echinocandin resistance but also the broader development of multidrug resistance across major antifungal drug classes.

674A

Understanding Diversity of Endohyphal Bacteria in Clinical Mucorales Isolates

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Fungal–bacterial interactions are increasingly recognised as important but understudied contributors to fungal pathobiology, virulence, and clinical outcomes. Members of the Mucorales are known to harbour endohyphal bacterial partners, particularly members of the Mollicute-Related or *Burkholderiaceae*-Related Endobacteria, commonly identified in environmental Mucorales isolates. Yet, for patient-derived isolates, the breadth, prevalence, and clinical relevance of these associations remain largely unexplored. To address this gap, we are taking combined computational and experimental approaches to investigate endohyphal bacteria (EHB) across both clinical and environmental Mucorales isolates. By mining publicly available sequencing datasets and analysing newly collected patient isolates from the UK, Mexico, and France, we aim to broaden the spectrum of bacterial associates present in medically relevant Mucorales species. Our holistic EHB detection strategy integrates metagenomic screening, PCR-based detection, fluorescence in situ hybridisation, and whole-genome sequencing to sensitively and specifically identify candidate endosymbionts across genera, including but not limited to *Mycetohabitans*. Initial findings reveal previously recognised symbioses as well as novel endohyphal species in clinical and environmental samples. These results suggest that endohyphal associations in clinical isolates are more widespread and diverse than currently appreciated, with potential implications for fungal physiology, virulence, and treatment response. Together, this work establishes a framework for systematic discovery of fungal–bacterial symbioses in clinical settings and lays essential groundwork for future mechanistic studies. Ongoing efforts include functional characterisation of select Mucorales-bacteria pairs, with the long-term goal of understanding how these interactions can shape pathogenic traits and may ultimately be manipulated for experimental and therapeutic purposes.

675A

Tribollium castaneum: a novel, ecologically-relevant arthropod model for human fungal pathogens

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Advances in human fungal pathogenesis are increasingly revealing a need for understanding the ecological context pathogens evolve in. Current *in vitro* models for fungal pathogen research are invaluable tools; however, they lack ecological relevance, suffer from limited sample sizes, and some even suffer from confounding batch effects. We present the flower beetle, *Tribolium castaneum*, as a novel model for studying fungal pathogenesis. *T. castaneum* naturally interacts with saprophytic species of fungi, including the fungal pathogens, *Aspergillus fumigatus* and *Aspergillus flavus*. We demonstrate that *T. castaneum* surmounts the shortcomings of current models, using three species of *Aspergillus*. *T. castaneum* infections display dose-dependent infections plus inter- and intra-specific responses to infection. Furthermore, we find *T. castaneum* survival correlates with mouse survival both between and within species. Additionally, we track fungal infection progress and report the transcriptional immune response of *T. castaneum*, relating it back to mammalian immune responses

676A

Invading in full force: substantial pressure from Botrytis cinerea infection cushions breaches plant surfaces

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Botrytis cinerea is a broad host-range plant pathogenic fungus that can develop two distinct structures for penetrating the plant surface. The unicellular appressorium (UA) facilitates the penetration of a single host cell, while the highly branched infection cushion (IC) penetrates at multiple sites leading to enzymatic destruction of the host surface. We hypothesized that the substantial enzymatic degradation of the host surface would make turgor pressure less important for IC-mediated penetration compared to UA-mediated penetration, where high pressures were previously reported. To investigate the contribution of directed turgor pressure in IC-mediated host penetration, we used a poly-dimethylsiloxane surface, with similar properties to the plant cuticle. By measuring the depth and contact perimeter of indentations made on the surface by *B. cinerea* ICs, we investigated the spatiotemporal dynamics of host penetration. We observed a sequential asynchronous penetration of several hyphal branches of the IC, that also increase in size as they penetrate more deeply, which might increase the contact surface with the plant host allowing for greater secretion of effectors and cell wall degrading enzymes.

Using a Hertzian contact model, we showed that substantial pressure of 6.4 bar is exerted on the surface, greater than the 5 bar of pressure exerted by the UA. This suggests that the more complex ICs may be more effective at penetrating host surfaces that could not be penetrated by UA.

To investigate this further, we used CRISPR-Cas9 mediated mutagenesis to analyze the relationship between cell wall structure of *B. cinerea* and pressure generation. In contrast to melanin-deficient mutants in other plant pathogenic fungi, the melanin-deficient pks12/pks13 mutant is still able to develop ICs, but applies significantly less force on the surface. Additionally, a reduced penetration force was observed in cda1 and cda3 mutants, characterized by reduced chitin deacetylation in the infection cushion. These results highlight how the modulation of the cell wall structure is important for plant pathogenic fungi to enable the proper direction of internal turgor pressure onto the penetration site, and how the more complex infection cushion not only allows for greater enzymatic degradation of the host surface, but also greater penetrative force.

677B

The C2-domain protein SppA contributes to virulence and resistance to FksA-targeting antifungals in *Aspergillus fumigatus*

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Aspergillus fumigatus is an opportunistic fungal pathogen responsible for severe infections known as invasive aspergillosis (IA) in immunocompromised individuals. Current treatment options for IA are extremely limited with only three approved classes of antifungal drugs. The recommended first line therapy is with the membrane targeting azoles, but reported resistance to these drugs is on the rise. In cases of poor clinical response to azoles alone, salvage therapy includes the use of the cell wall targeting echinocandins. These lipopeptide drugs inhibit the enzyme FksA which synthesizes the cell wall polysaccharide beta-1,3-glucan. Recently, a novel drug called ibrexafungerp, derived from the fungal terpene enfumafungin, was found to inhibit the same enzyme and has since entered clinical trials for use in IA. We performed a proteomic screen to identify proteins regulated by the transcription factor ZfpA during caspofungin treatment. The most highly upregulated protein was the C2 domain containing protein orthologous to the septal-associated protein Aspergillus oryzae SppA. Deletion of sppA rendered A. fumigatus highly susceptible to killing by both echinocandins and enfumafungin including in an OE::zfpA background which normally exhibits increased resistance to fungicical activity of these drugs. The ΔsppA mutant still forms chitin cross-walls, but is unable to properly plug the septal pores upon hyphal damage and cytoplasmic loss, similar to the ΔzfpA strain. Overexpression of sppA results in aberrant septa and large chitin patches in the hyphal cell wall although normal septa are also observed. SppA appears to be important during infection as its deletion attenuates virulence in multiple models of invasive aspergillosis using larval zebrafish.

678B

Genomic features of Crusty, a novel polyextremotolerant fungus with *Methylobacterium* symbionts that functions within biological soil crust consortia

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JF2 08-2F Crusty is a novel melanized polyextremotolerant fungus (*Neophaeococcomyces*) isolated from a biological soil crust that harbors *Methylobacterium* spp. endosymbionts called Pinky. Multiple lines of evidence suggest that Crusty and Pinky belong to a larger mutualistic consortium and that Pinky may support the growth of Crusty via shared algal partners whose growth is stimulated via phytohormone production. As an initial step towards functional investigation of this consortium, we are generating complete genome sequences of key partners. Here, we describe the Crusty genome and some of its unique features. The size of the assembled Crusty genome is 40.39 Mbp consisting of 21 scaffolds. The total number of predicted gene models is 11,316. A notable feature of the Crusty genome is the presence of a single predicted *Starship* element with an estimated length of 100.3 Kbp. Strikingly, the element is positioned about 70.3Kbp from a type I PKS cluster that exhibits similarity to *Aspergillus nidulans stcA* and is not well conserved in other Chaetothyriales. At least 37 annotated genes are present on the element, including duplicated copies of a protein kinase whose function is unknown and a single copy of a Zn(2)-C6 transcription factor related to Nit-4. The duplicated protein kinase genes reside within a four-gene duplication inside the *Starship* element. A second intriguing feature of the genome is the presence of 23 apparent homologs of a gene that includes a shared domain with *A. nidulans* OrsD as well as domains characteristic of DEAD/DEAH box helicases. Moreover, all 23 homologues of the OrsD/helicase are encoded by genes located at the ends of sequence scaffolds. Seven of the 21 scaffolds are bounded by homologues at either end, with eight additional scaffolds possessing homologues at one end. While the specific functions of the *Starship*-associated genes or the OrsD/helicase have yet to be determined, their unique presence in the Crusty genome suggests a potential role in fostering genome dynamics that enable the Crusty-Pinky symbiosis.

Parallel genomic analysis of four spontaneous albino mutants of Crusty provides further evidence of a dynamic genome, as two of the four mutants possess large deletions that encompass the *PKS1* gene encoding the polyketide synthase responsible for melanin production.

679B

Secretion of *Periglandula clandestina*-derived ergot alkaloids from host morning glory roots

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Ergot alkaloids are pharmaceutically and ecologically important chemicals produced by certain fungi, including *Periglandula clandestina* which is symbiotic with the common morning glory *Ipomoea tricolor*. Previous work indicates that ergot alkaloids produced by *P. clandestina* occur in very high concentrations in *I. tricolor* roots and suppress parasitic nematode populations. We hypothesize that ergot alkaloids are secreted from roots into the surrounding medium and devised a hydroponic system featuring 3D-printed net pots and Hoagland's solution to facilitate sampling of roots and associated growth medium. Half of the plants were treated with the systemic fungicide tebuconazole to suppress the symbiotic fungus. Plant tissues and growth medium were sampled regularly over six weeks, and ergot alkaloids were quantified by high-performance liquid chromatography. Our data demonstrate that ergot alkaloids, including lysergic acid α-hydroxyethylamide, ergine, and ergonovine, were secreted from roots in two phases: a flush of seed-inherited alkaloids, in both non-treated and fungicide-treated plants, followed by distribution and secretion of newly synthesized ergot alkaloids in non-treated plants only. Of seed-inherited ergot alkaloids transported to roots, approximately 12% were secreted from non-treated plants as opposed to only 4% in fungicide-treated plants. These data indicate a role for *P. clandestina* in secretion or an unintended consequence of fungicide treatment. Newly synthesized ergot alkaloids were detected only in non-treated plants and were secreted from roots at a rate similar to that of seed-inherited alkaloids. Quantitative PCR analyses of *I. tricolor* tissues detected *Periglandula* DNA most abundantly in hypocotyls but revealed an absence of *Periglandula* DNA from roots, indicating extensive transport of ergot alkaloids within the plant in addition to secretion. In additional experiments, ergot alkaloids that were externally added to the hydroponic solution were rapidly bound to roots or absorbed into them, indicating the values we measured in solution for symbiont-derived ergot alkaloids were only a portion of the quantity originally secreted. Considering the high concentration of ergot alkaloids in morning glory roots, secretion of even a small percentage may have significant ecological effects on the rhizosphere.

680B

The UPR regulated Phu1 protein connects endoplasmic ATP levels to stress tolerance and virulence

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The unfolded protein response (UPR) is a crucial virulence determinant of pathogenic fungi ensuring ER homeostasis under stress conditions. This is mediated by increasing expression of a diverse set of genes encoding conserved UPR factors including ER chaperones, foldases, lipid synthesis proteins, transporters and species-specific proteins with so far uncharacterized function. Comprehensive gene deletion screening of the UPR regulon in the corn smut fungus *Ustilago maydis*, identified Phu1, a predicted UDP-galactose transporter as essential for pathogenic growth, ER- and cell wall stress resistance. Deletion strains show constitutive UPR activity and repression of mating type gene expression, resulting in suppression of the pheromone response and strongly reduced filamentous growth. In addition, *phu1* deletion display aberrant chitin deposition and increased chitin levels in the fungal cell wall. Interestingly, expression of the *Homo sapiens* SLC35B1 and *Saccharomyces cerevisiae* Hut1 orthologs fully restored virulence and stress-resistance of *phu1* deletion mutants. While *S. cerevisiae* Hut1 was described previously as a UDP-galactose transporter, direct evidence for this molecular function is still lacking. By contrast, SLC35B1 is a well-characterized and highly specific ATP/ADP exchanger (AXER) in the ER membrane, whereas no transport of UDP-Galactose or UDP-Glucose was detectable [1, 2]. In conclusion, the functional conservation between *U. maydis* Phu1, *S. cerevisiae* Hut1 and human SLC35B1 strongly suggests that the observed phenotypes related to Hut1, Phu1 and orthologous proteins are rather connected to reduced ATP-levels in the ER than to UDP-galactose transport.

[1] Klein et al.,2018 Nat. Communications. https://doi.org/10.17632/b49tkfjcm5.1
[2] Gulati et al., 2025 Nature. https://doi.org/10.1038/s41586-025-09069-w

681B

Proteomics of programmed cell death in *Aspergillus fumigatus* conidia to design fluorescent viability reporter

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Airborne conidia of the human-pathogenic fungus *Aspergillus fumigatus* are the main cause of invasive pulmonary aspergillosis particularly in immunocompromised individuals. After inhalation, alveolar macrophages constitute the first line of defense by phagocytosing and intracellularly processing conidia; however, *A. fumigatus* can partially evade this immune response, making improved therapeutic strategies necessary. A major challenge in studying early host–pathogen interactions is the lack of reliable markers to distinguish viable from dead conidia in situ, as both exhibit only minimal morphological differences during the initial hours post-infection. Developing a reporter strain expressing a fluorescently tagged cell-death–associated protein would allow real-time visualization of conidial death by microscopy. To identify suitable candidate proteins for such a reporter system, we characterized programmed cell death (PCD) in *A. fumigatus* conidia. An in vitro cell death assay was established using multiple PCD-inducing compounds, treatment durations, and culture conditions. Among the tested inducers, hydrogen peroxide in nutrient-rich medium exhibited the highest killing efficiency on resting conidia, compared to amphotericin B and voriconazole. These cell-death inducing conditions were subsequently used for comparative proteomic analyses of resting and swollen conidia. The resulting data revealed a set of cell-death–associated proteins with differential abundance during PCD. Based on these findings, fluorescent reporter strains expressing a fluorescent protein fused to selected candidate proteins were generated. This integrated approach provides new insights into the molecular features of PCD in *A. fumigatus* conidia and establishes a foundation for the development of robust live/dead reporter strains. Such tools will facilitate in situ tracking of conidial death during host–pathogen interactions and may support the evaluation of antifungal interventions and the development of novel therapeutic strategies.

682B

Understanding lichen communication: Structural and functional characterization of *Peltigera* GPCRs

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Lichens are complex, cross-kingdom microbial communities that thrive in some of the most extreme environments on Earth. The core symbioses involving fungal mycobionts, photosynthetic partners (algal or cyanobacterial photobionts) and associated bacteria are difficult to analyze at a molecular level. In particular, the mechanisms that enable lichen-forming fungi to recognise and coordinate with their partners remain unclear. G-protein-coupled receptors (GPCRs) are key mediators of environmental sensing and signaling, and many lichenised mycobionts exhibit expanded and diversified GPCR repertoires. Notably, Pth11-like receptors and divergent Gα-subunits are differentially expressed during photobiont interaction, suggesting specialised roles in lichen establishment.

We analyzed the GPCR repertoires of 38 *de novo*-sequenced *Peltigera* mycobionts, creating the largest high-quality lichen fungal genome dataset of a single genus to date. Since fungal GPCRs often exhibit high sequence divergence, we used structure-based clustering to identify remote homologies and domain architectures that would be missed by sequence alignment alone. We further refined our analysis by modeling GPCR-Gα interactions and using interface prediction confidence scores to identify potential G-Protein coupling. Comparing these structural and interaction models with those of non-lichenized and pathogenic fungi reveals receptor families and coupling profiles that are enriched in lichens and could potentially be involved in symbiosis-specific signaling.

In parallel, we used a heterologous yeast expression system to characterize selected GPCRs. We have already observed reproducible receptor activation in this system and are currently narrowing down specific ligands. As the *Peltigera* mycobiont can presently not be isolated or genetically manipulated, this approach allows to functionally validate selected GPCRs and identify cognate ligands. This workflow links high-confidence structural models with experimental validation, providing tools to understand the chemical communication language that governs the establishment and maintenance of lichen symbiosis.

683B

Host suppression of stress signaling in the maize pathogen *Cochliobolus heterostrophus*

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A phenolic compound abundant in maize, ferulic acid (FA), has far-reaching effects on *Cochliobolus heterostrophus*, the Dothideomycete agent of Southern corn leaf blight. Exposure to FA suppresses phosphorylation of the stress-activated MAP kinase ChHog1, and promotes its sequestration to cytoplasmic foci. FA induces mRNA-containing stress granule formation, overlapping in part with the ChHog1:Gfp foci. FA suppresses ChHog1 activation even under osmotic stress, inhibiting dual phosphorylation, nuclear retention and the expression of a Hog1-dependent transporter gene, ChMST1 [1]. This apparently reversed signaling mode could promote survival during infection by mitigating over-activation of the stress response pathway. On the other hand, it seems unlikely that the inability to respond to stress confers a direct advantage to the pathogen. Rather, host-dependent post-translational regulation of the fungal stress-response pathway could co-exist with a fungal survival response. Comparison of such stress responses across fungal species will test how general they are.

[1] Zuchman et al. (2025) PLoS Pathogens. doi: 10.1371/journal.ppat.1013620

684B

Assessing the Ecological and Genomic Determinants of Facultative Pathogenicity Through *Colletotrichum siamense* on Strawberry

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Facultative plant pathogens are microbes capable of endophytic colonization that only transition to disease under specific host or environmental contexts. These pathogens represent a major, yet underappreciated, dimension of plant–fungal interactions, as their cryptic presence in healthy tissue complicates disease forecasting and challenges assumptions about host resistance. Currently, few systems enable controlled study of this transition. To address this gap, the pathogenicity of *Colletotrichum siamense*, a causal agent of anthracnose crown rot (ACR), was evaluated across a panel of differentially susceptible, field-grown strawberry cultivars. Despite striking differences in symptom severity, the frequency of C. *siamense* isolation was constant across genotypes, including those with apparent tolerance. This system isolates the host-genotype component of facultative pathogenicity and supports a genotype-by-environment model in which virulence emerges opportunistically rather than constitutively. Further, field evaluations indicate that nutrient stress and physical damage play substantial roles in triggering disease. Given this pattern of endophytism paired with variable pathogenicity, subsequent analyses sought to determine whether recent increases in ACR incidence and severity might be driven by genomic changes. Historically, *C. siamense* has been a relatively minor pathogen of strawberry, but its virulence and geographic range have increased markedly in recent years. To investigate genomic mechanisms underlying this shift, a hybrid assembly of the highly virulent isolate CT6-1 was generated. Comparisons of conspecific genomes identified an accessory chromosome (AC) present in all sequenced Rosaceae-infecting isolates. This AC is maintained at an elevated copy number (5–8x the core genome), representing the first documented case of extreme aneuploidy affecting fungal ACs and presenting a previously unrecognized form of genomic plasticity in *Colletotrichum*. Genomic evaluation identified predicted pathogenicity factors, suggesting that copy number escalation may potentiate virulence. Together, these findings position *C. siamense* on strawberry as a tractable system for dissecting facultative pathogenicity and highlight the possibility that extreme AC aneuploidy catalyzes the transition between endophytism and disease. The ecological and epidemiological implications of such symbioses suggest a previously unrecognized biological ubiquity.

685B

Fusarium oxysporum f. sp. *vasinfectum* race 4 (Fov4) *FNP1*, a non-ribosomal peptide synthetase gene, plays an important role in cotton Fusarium wilt

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Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *vasinfectum* (Fov), is one of the most destructive early-season cotton diseases worldwide. The recent emergence of the highly virulent Fov race 4 (Fov4) and its aggressiveness have raised significant concerns for the US cotton industry. Unlike other Fov races in US cotton production, which require root-knot nematodes to cause damage, Fov4 is known to infect cotton independent of nematodes. However, molecular mechanisms of Fov4 virulence in cotton are not clearly understood. Secondary metabolites are frequently identified as virulence factors in plant pathogens, and we analyzed the genomes of Fov1 and Fov4 using Fungal antiSMASH to identify a Fov4-specific nonribosomal peptide synthetase (NRPS) gene *FNP1*. To investigate its function, we generated *FNP1* knock-out mutant using CRISPR-Cas9 approach. Growth assays revealed that the mutants exhibit significantly attenuated hyphal production on media containing cotton roots as the sole carbon source, increased sensitivity to cell stress agents, as well as lagged spore germination. Furthermore, the mutant exhibited defect in cotton root rot virulence and significant decrease in Fusaric acid production. Microscopic observation of GFP-labeled *FNP1* deletion mutant showed impeded infection progression in cotton roots compared to the wild type (WT), which further explained the impeded virulence in *FNP1* mutant. RNA-seq analysis further revealed extensive transcriptional reprogramming in the *FNP1* mutant, with 1,336 differentially expressed genes and significant changes across six KEGG pathways, including secondary-metabolite biosynthesis, supporting a key regulatory role for *FNP1* in fusaric acid biosynthesis and broader metabolic networks. Gene complementation restored the observed defects, confirming that *FNP1* is critical for Fov4 virulence, hyphal development, Fusaric acid production, and stress responses.

686B

Distorted segregation and occurrence of non-parental allele type in a biparental population of wheat tan spot pathogen *Pyrenophora tritici-repentis*

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Tan spot (yellow spot) of wheat, caused by the necrotrophic fungus *Pyrenophora tritici-repentis* (Ptr), is an important foliar disease worldwide. Ptr produces three known necrotrophic effectors (NEs), Ptr ToxA, Ptr ToxB and Ptr ToxC, but several studies suggest the existence of additional virulence factors. To identify genomic region(s) associated with a novel necrosis-inducing virulence factor in race 3 isolate 331-9 on selected differential wheat lines, we developed a biparental fungal population and conducted genetic linkage mapping. The mapping population was developed by crossing 331-9 with the race 2 isolate 86-124 (a non-necrosis producer on the selected differential wheat lines), after both were genetically converted to heterothallic strains. Production of functional pseudothecia, asci, and ascospores from this cross was extremely low, indicating poor sexual compatibility between the two isolates. A total of 120 progeny isolates were collected and screened for the presence/absence of *ToxA* (associated with 86-124), *ToxC1* (present in 331-9), both mating-type genes, and several SSR markers across the genome. All loci showed significant deviations from expected Mendelian segregation, and a high proportion of non-parental allele types was also detected. These findings suggest preferential transmission of certain chromosomal regions, as well as possible chromosomal rearrangements during sexual reproduction. Phenotyping of selected progeny isolates on wheat differential lines confirmed the segregation of virulence associated with *ToxA* and *ToxC1*. Long-read genome sequencing of the parents and representative progeny is currently underway to understand the causes of the unexpected segregation patterns and the origin of novel allele types generated during sexual reproduction in this fungal pathogen.

687B

Functional characterization of the HSP70 domain-containing core effector of *Cercospora beticola*

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Plant pathogens utilize effector molecules to establish disease on their hosts. So-called “core effector” proteins are a subset of the total effector catalog that are consistently present across isolates of a species. Due to their role in host infection, core effectors are a key target for understanding pathogen virulence mechanisms. Using a core effector-focused strategy in *Cercospora beticola*, we identified Cb1131, an early-expressing heat shock protein 70 (HSP70) domain-containing effector. HSP70s are also of particular interest due to their established role in plant immunity. We demonstrate that Cb1131 suppresses BAX-induced cell death, PAMP-induced MPK3/MPK6 signalling, and localizes to chloroplasts in *Plants*. Interestingly, Cb1131 also showed >50% sequence homology to plant chloroplast HSP70s (cpHSP70s), potentially indicating functional mimicry of Cb1131 to host HSP70 proteins. To investigate its role in virulence, knockout mutants were generated in *C. beticola*. Furthermore, transgenic lines expressing Cb1131 are being generated in Arabidopsis using AtCpHSP70 mutant backgrounds to test for functional complementation and characterize their role in virulence. These lines will be further analyzed for validating Cb1131’s role in reactive oxygen species production, unfolded protein responses, and disease development through infection assays using Arabidopsis-compatible pathogens. These data will be discussed to highlight the HSP70 protein as a putative chloroplast-targeting effector to aid in disease establishment in *C. beticola*.

688B

Chromosome-scale metagenome assemblies reveal transposable element dynamics in *Peltigera* lichens

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Many fungi engage in diverse symbiotic relationships, ranging from pathogenic and commensal to mutualistic interactions. Lichens represent one of the most stable and ecologically successful mutualistic systems, typically formed between a fungal partner (mycobiont) and one or more photosynthetic partners (photobionts), such as green algae or cyanobacteria. In addition to this core partnership, lichens harbor diverse fungal and bacterial communities, forming a complex microbiome.

The genus *Peltigera* primarily forms symbioses with the cyanobacterial photobiont *Nostoc*, characterizing it as a representative cyanolichen. However, a few *Peltigera* species form tripartite associations, in which the primary photobiont is a green alga of the genus *Coccomyxa* and the secondary photobiont is a cyanobacterium (*Nostoc*). Despite more than two centuries of research, many genomic aspects of lichen symbiosis remain poorly understood. Recent advances in combining long-read sequencing methods enable the resolution of complex lichen metagenomes.

Here, we present 38 high-quality metagenome assemblies from eight *Peltigera* species associated with the cyanobacterial photobiont *Nostoc,* collected across five European countries. In addition to this core cyanolichen dataset, we have assembled three additional samples from two species forming tripartite associations involving *Coccomyxa* and *Nostoc,* allowing us to investigate why some *Peltigera* species engage in these additional algal partnerships while others remain exclusively cyanobacterial. In our dataset, 29 of the 38 mycobiont assemblies reached chromosome-scale resolution, each recovering a set of 13 chromosomes, with five assemblies attaining full telomere-to-telomere (T2T) completeness. Transposable element (TE) annotations showed variable abundance across species, which correlated with genome size variation, suggesting that species-specific genome expansions were likely driven by TE proliferation. In addition, we will present analyses of photobiont genomes and associated microbiomes to assess species-level variation and identify core members shared across samples.

This dataset represents the largest genomic resource currently available for a single lichen genus and provides a robust foundation for investigating the molecular basis of lichen symbioses. By jointly analyzing the mycobiont, photobiont, and associated microbiome, our study advances the genomic understanding of the genus *Peltigera* and contributes broadly to symbiosis research.

689B

Vaccine Development for *Cryptococcus neoformans*

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Currently, there are no fungal vaccines available. Treatment options are limited to just three classes of antifungals: polyenes, azoles, and echinocandins. This narrow arsenal underscores the urgent need for alternative approaches, including the development of vaccines, to prevent and control *Cryptococcus* infections in vulnerable populations. Previous studies in our lab have developed an antifungal vaccine candidate based on the heat-killed *C. neoformans fbp1*Δ mutant (HK-fbp1) that lacks the F-box protein Fbp1, a subunit of an SCF E3 ligase. The HK-fbp1 vaccine triggers superior Th1/Th17 protective immunity that can provide strong protection against *Cryptococcus* infections. However, it remains unclear what are the antigenic factors triggering protective immunity. Our recent studies have identified Cig1, a cytokine inducing mannoprotein regulated by Fbp1, playing a role in the immunogenicity of HK-fbp1. Nonetheless, vaccination with Cig1 offered only modest protection against wild type challenge compared to the HK-fbp1 vaccine itself, suggesting additional immunogens contributing to immune activation remain to be identified. In this study, we have further characterized Cig1 antigen by identifying Cig1 antigenic epitopes and testing their ability to induce the production of protective cytokines (IFN-γ, IL-17A) in HK-fbp1 primes CD4+ T cells in immunized mice. We are using these immunogenic Cig1 peptides to construct a Cig1 tetramer to define the importance of Cig1 antigen in HK-fbp1 vaccine mediated T cell stimulation in vitro and test protection in an in vivo mouse model of vaccination. We are also characterizing the potential role of other mannoproteins, including Fbp1 substrate Cmp1, in HK-fbp1 mediated immune protection. Our goal is to utilize identified antigens in the HK-fbp1 vaccine as adjuvants and to develop an antigen combination-based subunit vaccine against cryptococcosis and other fungal infections.

690B

Parallel comparative transcriptome analyses of bacterial–fungal interactions (BFI) reveal the determinants of BFI directionality

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Bacteria and fungi coexist in diverse environments, exhibiting either antagonistic or cooperative relationships over space and nutrients. Bacterial–Fungal Interactions (BFI) play a crucial role in shaping the structure and function of microbial communities. However, the underlying mechanisms remain largely unexplored. The knowledge gap persists as most studies have focused on specific model systems or individual pairwise interactions, lacking

comprehensive cross-combinatorial comparisons. In this study, we conducted a systematic screening of 828 bacterial–fungal pairings and selected three each of fungal and bacterial species displaying prominent interaction traits (e.g., antagonism, mutualism, neutrality). By comparing the transcriptomic dynamics induced by a range of microbial pairings, we have identified gene regulations unique to each interaction. The trends of gene expression across all samples revealed orthologous genes contributing to interaction traits. Genes involved in secondary metabolite production, oxidative stress responses, and vitamin B biosynthesis were regulated specific to the interaction type. We propose a dual interaction model in which fungal hyphae in contact with harmful bacteria release defensive metabolites, while concurrently fostering spatial and chemical interactions with beneficial or neutral bacteria. Our findings highlighted the commonly altered responses and the pronounced differences arising from subtle phylogenetic variations, enabling a deeper understanding of the mechanisms underlying the specificity of BFI. This contributes to elucidating the intricate dynamics and functional roles of microbial communities across diverse fields, including environmental science, agriculture, and biomedical applications.

691B

Expanding the fungal microbiome: databases, detection tools, and novel mycovirus–endobacteria co-occurrences

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Fungi harbor complex and poorly understood endohyphal microbiomes. Although the number of identified mycoviruses has rapidly expanded in recent years, their diversity, co-occurrence patterns, and functional impacts remain understudied. To address this gap, we developed a publicly available database of fungal-associated viruses that integrates taxonomic information with direct links to publications and genome assemblies. To facilitate the discovery of novel mycovirus associations in sequencing datasets, we constructed a custom GOTTCHA2 read-based classification bioinformatics workflow that provides high-accuracy taxonomic assignment of mycovirus reads from fungal sequencing projects. Complementary GOTTCHA2 databases and a full bioinformatics pipeline were also created to identify other diverse constituents of fungal microbiomes. We are using these workflows and tools to perform a comprehensive assessment of publicly available fungal sequencing data for classifications and co-occurrence patterns of members within these fungal microbiomes. In parallel, we identified and are characterizing two mycoviruses from *Benniella erionia* and *Podila minutissima* isolates using viral enrichment, transmission electron microscopy, and RNA sequencing. These viruses co-occur with endobacterial partners, and we are currently investigating their functional interactions with each other and with their fungal hosts. Overall, improved identification and characterization of fungal-associating bacteria and mycoviruses is essential for understanding fungal ecology and host–microbe interactions.

692B

Proteomic profiling of antifungal drug responses of *Aspergillus fumigatus* indicates drug-specific signatures and modes of action

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The human fungal pathogen *Aspergillus fumigatus* has become increasingly resistant to clinically used antifungals agents, in particular to azole-based drugs. This has led to high mortality rates in patients with azole-resistant invasive aspergillosis. The discovery of new antifungal agents, especially those with novel modes of action (MoA), is therefore urgently needed. However, due to the closer evolutionary relationship between cells of fungi and humans in comparison to bacteria, the number of fungal-specific targets for drug development is rather limited. This often leads to the rediscovery of drugs with known MoA. To address this challenge and to learn more about how antifungal agents affect fungal cells, we employed expression-based proteomics to construct a comprehensive drug-specific proteomic response library based on the profiling of 14 anti-*Aspergillus fumigatus* compounds. Central to this effort was the development of a robust, standardized workflow from cultivation to LC-MS/MS data evaluation. Our analysis revealed that the strongest proteomic responses were linked to processes involved in ‘detoxification’ and ‘defence’ against environmental insults. Our analysis allows to distinguish between synthetic drugs and natural products. Based on our response library, we successfully identified MoA-specific signature proteins, enabling the prediction of similarities between MoAs of compounds. Our strategy also uncovered drug-specific induction patterns of biosynthetic gene clusters (BGCs). We also validated our approach by using three additional drugs, including azalomycin F, a natural product with a predicted new MoA. Notably, we identified the drug-specific increase of poorly characterised transcription factors, which suggests complex regulatory circuits of antifungal drug resistance.

693B

Spp1-mediated processing of substrate proteins promotes plant defence suppression and virulence of fungal biotrophs

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In the smut fungus *Ustilago maydis*, the signal peptide peptidase (SPP) Spp1 is essential for plant defence suppression and establishment of a compatible biotrophic interaction with its host plant maize. SPPs are endoplasmic reticulum (ER)-resident intramembrane proteases, that cleave a subset of remnant signal peptides and transmembrane domain containing proteins, releasing them from the membrane for degradation or to exert a biological function. The conserved catalytic activity and generated cleavage products are crucial for the virulence function in *U. maydis*. Using a Co-IP/LC-MS approach from infected maize tissue, potential substrates of Spp1 were co-purified. Among these we identified the signal peptide (SP) of an effector protein, that is necessary for Spp1-dependent processing prior to effector secretion. Replacement of the Spp1-bound SP with an alternative SP abolished proper effector processing and significantly reduced virulence. In addition, expression of a predicted cleavage product is sufficient to partially rescue the virulence defect of the effector deletion strain. Another identified substrate is an ER membrane-anchored protein, that is required for virulence and depends on Spp1 for proper subcellular localisation. Direct transcriptional regulation via unfolded protein response elements (UPREs) links Spp1 function to biotrophic growth

of *U. maydis*. In silico analyses suggest that the direct UPR-dependent regulation and its virulence function are specifically conserved in biotrophic fungi. Consistently, Spp1 is dispensable for virulence in the hemibiotroph *Verticillium dahliae* but essential for the biotroph *Sporisorium reilianum* to cause disease. Collectively, our data suggest that Spp1 mediates communication between biotrophic fungi and their host plants in a previously unknown manner.

694B

Identification and functional validation of avirulence effectors in wheat rusts

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The identification of avirulence genes (*Avr*) in plant pathogenic fungi remains a major bottleneck for understanding and ultimately mitigating the diseases these pathogens cause. Accurate effector annotation is particularly challenging as these genes often lack sequence homologs, reside in rapidly evolving genomic regions, or represent single-exon genes, which leads to their exclusion by most annotation pipelines. Using EffectorGeneP, a self-training machine learning tool that leverages infection transcript data, we identify effector candidates and generate libraries to submit to a protoplast-based high-throughput screen that enables the characterisation of novel *Avr* genes. The power of this approach has been previously demonstrated with the increased number of isolated *Avr* genes in the wheat stem (black) rust fungus *Puccinia graminis* f. sp. *tritici*, contributing to the construction of an *Avr* gene atlas for the species. Here we report the identification of at least four *AvrSr26* loci through this approach, likely explaining why *Sr26* has yet to be overcome in the field. This not only constitutes a new *Avr* discovery for stem rust but also reveals a diverse and previously poorly annotated gene family. All *AvrSr26* variants were functionally validated by transient expression in wheat protoplasts, virus-mediated expression in intact wheat plants and agroinfiltration assays in *Nicotiana benthamiana*. In addition to this, we report our progress toward the identification of *Avr* effectors in *P. striiformis* f. sp. *tritici*, the causal agent of wheat stripe (yellow) rust. These advances mark an important milestone in dissecting stripe rust pathogenicity and demonstrate the broader applicability of our approach to other high-priority pathogens.

695B

Outcomes of competitive dynamics of *Fusarium graminearum* and *F. poae* on wheat heads

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Although *Fusarium graminearum* (*Fg*) is the pathogen responsible for most Fusarium head blight (FHB) outbreaks on wheat in North America, several other weaker pathogens (e.g., *F. poae* [*Fp*]) co-exist within the same head or field in certain agroclimatic settings. The implications of the competitive dynamics between *Fg* and *Fp* within the same head for FHB development and mycotoxin contamination remain poorly understood. Here, we evaluated whether pre-inoculation with *Fp* two days prior to *Fg* or co-inoculation of the two pathogens would impact *Fg* disease outcomes. We hypothesized that disease outcomes would be dependent on 1) the *Fg* population/chemotype (NA1/15-ADON and NA2/3-ADON), 2) the wheat variety (Alsen and Norm), and 3) wheat immune responses. Our preliminary results indicate pre- and co-inoculation of *Fp* with both NA1 and NA2 *Fg* strains resulted in significantly greater disease severity and deoxynivalenol (DON) toxin accumulation on cultivar Alsen heads compared to treatments of *Fg* alone. Similarly, on cultivar Norm heads, pre- and co-inoculation of *Fp* with the NA2 *Fg* strain caused higher FHB severity and DON contamination relative to NA2 *Fg* reference inoculation. For the NA1 strain treatments, no consistent statistically significant difference was noted in disease development and DON accumulation between pre- or co-inoculated heads as compared to the reference treatments of *Fg* strain inoculated alone. Analyses assessing the level of the plant defense phytohormones salicylic and jasmonic acids and expression patterns of their biosynthetic genes are underway to gain insight into the mechanisms underlying the observed disease outcome differences.

696B

Investigation of major surface proteins in cryptococcal immunogenicity

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Cryptococcus neoformans causes cryptococcosis that leads to lethal meningoencephalitis. *C. neoformans* enters the host through inhalation, replicates in the lung, disseminates to the blood stream, crosses the blood-brain barrier (BBB), and proliferates within the brain, causing lethal infection. Despite its disastrous global impacts, there is no cryptococcal vaccine, and the only approved treatment regimen against cryptococcosis is expensive and toxic and requires long duration. In order to fight this complex eukaryotic pathogen, we need to have deeper understanding of cryptococcal pathogenesis and identify critical immunogenic molecules to improve antifungal chemotherapy and guide vaccine development. Cryptococcal surface proteins play important roles in cryptococcal pathogenesis and immunogenicity. Five major surface proteins were among the top 50 most expressed cryptococcal genes in cerebrospinal fluid from human patients with cryptococcal meningoencephalitis infected with diverse isolates. Because they are glycosylated surface or secreted proteins that often act as ligands for receptors on host cells, they are strong candidates for protective antigens. The whole-cell cryptococcal vaccine *ZNF2*^{OE} strain exhibited increased exposure of surface proteins, indicating some surface proteins are likely protective antigens responsible for this protection. Based on these lines of evidence, some cryptococcal surface proteins must contribute to *ZNF2*^{OE}-mediated protection against cryptococcosis. Our lab has predicted major surface protein genes and constructed their corresponding overexpression and deletion mutants. These mutants will be screened for the genes involved in *ZNF2*^{OE}-mediated protection. To further determine the necessity and sufficiency of these antigens for *ZNF2*^{OE}-mediated protection, we will construct overexpression and deletion mutants of multiple responsible antigens and vaccinate the mice with those strains. This project will reveal novel protective antigens for cryptococcosis and provide a critical guidance for cryptococcal vaccine development.

697B

Signal Reliability and Nutritional Rewards Drive Divergent Evolutionary Trajectories in Nematode-Trapping Fungus *Arthrobotrys oligospora*

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Nematode-trapping fungi (NTFs) and nematodes cohabit diverse soil environments, engaging in a predator-prey relationship driven by the fungal requirement for nitrogen. The NTF *Arthrobotrys oligospora* functions as a facultative predator: under nutrient-replete conditions, it grows as a saprophyte; however, upon sensing low nitrogen and nematode signals (e.g., ascarosides), it initiates trap morphogenesis to develop specialized adhesive networks. This inducible strategy is considered an adaptation to conserve metabolic resources, ensuring energy-expensive traps form only when prey is present. Yet, the evolutionary forces and ecological trade-offs — specifically the balance between metabolic conservation and the sensory threshold required to initiate costly trap formation — remain experimentally underexplored. To investigate how nutritional reward and the predictive value of prey cues influence the evolutionary trajectory of these fungi, we employed a long-term experimental evolution approach. Here, we report findings from a 33-week snapshot where 12 replicate populations were evolved under three conditions: (1) Starvation Control; (2) Predation-Reward (continuous access to live prey); and (3) Interrupted-Induction (transient exposure to prey inducing traps sufficient to induce trap formation, followed by prey removal prior to capture). We observed high phenotypic divergence across the 36 evolved lineages. While the ancestor and Starvation Control lines largely retained inducible trap morphogenesis, spontaneous trap formation emerged as a distinct strategy in multiple lineages within both Predation-Reward and Interrupted-Induction groups. The emergence of this constitutive predatory lifestyle suggests that when prey is frequent, the need for rapid capture can override metabolic savings repressing trap production, driving a shift from a "reactive" to an "active" hunting strategy. The signal-exposed groups diverged in sensing strategies: unlike most Predation-Reward lines, the majority of Interrupted-Induction lines exhibited a secondary loss of pheromone sensitivity; maintaining expensive traps in response to transient, misleading signals would be maladaptive. In contrast, we identified a striking life-history trade-off between conidiation and predation in the Predation-Reward group. Analysis of conidiophore architecture indicated that investment in predatory traits limits reproductive potential. This trade-off was most pronounced in a divergent Predation-Reward lineage, which evolved a hyper-predatory phenotype characterized by robust spontaneous traps and heightened sensitivity, but severely reduced sporulation (forming single rather than multiple conidial clusters). Our results demonstrate that *A. oligospora* responsiveness to prey signals and its decision to produce traps rapidly evolve in response to selective pressures.

698B

IL-17 derepresses cytokine translation to induce immunity against candidiasis

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Candida albicans asymptomatically colonizes the oral cavity in most healthy individuals but can transition to a pathogenic state upon local or systemic immune compromise, leading to oropharyngeal candidiasis (OPC). During OPC, *C. albicans* invades oropharyngeal epithelial cells, which respond by secreting proinflammatory mediators that recruit and activate neutrophils. This response is amplified by IL-17, produced by various lymphocyte subsets. Human and animal studies confirm the central role of IL-17 signaling in host defense against OPC.

To elucidate oral epithelial responses to *C. albicans*, we performed bulk RNA-seq on infected human oral epithelial cells. Infection induced marked upregulation of transcripts encoding transcription factors such as c-FOS, c-JUN, NFκB1, and NFκB2, with immunoblot analysis confirming their phospho-activation. Consequently, genes encoding pro-inflammatory cytokines such as *IL-6*, *CXCL8*, *CSF2*, and *CSF3* exhibited massive mRNA induction—increasing by several hundred- to thousand-fold. However, their corresponding protein levels rose only modestly (2–4 fold), indicating substantial post-transcriptional regulation.

Using polysome profiling and SUrface SEnsing of Translation (SUnSET) assays combined with RNA-seq analysis of polysome-associated RNA, we found that *C. albicans* infection induces a broad translational repression in oral epithelial cells in vitro. This inhibition depended on the fungal adhesin/invasin Als3 and the toxin candidalysin. Mechanistically, infection activated host eukaryotic elongation factor 2 (EEF2) kinase, leading to EEF2 phosphorylation and subsequent arrest of translation elongation. This repression occurred independently of integrated stress response activation or formation of p-bodies/stress granules.

Notably, although the addition of IL-17 cytokine to infected cells did not reverse the global translation inhibition observed in polysome profiles, it acted synergistically to enhance the translation of a small subset of mRNAs including *NFKBIZ*, *MCPIP1*, *CXCL8*, and *CSF2*. Addition of IL-17 to *C. albicans* infected cells results in sustained activation of transcription factors, as well as selective de-repression of translation inhibition of the subset transcripts. Virtually all of the products of these transcripts are known to be required for the normal host defense against OPC. *C. albicans*-induced translational repression was verified in a murine OPC model.

In summary, we propose a model wherein *C. albicans* infection induces strong transcriptional activation in oral epithelial cells, counterbalanced by translational repression. IL-17 releases this repression for key immune genes, enabling a targeted inflammatory response. These results elucidate a pivotal mechanism by which the host response to an oral commensal is modulated until the fungus becomes pathogenic.

699B

A Toxic Paradox: Towards understanding how the blue mold fungus escapes patulin toxicity during host-microbe interactions

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Patulin is a polyketide lactone produced by at least 60 fungal species including *Penicillium griseofulvum*, *Aspergillus clavatus*, and the blue mold fungus, *Penicillium expansum*. Patulin was initially described as antibacterial but later was labeled a mycotoxin. *In vitro* studies show patulin has nephrotoxic, hepatoxic, and immunotoxic properties. Since *P. expansum* causes blue mold decay of apple fruit, processed fruit products (e.g. apple sauce, juice and butter) get contaminated with patulin which is a food safety concern. Many efforts have focused on understanding patulin biosynthesis, detection, and mitigation or detoxification. Unfortunately, the mechanisms that allow the fungus to evade patulin toxicity are not well understood. Recently, autotolerance to patulin was shown to be mediated in part by active efflux. Additionally, we hypothesize that multiple mechanisms are in place that may include decoy targets, sequestration, extra cellular biosynthesis, and metabolism. Thus, to understand how *P. expansum* can survive patulin exposure, we have implemented functional genetic and comparative genomic approaches. In this study, we screened a *P. expansum* random T-DNA library *in vitro* for patulin sensitivity. We treated spore suspensions from 313 random T-DNA isolates with purified patulin and measured colony growth. Nine isolates with reduced colony size, compared to the control, upon exposure to exogenous patulin were identified. The genomes of these isolates will be sequenced to identify the T-DNA insertion location and utilized in transcriptomic and metabolomic studies to elucidate patulin auto tolerance mechanisms.

700B

A tale of two Xylaria: exploring the genomic basis of opposing plant-fungal associations in shared hosts

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Fungi in the *Xylaria* genus (family Xylariaceae) are notable for their diverse host generalism and trophic plasticity, primarily as saprophytes and endophytes of plants but rarely as pathogens, however, the genetic signatures and molecular mechanisms driving these stable interactions remain largely unknown. While searching for *X. necrophora (Xn)*—the causal agent of taproot decline disease—on soybean plants originating from naturally infested plots, we isolated an endophytic strain of *X. multiplex* (*Xm* 22-34) from sterilized leaf tissue. *Xn* and *Xm* 22-34 were subsequently utilized for artificial inoculations on soybean, switchgrass and Arabidopsis seedlings to determine host-colonization and association states in vitro. Consistent with natural observations to date, the two *Xylaria* isolates produced host-dependent effects on seedling growth and health, with *Xn* causing negligible to modest reductions and *Xm* 22-34 promoting overall seedling vigor. Fluorescent microscopy of *Xn* and *Xm* 22-34 inoculated host roots also revealed the development of distinct mantle-like hyphal structures throughout the root system indicative of large-scale colonization. To investigate the genetic and potential epigenetic bases of these two association states, we generated high-quality genome assemblies for both *Xylaria* using Oxford Nanopore Technology long-read sequencing with methylation-aware basecalling. The genome size, structure and biosynthetic gene cluster content were found to be similar between the two fungi, suggesting other factors are likely involved in shifting host-association dynamics. Conversely, we hypothesize that regulatory mechanisms could be involved in modulating secondary metabolism and candidate effector expression that would regulate the establishment of host symbiotic states. This investigation also takes advantage of recent advances in direct modified-base prediction to compare the methylome landscapes of *Xn* and *Xm* 22-34. Methylation analysis revealed common patterns surrounding gene coding regions and strain specific patterns that could affect transcriptional regulation in both species. Overall, this work lays the foundation for downstream -omics and molecular investigations to pinpoint mechanistic variations governing pathogen and endophyte host-association states in *Xylaria* fungi.

702B

Decoding Phenazine Stress Responses in *Aspergillus calidoustus* During Protection by *Paraburkholderia edwinii*

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The composition of microbial communities is modulated by a number of factors, including the antimicrobial-compounds(AMC) secreted by several members of the community. Phenazines, a category of redox-active compounds produced by a wide variety of soil bacteria, are generally harmful to fungi. In spite of this, from a phenazine-rich niche, Dahlstrom & Newman (2022) reported the co-isolation of Aspergillus calidoustus with a protective bacterium, Paraburkholderia edwinii. Further-work also showed that the novel species *Paraburkholderia edwinii* can protect *Aspergillus calidoustus* from phenazine-1-carboxylic acid (PCA) by absorbing the toxin and sequestering it in a reducing environment. Our research builds on this base by identifying which *A. calidoustus* genes aid in survival when under PCA stress in the presence of the protective bacterium. We identified a group of genes that are particularly elevated during co-culture with P.edwinii during PCA exposure using RNA-seq. These include genes involved in membrane transport, ROS detoxification, stress response pathways, and a number of transcription factors.

We have also identified two specific candidate genes to focus on. The first, CDR1_2, has a high degree of similarity to Cdr1B, an ABC efflux transporter in *Aspergillus fumigatus* that is known to mediate azole resistance by expelling itraconazole and other azoles. The second potential candidate encodes a ROS-detoxifying enzyme, which may help *A. calidoustus* reduce oxidative stress brought on by phenazine exposure accompanied the protective partnership. We are now employing CRISPR–Cas9 with homology-directed repair (HDR) to create targeted knockouts in order to experimentally confirm these candidates. Using PEG-mediated transformation, editing constructs are introduced into fungal protoplasts to accomplish accurate gene replacement. The PCA-stress phenotypes of the resulting mutants will be evaluated alone and in co-culture with P. edwinii.

This study offers insight into how fungi survive in chemically harsh environments by defining the fungal genetic responses to phenazine stress in the presence of a protective bacterial partner. In a larger sense, these results may shed light on how fungal-bacterial interactions play a role in the development or alteration of antifungal resistance.

703B

A link between calcineurin and the amino acid permease, BycA, for virulence in *Mucor*

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Mucormycosis is a recently emerging fungal infection found mainly in immunocompromised individuals. Fungi from the order Mucorales are the causative agent of mucormycosis. Mucorales fungi are highly resistant to antifungal drugs, and treatment options remain limited. Calcineurin, a Serine/Threonine phosphatase, is a well-known virulence factor in many human pathogenic fungi that is required for invasive hyphal growth in the model Mucorales *Mucor*. Calcineurin is therefore an attractive antifungal drug target; however, it is highly conserved in humans and fungi, making direct inhibition impossible. Thus, this study is to investigate the specific fungal downstream of calcineurin, the amino acid permease, BycA that is absent in mammalian cells to alternatively target calcineurin. Calcineurin is a negative regulator of *bycA* which inhibits invasive hyphal growth by triggering yeast growth. We hypothesize that a transcription regulator(s) (TRs) in the downstream of calcineurin regulates the expression of *bycA* during invasive hyphal growth. We have identified eight candidate TRs downstream of calcineurin via comparative phosphoproteomics and have successfully generated either knockout or knockdown mutants of each candidate. In addition to this, we have identified eight additional candidate TRs that bind to the *bycA* promoter by utilizing the yeast one-hybrid system and further study is in progress to generate deletion mutants to investigate *bycA* gene expression and characterize the phenotypes. Through this study, we aim to identify a TR(s) that is involved in the regulation of the expression of the *bycA* gene, to alternatively target calcineurin and control Mucormycosis infections.

704B

The core and accessory gene repertoire of rust fungi (pucciniales): Genomic insights into their adaptive evolution

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The order Pucciniales (rust fungi) comprises an ecologically diverse group of obligate biotrophic plant pathogens that infect crops such as cereals, soybean, and coffee. These fungi typically undergo rapid evolutionary changes driven by host-selective pressures; however, the mechanisms underlying their genetic adaptability remain poorly understood. In this study, we characterized core, accessory, and singleton gene repertoires of Pucciniales. Our analysis utilized publicly available high-quality genome assemblies from 12 Pucciniales species, including the soybean rust *Phakopsora pachyrhizi*. We observed striking variation in the distribution of core genes across Pucciniales genomes, with core gene frequencies ranging from 23.74% (*Puccinia graminis*) to 34.41% (*Puccinia triticina*). On average, accessory gene frequency (44.61%) was larger than core genes. The highest proportions of accessory genes (>55%) occurred in *Melampsora* spp and *P. pachyrhizi*. The myrtle rust fungus, *Austropuccinia psidii*, stood out for having 52% of its genes classified as singletons. In contrast, *Melampsora* species and *P. pachyrhizi* exhibited the lowest frequencies of singleton genes, ranging from 5.07% to 12.75%. A PCA analysis revealed that the accessory gene composition of Pucciniales formed three major clusters, reflecting distinct evolutionary trajectories among *Melampsora* spp, *P. pachyrhizi,* and *Puccinia* spp. We identified multiple expansions and contractions of genes within the 119 core and 365 accessory orthogroups across Pucciniales. The *P. pachyrhizi* genome, specifically, displayed 40 expanded core and 62 expanded accessory orthogroups. The expanded core and accessory orthogroups of *P. pachyrhizi* included primarily G25 CAZyme family, S8/S53 Peptidase, Homeobox transcription factors, Ca2+/cation antiporter (CaCA) family, and effector genes with unknown functions. As expected, transposable elements (TE) contributed intensively to the expansion of both core and accessory components in *P. pachyrhizi*, with 582 accessory genes localized within TEs. We conclude that rust fungi exhibit substantial variation in the size and composition of their core, accessory, and singleton gene repertoire. This highly variable repertoire was likely shaped by gene gains and losses through TE activity, particularly in *P. pachyrhizi*. Our study sheds light on the genomic and evolutionary processes influencing adaptation in Pucciniales.

705B

Study of Virulence and Fungicide Resistance in *Fusarium oxysporum* f. sp. *mori*

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Fusarium oxysporum f. sp. *mori* is the phytopathogen responsible for causing Fusarium wilt in blackberry crops in the United States. Notably, this fungus has also been detected in Michoacán, Mexico, the world's leading blackberry producer. Field management of Fusarium wilt primarily relies on fungicide applications; however, prolonged and frequent use of these chemicals has led to the emergence of *F. oxysporum* resistant strains. Fungicide resistance is defined as the acquired and inherited insensitivity of a fungal species to a specific antifungal or fungicidal compound, which significantly compromises disease control. In this study, *F. oxysporum* f. sp. *mori* isolates were obtained from blackberry plants exhibiting vascular wilt symptoms in Mexico. The isolates were morphologically and genetically characterized, and their pathogenicity was assessed by testing their ability to colonize and induce symptoms in both blackberry and strawberry plants. Additionally, fungicide sensitivity assays were performed to evaluate the isolates' resistance to the most commonly used fungicides for controlling *Fusarium* species and *F. oxysporum* f. sp. *mori* in the field.

706B

Mariner-Based Transposon Mutagenesis Identifies *Paraburkholderia edwinii* Genes Required for Protecting *Aspergillus calidoustus* Under Phenazine Stress

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The soil bacterium *Paraburkholderia edwinii* possesses the ability to protect the filamentous fungus *Aspergillus calidoustus* from fungistatic, redox-active molecules called phenazines. Phenazines are produced by bacteria within microbial communities and have an effect on shaping the species-level

composition of microbial communities found in various environmental and healthcare settings. *P. edwinii* has the ability to form aggregates within the mycelium of *A. calidoustus*, where it sequesters and reduces a common phenazine called phenazine-1-carboxylic acid (PCA) into a less toxic form. This bacterial protection response is triggered by the acidic microenvironment created by *A. calidoustus* during PCA stress. The genes underlying this unique protection response remain mostly unknown. To investigate the genes that *P. edwinii* utilizes to sequester and reduce PCA, an axenic mariner-based transposon mutagenesis assay was performed. Here, transposon mutants are plated onto a pH adjusted agar plate containing PCA, mimicking the presence of the fungus, which triggers the bacterial protection response. On this medium, the wild-type bacteria appear yellow, indicating the reduction of PCA into its hydroquinone form, which produces a yellow pigment. Transposon mutant colonies that appear as pale-yellow/white serve as an indicator of deficiencies in PCA sequestration or reduction. The transposon mutants that show deficiencies in sequestration or reduction are then subjected to a co-colony protection assay with *A. calidoustus* to test the bacterium's ability to protect the fungus from PCA toxicity. The candidate mutants that show phenotypic differences in the protection of *A. calidoustus* are then subjected to whole genome sequencing to identify and analyze the disrupted genes. This study aims to identify the bacterial genes that are responsible for the sequestration and reduction of PCA. Preliminary results have identified genes involved in metabolic, membrane, and transport functions as being critical to PCA reduction and sequestration. The knowledge gained from this research will aid in understanding bacterial-fungal interactions in competitive microbial environments.

707B

A co-evolved peptide-GPCR system senses host entry to drive fungal infection

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Interactions between fungi and their hosts rely on precise sensing mechanisms for environmental cues, signaling molecules, and nutrients detection, often mediated by G-protein coupled receptors (GPCRs). In plant-associated fungi, GPCRs play a crucial role in sensing host-derived signals that trigger transitions lifestyles. Yet, the precise mechanisms by which fungal GPCRs recognize and respond remain largely unexplored. Based on recent developments in structure prediction, we investigated the presence of seven-transmembrane (7TM) proteins in the plant pathogen *Ustilago maydis*. Our analysis identified potential GPCRs candidates, one of them being previously reported as an important virulence factor. Interestingly, a secreted protein is neighboring this GPCR candidate on the genome. This secreted protein, called Pit2, is then processed by plant proteases during host infection and we demonstrated that one of its cleavage products, a 9 amino acid peptide-like pheromone contained within its core, activates the G-protein-coupled receptor promoting fungal in planta expansion (Gpe1). Our results show that this GPCR acts as a molecular gatekeeper, detecting host entry by recognizing a highly hydrophobic peptide motif. We solved the crystal structure of Pit2 at 2.3 Å resolution and show that Pit2 forms a tightly intertwined homodimer that buries the conserved WWFGFTGSL motif in its dimer interface, supporting a scaffold role that shields the ligand before host-dependent release. AlphaFold3 modelling of the Gpe1–peptide complex implicates residues in Transmembrane Helix 1 and Extracellular Loop 2 in ligand binding confirmed by structure-guided mutagenesis of these residues compromises both receptor activation and virulence in maize plant infections. Comparative analyses also reveal that the Gpe1/Pit2 module is conserved in related smut fungi, with species-specific peptide–receptor pairs that limit cross-activation. Furthermore, live-cell imaging in axenic cultures shows in peptide presence it triggers Gpe1-dependent changes in endomembrane dynamics and promote fungal proliferation by enhancing cell division. Together, our findings reveal a co-evolved mechanism between fungus and host that encodes environmental context into a protein scaffold, establishing a novel paradigm for host-dependent signaling with implications for inter-organismic communication.

708B

An OMICS view of the interaction established between the commensal bacterium *Lactobacillus gasseri* and the pathogenic yeast *Candida glabrata*

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The success of *C. glabrata* as a human commensal and pathogen depends on its ability to cope with a competing bacterial microflora that includes, in the vaginal and in the GI tract, the poorly characterized *L. gasseri* bacterial species. While in co- culture (both in planktonic or in biofilms) we found that *L. gasseri* reduces growth rate, viability and pathogenesis of *C. glabrata*, a phenotype that is, in part, prompted by the presence of acetate that enhances bacterial virulence towards the yeast. To better understand this result and foster the development of *L. gasseri*-based anti-*Candida* treatments, we explored a range of OMICS analyses that scrutinize, in detail, this bacteria-yeast interaction. In this work we will discuss those results, that allowed us to put together the landscape of how these two species interact, including the identification of metabolites produced by the bacterium that showed anti-*Candida* effect. Gathering results from phenOMICs experiments (undertaken in planktonic and in biofilms) we could also identify a set of *C. glabrata* mutants highly sensitive to the presence of *L. gasseri*. Leveraging these results, we chemically inhibited one of the signalling pathways determining tolerance of *C. glabrata* to *L. gasseri* resulting in a prominent loss of competitiveness of the yeast in a co-culture with the bacterium.

709B

Generalist pathogen *Botrytis cinerea* shows host-specific responses to closely related legumes

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Botrytis cinerea is a broad host-range pathogen that infects over 1,400 plant species, yet the molecular mechanisms enabling it to infect diverse hosts are not understood. Recent evidence has shown that *B. cinerea* can alter its gene expression to create host-specific programs to facilitate broad range infection. While this indicates *B. cinerea* senses and responds differently to diverse hosts, it is unclear how different the induced virulence mechanisms may be when infecting closely related hosts. To investigate how this generalist pathogen responds to closely related hosts, we inoculated 72 genetically diverse *B. cinerea* isolates onto two legume species, common bean (*Phaseolus vulgaris*) and cowpea (*Vigna unguiculata*). Lesion formation was strongly driven by pathogen isolate, with limited effects of host species, suggesting that *B. cinerea* virulence is broadly conserved across these hosts. In contrast, transcriptomic profiling of *B. cinerea* during infection revealed extensive variation in gene expression across isolates and hosts. *Most B. cinerea* genes were differentially expressed across isolates, and a large fraction exhibited significant host-by-isolate interactions, indicating widespread fine-tuning of the pathogen transcriptome depending on host background. Differential expression and co-expression analyses identified host-specific reprogramming of

known and novel virulence pathways. Analysis of genes differentially expressed on either host showed host specificity of many cell wall modifying enzymes and specialized metabolic biosynthetic enzymes. Co-expression networks for an NRPS (non-ribosomal peptide) biosynthetic gene cluster showed strong host specificity across isolates, resulting in specific modifications to the network when infecting either host. Together, these results suggest that while *B. cinerea* maintains robust virulence across closely related hosts, it fine-tunes its underlying molecular strategies in a host-dependent manner.

710B

Parastagonospora nodorum necrotrophic effector SnTox267 targets a wheat susceptibility pathway to facilitate mesophyll colonization and drive in planta infection

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Parastagonospora nodorum is a necrotrophic fungal pathogen that causes septoria nodorum blotch (SNB) of wheat. *P. nodorum* secretes multiple necrotrophic effectors that exploit host susceptibility pathways. Among them, SnTox267 targets three wheat genes: *Snn2*, *Snn6,* and *Snn7*, with each playing a critical role in susceptibility and disease development. A previous study determined that *Snn2* and *Snn6* are likely components in the same pathway, and both dominant genes are required to induce light-dependent programmed cell death (PCD). However, the SnTox267-Snn7 interaction was shown to use a light-independent PCD pathway to amplify susceptibility. Current evidence indicates that *Snn2* and *Snn6* are also essential for *Snn7*-mediated PCD, however it remains unclear whether they are components of the same or distinct signaling pathways. SnTox267 contains a predicted β-barrel structure, like that verified in SnTox3 and predicted in SnTox5. Because SnTox3 interacts with host PR proteins, and SnTox5 facilitates mesophyll colonization, the structural similarity suggests that SnTox267 may also have an additional role beyond PCD induction. However, SnTox267 is one of the least functionally characterized of the known necrotrophic effectors, and its precise role in wheat colonization and additional modes of action are unknown. To address these gaps, we compared macroscopic lesions and microscopic in planta colonization, complemented by oxidative burst assays, to determine how *P. nodorum* uses SnTox267 to complete its life cycle and to identify additional roles beyond PCD induction. These assays showed that SnTox267, as well as both *Snn2* and *Snn6*, are required for *P. nodorum* colonization of the wheat mesophyll cells, resulting in a strong oxidative burst and the development of coalescing necrotic lesions. Additionally, when *Snn7* is present with *Snn2* and *Snn6*, *P. nodorum* isolates expressing SnTox267 colonized the mesophyll at earlier timepoints relative to lines only harboring Snn2 and Snn6, resulting in a sustained oxidative burst and earlier macroscopic lesion development. Collectively, the results showed that the SnTox267-*Snn2/Snn6* pathway interaction facilitates mesophyll colonization and is critical for *P. nodorum*'s infection cycle, while *Snn7* serves to amplify susceptibility, resulting in more effective colonization.

711B

A candidate effector modulates virulence dependent on temperature and host phosphate status by regulating root colonization

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The genus *Colletotrichum* includes both devastating pathogens and beneficial endophytes. The plant-associated species *C. tofieldiae* (Ct) has diverse lifestyles, ranging from pathogenic to mutualistic, depending on the strain and/or environment. Strain Ct3 is a pathogen at 22°C, but at 26°C, it instead promotes plant growth in the model plant *Arabidopsis thaliana*. To investigate the genetic basis of this lifestyle transition, we performed *in planta* transcriptome profiling of Ct3 at 22°C and 26°C. We found that effectors were enriched in the set of down-regulated genes, and in particular, a predicted effector gene, which we here call *Necessary for Full Colonization 1* (*NFC1*), was expressed at 22°C under high phosphate conditions, but not under low phosphate conditions or at 26°C. We next performed a time-course inoculation with Ct3 and found that *NFC1* was expressed especially strongly in the early infection stage. We therefore generated a targeted knock-out of *NFC1* in the Ct3 background, and we found that the *nfc1* mutant had reduced virulence and root fungal biomass compared to wild-type Ct3, potentially indicating an important role in colonization. Given the expression profile of *NFC1*, we hypothesized that its expression, and thus contribution to virulence, might depend on the plant phosphate starvation response (PSR). Surprisingly, we found that the phenotype of *nfc1* in *phr1phl1* plants disrupted in the PSR regulators mimicked wild-type plants, indicating that the PSR regulators do not play a role in regulating *NFC1*. However, in the constitutively phosphate-starved phosphate transport facilitator mutant *phf1*, *NFC1* expression was dramatically reduced even under high environmental phosphate conditions, indicating that the plant phosphate status is an important determinant of *NFC1* expression level. Interestingly, in *pho1* mutants–which exhibit shoot phosphate deficiency despite root sufficiency–the *nfc1* mutation resulted in root colonization and a plant growth phenotype similar to that of wild-type Ct3. Considering that *NFC1* expression is attenuated under low Pi conditions, this finding suggests the existence of a shoot-to-root signal transduced independently of the PSR regulators PHR1/PHL1. Together, these data identify *NFC1* as a candidate effector gene that functions in root colonization, with expression level dependent both on temperature and on host phosphate status. Next, we intend to apply yeast two-hybrid to identify the host target of NFC1 and *in planta* RNA-sequencing of the *nfc1* mutant and a complementation line, in addition to wild-type Ct3, in wild-type and *phf1* plants to identify the host response to *NFC1*.

712B

Copper homeostasis links plant colonization and trap formation in the nematode-trapping fungus *Arthrobotrys flagrans*

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Plant-parasitic nematodes are major agricultural pests responsible for over US$150 billion in annual crop losses. Their management depends largely on synthetic nematicides, whose environmental impact and increasing regulatory restrictions highlight the need for sustainable alternatives. Nematode-trapping fungi (NTF) offer a promising solution. These soilborne fungi grow saprophytically, but can switch to a predatory lifestyle by forming specialized traps. Species such as *Arthrobotrys flagrans* and *A. oligospora* show strong biocontrol potential against root-knot nematodes such as *Meloidogyne incognita* or *M. javanica*, and have been linked to enhanced nutrient uptake and growth in tomato plants. In our group, we are investigating the ability of *A. flagrans* as a fungal endophyte to control the plant parasitic nematode *M. javanica*. RNA-seq analyses revealed that interaction with *Arabidopsis thaliana* or *Solanum lycopersicum* induced *A. flagrans* genes associated with copper starvation. We investigated fungal growth and transcriptional response under varying copper conditions and our results interestingly revealed a strong induction of copper-deficiency genes not only during plant colonization but also during trap formation. In particular, we found a putative small cluster of three genes, containing two X325 LPMO-like proteins and the

copper transporter CtrC, which is specially upregulated during both, plant colonization and trap formation. On top of that, one of the X325 proteins, named CblB, whose putative orthologue in *Cryptococcus neoformans* (Cbi1) had been found to be involved in copper uptake and virulence, was strongly induced in all conditions analyzyed. We are currently molecularly dissecting the copper-dependent mechanisms underlying root colonization and trap development, with a particular focus on this gene cluster during the interaction with *M. javanica*. Our results suggest that copper limitation is a shared feature of plant colonization and nematode predation and that copper homeostasis could be central to the NTF biology and thus, to the fight against plant parasitic nematodes.

713B

Understanding the mechanisms of antifungal response in an emerging fungal pathogen, *Basidiobolus*

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Fungal infections are a major global health threat, driven by the rise of antifungal resistance and emerging pathogens. Current antifungals primarily target conserved cell membrane or cell wall synthesis pathways in the most critical fungal pathogens. However, a distantly related genus in Zoopagomycota, *Basidiobolus*, has demonstrated the potential to become an increasing threat. This fungus is globally distributed as a herptile commensal, saprobe, and occasional mammalian pathogen.

A review of 171 cases since 1954 indicates a shift in cases occurring farther from equatorial regions and a rising prevalence of gastrointestinal basidiobolomycosis, the form of infection with the highest mortality rate. Case prevalence has also shifted from humid, tropical regions to arid regions. Both polyenes and echinocandins are consistently ineffective against *Basidiobolus*, leaving azoles as the only viable treatment.

This project investigates functional antifungal response in *Basidiobolus* using in-vitro assays for the three most common treatments, amphotericin B, itraconazole, and fluconazole. The isolates used include a selection of type, environmental, and clinical isolates. Across all isolates and antifungals tested, IC_{50} values exceeding clinical breakpoint concentrations established for other fungal pathogens, indicating potential antifungal tolerance.

To investigate the underlying mechanisms behind this response, amphotericin B was selected for transcriptomic analysis due to its effects broadly in oxidative and membrane damage rather than acting on a single enzyme target. Differential gene expressions between control and IC_{50} amphotericin B conditions will identify membrane remodeling and stress response in *Basidiobolus*. This will provide insights into the genetics and genomic mechanisms of antifungal response in a non-Dikarya fungal pathogen.

714B

Metabolic plasticity and virulence of *Cryptococcus neoformans* are regulated by mitochondrial homeostasis

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For microbes to establish themselves in a host, they must be able to assimilate the locally available nutrients. Nutrient assimilation is a fundamental process required for the growth, development, reproduction, and survival of pathogens. However, host niches are dynamic environments where nutrient availability can vary. For instance, nutrients such as iron are often denied by host defense mechanisms. Therefore, microbes must adapt to these microenvironments by regulating nutrient uptake mechanisms and metabolism. This metabolic plasticity is a key feature that enables microbes to thrive during host-microbe interactions. The mitochondrion is a versatile organelle that plays key roles in diverse processes, including metabolic adaptation, metal homeostasis, plasma membrane and cell wall integrity, stress response, and thermotolerance, in addition to its role in generating energy. Consequently, mitochondrial fitness is essential for fungal pathogens. *Cryptococcus neoformans* is a fungal pathogen responsible for over 180,000 HIV-related deaths each year, primarily in immunosuppressed individuals. In this work, we analyzed *C. neoformans* metabolic plasticity when grown with non-fermentable carbon sources and their impact on virulence and mitochondrial homeostasis. Growth on non-fermentable carbon sources resulted in increased thermotolerance, glucuronoxylomannan (GMX) content in the capsule, melanization rate, urease activity, and biofilm formation. Moreover, cells grown on non-fermentable carbon sources manifested increased mitochondrial number, activity, and virulence. Conversely, mutants of the master regulator of mitochondrial biogenesis, known as the HAP complex, the catalytic subunit 1 of protein kinase A, or yeast cells grown in media supplemented with antioxidants, exhibited decreased ability to use alternative carbon sources. This metabolic impairment also impacted capsule formation, melanin synthesis, urease activity, mitochondrial number, and virulence. Overall, our results indicate that mitochondrial biogenesis plays a significant role in regulating virulence via the PKA pathway, suggesting that targeting mitochondrial homeostasis could be a potential strategy for treating cryptococcosis.

715B

Genetic Basis for a Bacterium Acting as a Toxin Sponge to Protect its Fungal Partner from Phenazines

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Phenazine-1-carboxylic acid (PCA) is a bacterially produced redox-active antifungal that is abundant in many environments. It was recently discovered that PCA-susceptible fungi, such as *Aspergillus calidoustus*, can gain access to PCA-abundant environments by physically associating with native phenazine-tolerant bacteria, such as the novel species *Paraburkholderia edwinii*. *P. edwinii's* protection response is characterized by the sequestration and reduction of PCA, rendering PCA less toxic to the fungus. Although *P. edwinnii's* protection response has been characterized, little is known about the molecular mechanisms involved. The deletion of the transcriptional repressor, *hrcA*, in *P. edwinii* enhances the protection response and allows for increased fungal growth, suggesting HrcA's involvement in protection response pathways. An RNA-seq comparing the Δ*hrcA* and wild-type strains of *P. edwinii* revealed that putative Csu-type pilus subunits (*csuA* and *csuB*) are upregulated in the Δ*hrcA* background. *csuA* and *csuB* are the first and last genes in a putative chaperon-usher pathway operon, respectively. The Δ*csuA* deletion strain and the double deletion strain Δ*csuA* Δ*csuB* are defective in protecting the partner fungus from PCA, as demonstrated by a significant decrease in total co-colony area. Moreover, the role of CsuA and CsuB is specific to the protection of the fungal partner and not a result of a bacterial growth defect in our conditions. Preliminary data indicates that Csu-type pili are involved in both fungal association and PCA detoxification. The Csu-type pili are required for bacterial adhesion to *A. calidoutus* spores in the presence of PCA, indicating these pili play a role in fungal partner association. Additionally, the Δ*csuA* and Δ*csuA* Δ*csuB* strains failed to produce the same low redox

potential that wildtype uses to detoxify PCA, suggesting a defect in reducing toxic molecules. Understanding how *P. edwinii* utilizes Csu type-pili to associate with and protect its fungal partner from PCA will further our knowledge of the mechanisms used by protective microbial partnerships to gain entry to AMC-abundant communities.

716C

Kinase-regulated development of plant surface penetration structures in B. cinerea and M. oryzae

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Botrytis cinerea and *Magnaporthe oryzae* are distantly related plant pathogenic fungal species that develop distinct penetration structures for breaching the plant host surface. Where *M. oryzae* develops a highly melanized, single-celled appressorium, *Botrytis cinerea* can develop two distinct structures for penetrating the host surface: a unicellular appressorium with little melanization that penetrates a single host cell, and a branched infection cushion that penetrates multiple sites and enzymatically breaks down the host surface. Homologous regulatory kinases have been identified in multiple pathogenic fungal species (including *M. oryzae* and *B. cinerea*), such as the master regulator MoPmk1/BcBmp1, and autophagy regulating kinase ATG1. This suggests the presence of a conserved regulatory pathway underlying fungal appressorium formation. We aimed to infer and compare the kinase regulatory networks in both species, to find shared regulatory pathway elements that are likely conserved in other fungal plant pathogens, and to investigate differences between the regulation of appressorium and infection cushion development. To investigate the role of kinase signaling in the appressorium formation in *M. oryzae*, time-dependent phospho-proteomics data was integrated with homology-derived kinase motifs to infer a kinase signaling network. Experimental protocols were developed to also obtain dynamic phospho-proteomics data for both penetration structures formed by *B. cinerea*, which allows for the comparison between the inferred networks between the two species. Our results show that in both species, a similar core set of kinases is activated during the late stage of appressorium formation, including MoPmk1/BcBmp1, Hog1, Gsk1, and Yak1. This suggests that phosphorylation of kinases and target proteins may be shared across evolutionary time, with key nodes differentiating the infection strategies employed by *B. cinerea*.

717C

Beyond Single-Pathogen Paradigms: A Meta-Pathogen Approach to Understanding Mycotoxin Production in Fusarium Head Blight

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Fusarium head blight (FHB) represents one of the most economically devastating diseases of wheat worldwide, with over 20 filamentous fungal species implicated in disease development and mycotoxin contamination. In European wheat production, seven predominant *Fusarium* species, including *F. graminearum*, co-occur and produce a complex array of mycotoxins—type A and B trichothecenes, zearalenone, enniatins, and beauvericin—that pose significant threats to food safety and public health.

Traditional research has approached FHB through a reductionist "one pathogen-one disease" framework, investigating individual species in isolation. However, this paradigm has proven inadequate for predicting mycotoxin accumulation patterns and developing effective control strategies in field conditions where multiple species coexist and interact. The dynamic interplay among co-occurring *Fusarium* species within the shared ecological niche of wheat ears likely generates emergent properties in mycotoxin regulation that cannot be predicted from single-species studies.

This research introduces a novel meta-pathogen conceptual framework that treats the seven most prevalent European *Fusarium* species as a single, integrated pathogenic system. By examining the molecular mechanisms governing mycotoxin production within this meta-*Fusarium* complex, we aim to elucidate how species interactions influence toxin profiles and accumulation dynamics. This holistic approach promises to reveal regulatory networks and interaction effects that are invisible to single-species analyses, ultimately providing a more robust foundation for developing integrated mycotoxin management strategies in wheat production systems.

718C

Rho-GDP dissociation inhibitor modulates aflatoxin biosynthesis and virulence in *Aspergillus flavus*.

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Aspergillus flavus affects human health in two main ways: mycotoxicosis and invasive disease. The major mycotoxin produced by *A. flavus*, aflatoxin (AF), produces hepatotoxicity, nephrotoxicity, and immune suppression when ingested at high doses. AF biosynthesis is regulated via complex transcriptional and post-transcriptional mechanisms, with the pathway-specific transcription factor *aflR* playing a central role. Transcriptomic comparisons under AF-conducive and non-conducive conditions identified a clade of genes with a similar transcription profile to that of *aflR*. Among these, a putative Rho-GDP dissociation inhibitor (Rho-GDI; GeneID G4B84_005214) was characterized by gene deletion and shown to regulate AF production and virulence in *Aspergillus flavus*. The encoded protein, RdiA, shared 45% identity to Rdi1p in *S. cerevisiae*. Deletion of *rdiA* resulted in increased septation intervals, altered hyphal branching, and severely decreased AF production. The Δ*rdiA* mutant phenotypes are more like the *rdi1*Δ mutant of *Cryptococcus neoformans* and the *bem4*Δ mutant in *S. cerevisiae*, including severe growth defects on minimal medium, moderate defects on complete medium, and cold sensitivity. Interestingly, the *A. flavus rdiA* gene appears at least partially to rescue the cold-sensitive phenotype of the *S. cerevisiae bem4*Δ mutant, suggesting a potential genetic link with RasA, a known regulator of AF biosynthesis. Consistent with findings in *C. neoformans*, the *A. flavus* Δ*rdiA* mutant displayed significantly attenuated virulence in an invertebrate model. Due to the shared phenotypes with the *C. neoformans rdi1Δ* mutant, we will

complement the Δ*rdiA* mutation in *A. flavus* and use the *A. flavus rdiA* gene to complement the *rdi1*Δ mutation in *C. neoformans*, thus confirming the identity and function of *rdiA*.

719C

Genomic, transcriptomic and molecular study of lichenization in the model lichen *Xanthoria parietina*

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Lichens represent complex multipartite symbioses consisting of lichen fungal symbionts (LFS), photosynthetic partners (chlorophyte algae or cyanobacteria), and diverse microbial communities including bacteria and yeasts that colonize the thallus after its formation through lichenization. The common lichen species *Xanthoria parietina,* that associates with the alga *Trebouxia sp.*, has been extensively investigated using metagenomic and metatranscriptomic approaches, establishing it as a model system for understanding lichen symbiosis and lichenization. Comprehensive analyses revealed putative immunity-related proteins alongside lichen-specific, structurally conserved secreted proteins exhibiting similarity to fungal pathogen effectors. These findings suggest that effector-like proteins may play crucial roles in modulating host immunity during symbiosis establishment and maintenance. To explore the evolutionary conservation and functional mechanisms of these effectors, referred to as Xanthoria effector proteins (Xep), I investigated their evolution and distribution across fungi using comparative phylogenomics approaches. Among the candidate effectors identified, Xep4 encodes a thaumatin-like effector which is a class of pathogens effectors known to target plant chloroplast protein called rubisco activase (RCA) to modulate the host response and increase plant susceptibility. Heterologous expression of Xep4 in *Nicotiana benthamiana* demonstrated that this effector is computationally predicted and targeted to the chloroplast, and its localization depends on the protein's chloroplast targeting peptide (cTP). Additionally, yeast-2-hybrid and co-immunoprecipitation-mass spectrometry (IPMS) analyses revealed that Xep4 and *Trebouxia sp.* RCA interact, indicating that the protein-protein interactions might be crucial for lichenization. Finally, I will present further work conducted on *Chlamydomonas reinhardtii*, the model chlorophyte alga closely related to lichen algal symbionts, which deepens our understanding of the role of Xep in lichen symbiosis.

720C

Host-Fungal Interactions at the Base of Animal Evolution

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Candida albicans is a common human fungal pathogen capable of causing severe disease in immunocompromised individuals. While typically commensal, it can become opportunistic and cause severe infections in immunocompromised patients. Despite its clinical relevance, the innate and mucosal immune responses that control *C. albicans* infection remain incompletely understood. To investigate how fungal pathogens shape mucosal immunity, we established a novel infection model using *C. albicans* and *Hydra*, an early-diverging metazoan with a simple but robust innate immune system. We characterized the progression and resolution of infection over a seven-day time course, monitoring morphological changes and injury using a quantitative *Hydra* Health Index. *C. albicans* induced dose-dependent pathology that varied among *Hydra* species, yet all species exhibited recovery by day seven. Through analysis of infection stages and recovery dynamics, we aim to identify innate immune regulators and pathways activated in response to fungal challenge using RNA-Seq. Additionally, we are examining the gene expression patterns of *C. albicans* in response to *Hydra*'s innate immune interventions over the course of infection. Leveraging *Hydra* as a whole-animal mucosal model provides a powerful system for dissecting *Candida–host* interactions, enabling the study of innate defenses in the absence of adaptive immunity and offering insight into evolutionarily conserved mechanisms of mucosal immune protection.

721C

Intracellular metabolism of the fungal pathogen Histoplasma capsulatum

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The fungal pathogen Histoplasma capsulatum infects and proliferates within host phagocytes, turning these normally fungicidal immune cells into permissive host environments. Within the phagosomal compartment of host cells, Histoplasma must acquire suitable resources for proliferation. However, the host molecules used by Histoplasma for energy and biosynthesis are largely undefined. Initial genetic studies implicated amino acids as the primary source of nutritional carbon used by Histoplasma within the phagosome. Histoplasma is unable to grow on proteins unless they are first digested by proteinases suggesting that amino acids are acquired either as peptides or free amino acids. To fully define the intracellular metabolism of Histoplasma, we integrated genetic and transcriptional studies with targeted metabolomic profiling of macrophages and intracellular Histoplasma yeasts. Our results reveal that not all amino acids are sufficiently present within the phagosome, indicating that Histoplasma uses select metabolic pathways for intracellular proliferation. Metabolite analysis of intracellular yeasts shows cysteine, leucine, and phenylalanine are consumed by Histoplasma within the phagosome. 13C-metabolic flux analysis showed significant carbon flux through the mannitol pathway and the methylcitrate cycle, which may facilitate NADPH generation and detoxification of branched chain amino acid intermediates, respectively during amino acid catabolism. Together these results provide a framework for understanding how Histoplasma acquires and metabolizes nutritional molecules to proliferate within the phagosome of host cells during infection.

722C

Genome-wide association mapping study-based identification of a candidate effector region in Cercospora sojina linked to Rcs2-mediated avirulence

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Cercospora sojina Hara is a destructive foliar fungal pathogen of soybean that causes frogeye leaf spot (FLS) disease, which can cause yield losses up to 60% under favorable conditions. C. sojina is primarily managed through fungicide application and planting genetically resistant cultivars. To date, three resistance genes, Rcs1, Rcs2, and Rcs3, have been identified and characterized in C. sojina. Both Rcs1 and Rcs2 have been overcome by C. sojina, leaving Rcs3 as the only reliable resistance gene. According to the gene-for-gene model, genetic resistance is a result of the interaction between effectors secreted by the pathogen and the corresponding host resistance protein. However, the effectors that interact with Rcs resistance genes have not yet been identified. Therefore, the objective of this study is to identify genes in C. sojina that encode effectors interacting with the resistant gene Rcs2.

We collected 52 isolates of C. sojina from different regions of Tennessee and combined them with 57 isolates that were collected before 2010 and 13 isolates collected in 2019 to develop a genome-wide association study (GWAS) population. We generated whole genome sequences for 122 isolates using the Illumina sequencing platform and identified 24,224 SNPs through variant calling using the Race 15 reference genome. Principal component analysis clearly showed that isolates grouped into six genetically distinct subpopulations and admixture was detected among them. The linkage disequilibrium (LD) analysis of 122 isolates showed relatively low LD decay with 244,790 bp for r2 =0.2, suggesting a clonal lineage. Currently, we are phenotyping this population on soybean lines Kent (Rcs2 differential line), Davis (resistant control), and Blackhawk (susceptible control). To date, we have phenotyped 61/122 isolates and found that 44 are avirulent and 17 are virulent on Kent. Subsequently, GWAS analysis identified a

significant marker-trait association on the C. sojina chromosome CP036211.1 spanning a 703 kb region containing 188 genes. We will continue to phenotype the rest of the C. sojina GWAS population to further delineate the candidate region. Molecular characterization of C. sojina effector genes will not only improve our understanding of the C. sojina-soybean interaction but will also facilitate breeding for genetic resistance through genetic engineering.

723C

Investigating the role glutathione degradation by *Magnaporthe oryzae* during rice blast disease

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Rice blast, caused by the hemibiotrophic fungus *Magnaporthe oryzae*, is the most devastating disease affecting rice cultivation worldwide. During infection, the fungus encounters reactive oxygen species (ROS) from a host-derived oxidative burst, a key component of the plant's innate immune response, along with nutrient starvation. To establish infection, *M. oryzae* must neutralize ROS to prevent the activation of stronger plant defenses. However, the mechanisms underlying fungal adaptation to these stresses during sustained *in planta* growth remain unclear. Here, we employed reverse genetics to investigate the role of the glutathione (GSH) system in fungal stress adaptation and pathogenicity. GSH serves as a major antioxidant, protecting *M. oryzae* from host-derived ROS, and functions as a sulfur and nitrogen source under nutrient-limiting conditions. Its degradation primarily occurs via the γ-glutamyl cycle, initiated by γ-glutamyl transpeptidase and completed by dipeptidase-mediated cleavage of Cys-Gly. Additionally, an alternative degradation pathway involving the Dug1-Dug2-Dug3 complex has been characterized in *Saccharomyces cerevisiae*, where Dug1 functions independently as a dipeptidase. While the *DUG1, DUG2* and *DUG3* gene homologs exist in *M. oryzae*, the complex function remains uncharacterized. To elucidate its role, *M. oryzae DUG1, DUG2* and *DUG3* were disrupted separately using the PCR-based split marker technique, replacing the coding region with *ILV2* conferring sulfonylurea resistance. Single gene deletion of *DUG1, DUG2 and DUG3* knockout mutants exhibited reduced virulence in whole-plant inoculation assays. Live-cell imaging of optically clear detached rice leaf sheaths of *Δdug1* revealed impaired biotrophic growth, with restricted cell-to-cell movement of invasive hyphae. Additionally, *DUG1* was found to be required for maximal conidiation while *DUG2 and DUG3* are essential for conidiation. Thus, the Dug1-Dug2-Dug3 complex is required for *M. oryzae* growth in host rice cells, and we are testing the hypothesis that this is due to a role in connecting GSH homeostasis with Sulfur and Nitrogen source availability. Taken together, our findings underscore the critical role of glutathione degradation during biotrophic growth, highlighting its potential as a target for antifungal strategies against rice blast and other plant diseases.

724C

Accessory gene cluster confers high copper tolerance in the cross-kingdom pathogen *Fusarium oxysporum*

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Fungi colonize different niches, from soil and plants to humans, using homeostatic regulatory mechanisms to rapidly respond to changing conditions. Copper is an essential micronutrient which is extremely toxic at high concentrations. Since it is utilized as an agronomic fungicide and employed by macrophages as an antimicrobial weapon through its accumulation in the phagosome, fungi face selective pressure to increase copper tolerance both in agricultural and clinical settings. In fungi, copper excess is sensed by the conserved copper-binding transcription factor Ace1, which activates a battery of genes for copper detoxification [1]. Loss of Ace1 leads to severe copper sensitivity and attenuation of virulence in human pathogens [2]. Here, we surveyed copper tolerance in different isolates of *Fusarium oxysporum* (*Fo*), a fungal pathogen that causes vascular wilt disease in many important crops and opportunistic infections in humans [3]. Several *Fo* isolates were highly copper tolerant compared to other plant or human pathogens such as *Fusarium*

graminearum or *Aspergillus fumigatus*. We found that high copper tolerance correlated with the presence of two neighboring accessory genes encoding a multicopper oxidase and a copper-binding transcription factor homologous to Ace1. Spontaneous loss of the accessory chromosome carrying both genes resulted in increased copper sensitivity, while introduction of either both genes or the multicopper oxidase gene alone was sufficient to confer high copper tolerance to strains naturally lacking the genes. Our results demonstrate a key role of this accessory gene group in copper tolerance and reveal a genetic mechanism for rapid adaptation of *Fo* to high copper environments found in agricultural settings or in the human host.

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725C

Substantial overlap in virulence-related genes and traits across strains of two Aspergillus species with differing clinical relevance

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Pathogenicity has evolved multiple times across the genus *Aspergillus*; however, the identification of discrete, causal genomic elements remains elusive. Most efforts to identify pathogenic elements have concentrated on the highly prevalent clinical pathogen *A. fumigatus*, resulting in the identification of dozens to hundreds of genomic features associated with pathogenic potential. These elements, however, are present in both pathogenic and non-pathogenic species. This complication stems from the fact that "pathogenicity" is not a measurable trait outside of the host-pathogen dynamic. For organisms like *A. fumigatus* that are not obligate pathogens, the genetic determinants of virulence are likely exaptations, and therefore refractory to tidy summarization. We previously reported that the conceptualization of "pathogenicity" as a binary trait at the species level is misguided, as evidenced by the pathogenic heterogeneity observed among strains of the "nonpathogenic" species *A. fischeri*.

Now, we extend this examination by comparing *in vivo* and *in vitro* measures of virulence among 16 strains each of *A. fumigatus* and *A. fischeri*. We find that strains exhibit species-specific growth characteristics in isolation, but that this distinction breaks down when strains are cultured in the presence of murine macrophages. Moreover, interspecific overlaps in virulence occur in a murine model of pulmonary aspergillosis, where three strains of the "non-pathogen" effected lethality rates of >50% while three strains of the "pathogen" were among the least virulent of all 32 strains tested. Thus, while certain traits appear species-distinct, strain heterogeneity can result in overlapping pathogenic potentials. Importantly, we could not statistically attribute these differences in pathogenic potential to specific genomic elements or secondary metabolites. These results further support the notion that the pathogenic potential of an opportunistic pathogen cannot be viewed either as a binary trait or as one that is strictly species-specific. Rather, the pathogenicity of any strain likely involves complex interactions among myriad genomic and ecological factors that only coincidentally converge to result in pathogenicity

726C

Single-cell profiling reveals tissue-protective and IFN-gamma-driven hyperactivated neutrophils during *Pneumocystis*pneumonia

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Pneumocystis jirovecii pneumonia is an opportunistic infection that can cause severe pneumonia and respiratory failure in immunocompromised patients, particularly those with T-cell dysfunction. Mortality remains high because respiratory decline reflects not only fungal persistence but also the magnitude and nature of host inflammatory responses. Neutrophils are rapidly recruited to the infected lung and are contribute to early fungal containment, yet their activity can also promote barrier disruption and hypoxemia. The specific neutrophil states that contribute to protection versus immunopathology remain unclear.

Using scRNA sequencing of Ly6G$^+$ neutrophils isolated from *P. murina*-infected mouse lungs, we generated a transcriptional atlas and resolved six developmental lineages emerging from a promyelocyte-like population. Diffusion and trajectory mapping revealed two dominant neutrophil fates. One trajectory gives rise to a mucosal defense-oriented, tissue modulating population enriched for antimicrobial effectors, regulated matrix remodeling programs, and monocyte and macrophage recruiting chemokines, consistent with barrier focused pathogen control and restrained cytotoxicity.

In contrast, a second trajectory exhibits progressive IFN-gamma-driven activation, marked by induction of inflammatory cytokines, chemokines, and antimicrobial and cytotoxic programs including Nos2, Gbp-family IFN-stimulated genes, and Cd274 (PD-L1). This lineage culminates in a terminal hyperactivated state with increased STAT1–IRF1 signaling, IFN-stimulated genes, and effector functions involving ROS generation, inflammasome-related signaling, and degranulation. The combination of sustained cytokine responsiveness, high chemokine output, cytotoxic granule activity, and transcriptional features associated with lytic inflammatory death pathways, including NETosis and pyroptosis, suggests a neutrophil population capable of amplifying inflammation, prolonging tissue residence, and contributing to epithelial injury.

Together, these findings show that cytokine dysregulation during *Pneumocystis* pneumonia drives neutrophils toward divergent functional outcomes: one supporting fungal control and one exacerbating tissue damage through sustained IFN-gamma-driven activation and cytotoxic effector programs. Defining these discrete neutrophil states provides a mechanistic framework for targeted immunomodulation in fungal pneumonia.

727C

Temperature-Driven Shifts in Cypress Immune Responses to Seiridium cardinale infections: Insights into Climate Change Scenarios

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Cypress Canker Disease (CCD) is a pandemic affecting Cupressaceae worldwide. Ascomycete fungi in the genus *Seiridium* are the causal agents, with *S. cardinale* being the most aggressive species. On the host side, *Cupressus* species are the most affected. Understanding the molecular basis of *C. sempervirens* resistance is essential for ensuring forest resilience under climate change scenarios. For this reason, we adopted an integrated Dual RNA-seq experimental framework. A first experiment aimed to characterize the response of resistant and susceptible phenotypes to *S. cardinale* infection, while a second experiment tested how thermal conditions influence host and pathogen transcriptional responses. Three temperature regimes were selected for indoor inoculations (32–30 °C, 22–20 °C, 15–10 °C), and outdoor inoculation trials were conducted during summer 2024 in Berkeley (mild climate), Half Moon Bay (cool coastal climate), and Davis (hot interior climate). We annotated the genomes of *C. sempervirens* and *S. cardinale*, and performed Differential Expression (DE) analyses. Clear transcriptional differences emerged between inoculated resistant and susceptible plants. Disease-resistance gene categories were strongly up-regulated in resistant clones, but down-regulated in susceptible ones. Gene Ontology (GO) enrichment indicated that resistant plants activated biological processes associated with: defense response, signaling, response to stress, cell communication, signal transduction, phosphorylation, and cellular response to stress. These findings are consistent with the activation of immune pathways. We then compared transcriptomic profiles between mock-inoculated and pathogen-inoculated plants. After quantifying overlaps in DEG sets, we focused on genes uniquely expressed in each condition. Pathogen-inoculated plants showed substantially higher activation of defence-related GO categories. Defense response biological processes displayed up to ~15-fold stronger expression compared to mock. Other GOs as response to stress, phosphorylation, and signaling, were also higher in pathogen-inoculated plants. Finally, comparisons across temperature regimes revealed that thermal variation modulates key defence pathways, suggesting that climatic conditions may influence host–pathogen interactions and the effectiveness of resistance mechanisms. Overall, our multi-layer approach identifies the major functional gene groups driving resistance in cypress, clarifies how wound and pathogen responses diverge, and provides the first evidence that temperature alters the expression trajectory of core defense pathways in this forest pathosystem.

728C

Searching for virulence factors of the plant pathogen *Ascochyta rabiei* using *Agrobacterium tumefaciens*-mediated transformation

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Ascochyta rabiei causes Ascochyta blight, an important foliar disease of chickpeas (*Cicer arietinum*). Chickpea Ascochyta blight is difficult to manage due to lack of highly resistant cultivars. In this study, we seek to identify virulence factors of the pathogen. Random mutagenesis through *Agrobacterium tumefaciens*-mediated transformation of the wild type *Ascochyta rabiei* strain AR628 was performed to create an insertional (knockout) mutant library with hygromycin resistance to enable selection. Subsequently, a total of 94 mutants were screened for reduced virulence on highly susceptible chickpea cultivar Spanish White under controlled conditions using a mini-dome assay. Experimental design included the wild type strain and a water mock for positive and negative controls and each individual mutant trial inoculation with three biological replicates. Disease severity was scored on a 1 to 9 scale with 1 as no lesions and 9 as plant death. The wild type controls produced a disease severity score of 7, and 10 mutants showed significantly reduced virulence, including 2 mutants which completely lost virulence. All 10 mutants are being screened on 4 chickpea cultivars with various levels of resistance to capture variation in virulence among the mutants. We are currently performing genome sequencing to identify the genomic locations of the insertions, and plan to use targeted mutations to confirm the functions of mutated genes. Furthermore, the genome sequence information will allow us to assemble the whole genome of the strain AR628, permitting genomic approaches to identify additional virulence factors in an effort to understand the virulence mechanisms of *Ascochyta rabiei*.

729C

Interactions of nematophagous fungi with *Steinernema* nematodes

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Nematophagous fungi can compensate for nutritional deficiencies in their environment by enticing, trapping, and digesting nematode worms. When the fungi lack nutrients and sense the presence of nematodes, they make a decision to begin a complex process of morphogenesis and specialized development, producing traps the fungus can use to immobilize and then consume the worms. Our existing mechanistic understanding of the interactions between nematophagous fungi and their nematode prey comes largely from studies using *Caenorhabditis elegans*, a free-living soil nematode widely studied in the laboratory. We are expanding these studies to use a newly emerging laboratory research organism, the entomopathogenic nematode *Steinernema hermaphroditum*. Nematodes are possibly the most numerous and diverse animals on Earth, and the last common ancestor of *S. hermaphroditum* and *C. elegans* lived hundreds of millions of years ago. Despite this extreme evolutionary distance between these two potential prey species, signals from *S. hermaphroditum* trigger trap development in the nematophagous fungus *Arthrobotrys oligospora*, which then proceeds to trap and consume the worms. We are testing known worm and fungal genes that mediate interactions between *A. oligospora* and *C. elegans* to see if their functions remain relevant to the interactions of the fungus with this distantly related prey, and will seek to discover new contributors to fungus-worm interaction that have not been identified as relevant to the fungus's interactions with *S. hermaphroditum*'s distant relative *C. elegans*.

730C

Host–pathogen interactions in an alternative model: elucidating *Candida albicans* virulence *in vivo*

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Candida albicans is a common opportunistic fungal pathogen that asymptomatically colonizes most humans. Although typically a benign commensal, dysbiosis caused by antibiotic use, immune dysfunction, or epithelial barrier disruption can trigger fungal overgrowth and infection, ranging from superficial mucosal disease to life-threatening systemic candidiasis. New preclinical infection models are needed to dissect *C. albicans* pathogenesis *in vivo* across distinct stages of infection and with different measurable host outcomes. We previously established the planarian *Schmidtea mediterranea* as an invertebrate host for studying host–pathogen interactions during *C. albicans* infection. *S. mediterranea* relies entirely on conserved innate immune mechanisms capable of overcoming infection with pathogenic microorganisms, including bacteria and fungi. Planarians' remarkable regenerative capacity and accessible stem cell populations make this organism a tractable model to analyze early immune responses, tissue repair, and pathogen clearance *in vivo*. This model supports simultaneous analysis of fungal virulence and host transcriptional responses, providing valuable insights into infection dynamics. I will present our newest data generated using this model, which will include optimization of wild-type *C. albicans* infection of *S. mediterranea*, dual-species transcriptomic profiling, and *C. albicans* mutant strain screening.

731C

Fungal Mycobiome and Secondary Metabolites in Corn

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Mycotoxins are secondary metabolites produced by fungi that contaminate human food and animal feed. They also are important components in the unseen microbial warfare that determines which fungi can colonize a substrate. In corn, *Aspergillus* spp. and *Fusarium* spp. are common fungal toxin-producing contaminants, but they are not the only fungi present. The relationship between fungal mycobiome and mycotoxin levels in corn is not well understood. So, we evaluated grain from 12 plants growing at two locations for the presence of up to 1200 secondary metabolites, and coupled the chemical studies with a mycobiome assessment with ITS (rRNA Internal Transcribed Spacer) sequences. Our objective was to identify fungal communities on individual corn ears, and their association with the secondary metabolites there. Our working hypothesis was that metabolite presence and quantity varied with composition of the mycobiome.

DNA was extracted from corn grain and sequenced. Fastq sequences were processed through the mothur pipeline to assign sequences to a fungal taxon at the genus level. Secondary metabolites were detected by LC-MS. R Studio was used to analyze the presence and relative abundance of fungal genera and secondary metabolites.

All of the corn ears were contaminated with fungi and secondary metabolites. *Fusarium* spp. and its toxins were found in 11/12 corn ears, with the abundance of fungi and amount of toxin detected weakly correlated. *Aspergillus* spp. were recovered in significant numbers from 3/12 ears and aflatoxin was detected in 2/3 ears. Fungi in the *Sarocladium* genus were ubiquitous and may be reducing colonization and mycotoxin production by *Aspergillus* and *Fusarium*. *Fusarium, Aspergillus* and *Penicillium* spp. were detected in corn that lacked the toxins they could synthesize.

Corn ear mycobiomes are diverse and complex, with interactions amongst the fungi present likely responsible for the mycotoxins produced. Mycotoxin contamination required that the mycotoxin-producing fungi be a significant portion of the mycobiome. Further mycobiome characterization and exploration at the species level could be used to identify toxin-producing fungi present and assist in their management. Managing the toxin producers and the amount of toxin produced will increase the value and safety of crops. Characterizing the fungal community can be used to identify previously poorly studied genera, *e.g.*, *Sarocladium*, that may function as biocontrols of toxin production.

732C

Functional Characterization of Pathogenicity Drivers in *Sphaerulina musiva* to Inform Split CRISPR-Cas9 Gene Drive Engineering

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The fungal pathogen *Sphaerulina musiva* poses a major threat to hybrid poplar plantations across North America, yet the genetic basis of its pathogenicity remains largely unresolved. To address this challenge, we are pioneering advanced genetic tools to dissect and ultimately disrupt virulence pathways. We are developing a split CRISPR-Cas9 gene drive system designed to bias inheritance during sexual reproduction, with the long-term goal of directing this platform toward key pathogenicity determinants identified through functional studies. As a proof-of-concept, a gene drive strain has been constructed targeting a selectable locus to enable high-throughput screening and validation of drive function. Work is underway to establish *in vitro* and *in vivo* mating assays using poplar leaf agar and tissue cultures to assess drive transmission and progeny segregation with gene drive strains.

In parallel, we are characterizing the roles of global transcriptional regulators CreA and LaeA in shaping secondary metabolism and virulence in *Sphaerulina musiva*. Our work has focused on targeted mutant strains, which show significant impacts on virulence, particularly in the development and progression of stem cankers on poplar hosts. To uncover the underlying mechanisms, we are conducting transcriptomic profiling directly from infected plant tissues, enabling us to capture host-pathogen interactions *in planta*. These datasets are revealing downstream regulatory networks that contribute to pathogenic development and will provide a set of candidate pathways for future gene drive-based attenuation strategies.

733C

Cercospora cf. flagellaris can infect *Nicotiana tabacum* and cause tobacco greenspot—a disease associated with latent *C. nicotianae* infections.

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In recent years, the wrapper leaf tobacco industry has frequently witnessed a phenomenon in which cured leaves retain green areas (spots) that appear to be recalcitrant to the curing process. Numerous hypotheses have been proposed to explain this occurrence, ranging from suboptimal curing conditions (abiotic factors) to the possible involvement of phytotoxins produced by fungal pathogens (biotic factors). Our recent observations regarding *Cercospora* diseases of soybean led to the hypothesis that greenspot of tobacco may be caused by multiple *Cercospora* species. To test this hypothesis, we inoculated Connecticut Broadleaf tobacco with various *Cercospora* isolates in greenhouses and growth chambers and observed symptoms after air curing. In these experiments, we were able to recapitulate green spot symptoms seen during commercial curing with strains of *Cercospora* isolated from tobacco. We then evaluated sequenced *Cercospora* strains isolated from soybean and found that they also caused greenspot symptoms during curing. In some cases, soybean isolates of *Cercospora* caused even more severe greenspot symptoms than strains originally isolated from tobacco plants. These results, along with genomic analyses of *Cercospora* isolated from soybean, suggest that the organism commonly associated with frogeye leaf spot on tobacco (*C. nicotianae*) may have hybridized with another *Cercospora* species (*C.* cf. *flagellaris*) that has been rapidly expanding its host range in the past 10-20 years. Our working hypothesis is that some *Cercospora* strains are arrested in the latent phase of infection (biotrophy) during pathogenesis on tobacco, and their effectors and related components of pathogenesis block the degradation of chlorophyll, leading to the presence of persistent green spots during curing.

734C

Macrophage phagocytosis of *Coccidioides* promotes its differentiation into the parasitic form

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Coccidioides is an endemic fungus that is increasing in prevalence and can cause life-threatening disease in otherwise immunocompetent people. In the environment, the spores (arthroconidia) develop into hyphae, yet when they are inhaled by a mammalian host, they develop into a unique form called the spherule. The transition to spherule can be triggered *in vitro* with elevated temperatures and high CO_2 levels, but the signals that trigger *Coccidioides* spherulation *in vivo* are not known. We used live imaging to investigate how macrophages affect the fate of *Coccidioides* arthroconidia. Under tissue culture conditions, arthroconidia quickly developed into hyphae. The addition of macrophages promoted spherule development and delayed hyphal formation, indicating that innate immune cells can influence *Coccidioides* development into the pathogenic form. Exposure of arthroconidia to macrophage supernatants was not sufficient to stimulate spherulation; rather, this transition was dependent on phagocytosis of arthroconidia by macrophages. Transcriptomics analysis of *Coccidioides* co-cultured with macrophages revealed a signature concordant with spherules grown *in vitro* and allowed the identification of a core set of spherule-specific transcripts. In addition, we identified *Coccidioides* transcripts with significantly higher abundance in the presence of macrophages compared to *in vitro* spherules, suggesting these factors may be needed to survive and thrive in the presence of innate immune cells. This work lays a foundation for uncovering host-pathogen signaling as well as *Coccidioides* factors that are critical for pathogenesis.

735C

Comparative transcriptomic analysis of *Microbotryum superbum* in pathogenic and endophytic lifestyle during infection of *Dianthus seguieri* plants

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Microbotryum species offer a unique model not often seen in genetically more homogeneous agriculturally propagated crop hosts: the diversity of *Microbotryum*/Caryophyllaceae associations provides a unique opportunity to test the predictive power for host shifts and hence, to anticipate emerging infectious diseases. Here, we study *Microbotryum superbum* as both a pathogen and as an endophyte using RNA-seq analysis. Buds and meristematic tissue were collected from symptomatic and asymptomatic *Dianthus seguieri* plants infected by *M. superbum*. The fungal genes g7163.t1 (GO:0016985), g8595.t1 (GO:0004252), and g165.t1 (GO:0016829), related to mannan endo-1,4-beta-mannosidase activity, prostanoid metabolism, and lipid metabolism, respectively, are upregulated in asymptomatic plants and downregulated in symptomatic plants. These genes play an important role in the establishment and dissemination of fungi within the host, modulating the host's immune response to promote fungal survival. The gene g6909.t1 (GO:0004252) upregulated in asymptomatic plants refers to serine-type endopeptidase activity, which is involved in protein degradation. In fungi that can switch between endophytic and pathogenic lifestyles, genes associated with this GO term can be a critical determinant of which lifestyle is adopted. In asymptomatic plants, the "ribonucleoprotein complex" and "ribosome structural constituent" pathways were enriched, and they are crucial for fungal growth and development. In symptomatic meristematic tissue, a high enrichment of the "oxidoreductase activity" pathway was observed, which is involved in fungal development, such as the transition from mycelium to fruiting body and host-pathogen interactions. When comparing symptomatic and asymptomatic buds, a high enrichment of the "guanine nucleotide exchange factor activity" pathway is observed. This pathway plays a significant role in the complex molecular communication between the fungus and the host plant, regulating the transition between a beneficial endophyte or a potentially pathogenic lifestyle, which affects the plant immune response and helps the endophyte establish a persistent and asymptomatic colonization. Our results showed important fungal genes and pathways that exhibit differential regulation during *M. superbum* infection in *D. seguieri* plants, which will be further characterized to better understand the implications of these genes on *M. superbum* lifestyle changes for both the host and the parasite.

736C

Evidence that Entomophthora muscae controls the timing of host death via its own circadian clock

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Timing in the natural world is a matter of life or death, consequently, nearly all life on Earth has evolved internal circadian clocks. Many behavior-manipulating parasites exhibit striking daily timing, but whether this is clock-driven has remained unclear. Here, we leveraged the laboratory-tractable zombie fruit fly model, *Drosophila melanogaster* infected by the behavior manipulating fungus *Entomophthora muscae*, to tackle this long-standing mystery. Using an automated behavioral paradigm, we found that the timing of death of wild-type flies continues to occur with ~21 hour periodicity in the

absence of environmental cues. Surprisingly, we also discovered that light is required within the first 24 hours of exposure for *E. muscae* to infect and kill flies. Experiments with circadian and photoreception mutants revealed that death is independent of host genotype, suggesting the fungus—not the host—drives this rhythm. Transcriptomic analysis of *in vitro* grown fungus revealed that *E. muscae* maintains rhythmic gene expression independent of the fly host that peaks at sunset and has a free-running period of ~22 hours. Among cycling genes, we identified a transcript encoding a protein with high homology to *white collar-1*, the blue light sensor and core component of the molecular oscillator in the model ascomycete fungus *Neurospora crassa*. Altogether, our findings suggest that *E. muscae* has an endogenous circadian clock that it uses to control the timing of host death. This study provides evidence that a fungal clock can influence fly outcomes, pointing to a new mechanism by which parasites temporally coordinate host manipulation.

737C

Endolevanase: A Host-Specific Hidden Weapon of *Fusarium graminearum* Provoking Fusarium Head Blight in Wheat

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Graminan- and levan-type fructans are primarily stored within specialized cellular organelles known as vacuoles in wheat. These fructans serve diverse functions, including osmotic regulation, serving as a reservoir for energy, and possibly acting as signaling molecules to elicit the plant's immune response upon subsequent pathogen challenges. We propose that upon breaching the initial physical barrier - the plant cell wall, *Fusarium graminearum* may invade the underlying plant tissues through the **secretion of a cocktail of enzymes**, notably **endolevanases**. These enzymes likely play a pivotal role by **degrading graminan-type fructans**, potentially utilizing them as a carbon source to support the proliferation of the pathogen within the host. Despite its critical implications, the presence and functional significance of endolevanase in the pathogenicity of *F. graminearum* **remain understudied**.

Through protein-carbohydrate docking, we identified a gene that encodes a **novel endo-levanase** in *F. graminearum*. Intriguingly, this gene remained dormant when *F. graminearum* PH-1 was cultured in a minimal medium supplemented with high DP levan as an inducer. However, the gene was activated by a combination of **high DP levan and wheat plant extract**, while lettuce extract failed to induce its expression. This observation suggests the involvement of **specific host-derived metabolic compounds** in endolevanase activation in *F. graminearum*. Further, we successfully **heterologously expressed** the endolevanase in *Pichia pastoris*, where it exhibited high activity, efficiently degrading high DP levan into levan-derived oligosaccharides (LOS). Moreover, we demonstrated the **synergistic action of endo and exo-levanases** in levan degradation by the pathogen. Next, we aim to employ **CRISPR-Cas technology** to knockdown this gene, thus elucidating its role in the pathogenicity. These findings hold promise for **controlling *Fusarium* head blight** by targeting such crucial genes involved in pathogenicity.

738C

Exploiting FgGMTV1 for Biocontrol of Fusarium Head Blight in Wheat

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Fusarium graminearum is a devastating fungal pathogen responsible for economically significant diseases, including Fusarium head blight (FHB) of wheat and barley, for which conventional control strategies remain largely ineffective. A recently identified mycovirus, *Fusarium graminearum* gemytripvirus 1 (FgGMTV1), can markedly reduce the pathogenicity of *F. graminearum*. FgGMTV1 is a gemycircularvirus with a tripartite, single-stranded circular DNA genome, requiring segments A and B for replication and hypovirulence induction, while segment C counteracts this effect by restoring virulence. In this study, FgGMTV1 DNA-A, B, and C, along with a DNA-A mutant carrying a premature stop codon in the Rep gene (A-mRep), were cloned into the binary vector pAI101. Agroinfiltration of *Nicotiana benthamiana* leaves with these constructs failed to initiate replication in plant cells. However, replicating viral DNA was detected in *F. graminearum* mycelia transformed with linearized 1.1-mers of DNA-A, B, and C. These transformants exhibited altered morphology, reduced growth, and diminished conidia production. Wheat leaf assays further demonstrated that the transformants significantly reduced the virulence of U.S. isolates of *F. graminearum*, suggesting potential utility in controlling FHB and related diseases. Ongoing experiments on wheat spikes will determine whether FgGMTV1 also reduces production of the mycotoxin deoxynivalenol (DON). In addition, a metatranscriptomics virome survey is underway to identify co-infecting mycoviruses in the transformed fungal isolate that exhibits hypovirulence.

739C

Epigenetic rewiring of the low-oxygen response by an Inhibitor of Growth (ING) family Protein drives *Aspergillus fumigatus* disease progression

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Adaptation to dynamic oxygen microenvironments in the lungs is critical for *Aspergillus fumigatus* disease progression. Higher oxygen levels are available in the large airways, but decline progressively in the alveoli, eventually leading to severe hypoxia in deep tissues. While epigenetic mechanisms are known to play an essential role in low-oxygen adaptation in mammals, their function in fungi is ill-defined. To address this gap in knowledge, we screened mutants in chromatin regulatory genes from the *Neurospora crassa* deletion library and identified an Inhibitor of Growth domain-containing Protein (IngA) required for low oxygen growth. The IngA ortholog in *A. nidulans* is part of the RpdA-L histone deacetylase complex. Loss of *ingA* in *A. fumigatus* resulted in stunted biofilms at both 21% and 10% atmospheric oxygen levels. A significant reduction in the Δ*ingA* submerged biofilm biomass at 10% oxygen was observed compared to wild-type and complemented strains.

Under hypoxic conditions (0.2% oxygen), both shaking batch cultures and submerged biofilms of Δ*ingA* exhibited a severe growth defect. Our results suggest that Δ*ingA* is metabolically limited, as it cannot grow on plates containing ethanol and 2-deoxy-D-glucose under normoxic conditions. Moreover, transcripts of genes encoding proteins involved in ribosome biogenesis and protein translation are significantly increased in Δ*ingA* in hypoxic conditions. We are currently testing the hypothesis that the growth failure of Δ*ingA* in hypoxic conditions arises from its inability to sense oxygen changes and adjust protein translation.

We also observe that IngA is a positive regulator of ergosterol biosynthesis, and Δ*ingA* is less susceptible to voriconazole. Critically, the loss of *ingA* results in a striking reduction in fungal burden and attenuated virulence in the triamcinolone murine model of invasive aspergillosis. Histological analysis reveals that the mutant can grow in the larger airways but cannot grow in deep tissues, where oxygen levels are much lower. Therefore, while IngA is not required for disease initiation, it is essential for disease progression in low-oxygen environments. As antifungals are typically administered after disease establishment in patients, identification of novel disease progression factors is critical for developing new antifungals to treat this deadly disease.

740C

Lichtheimia: An Emerging Fungal Pathogen in Human Mucormycosis – Insights from Lung-on-Chip and Invertebrate Models

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Lichtheimia is a thermotolerant fungal genus and a leading cause of mucormycosis, a life-threatening angioinvasive infection. Its importance is underscored by its high mortality, especially in immunocompromised hosts, and challenges in rapid diagnosis and treatment. Emerging as the second most prevalent agent in some regions, it presents a significant clinical burden. The genus currently consists of eight species, but this number is likely to increase as taxonomic efforts continue to define new species. Recent advancements in pathogenesis research have been propelled by innovative infection models. Invertebrate models, such as *Galleria mellonella* and *Drosophila melanogaster*, provide a high-throughput, ethically accessible system for virulence studies and antifungal screening. Complementarily, human lung-on-chip models offer a sophisticated, physiologically relevant platform to dissect host-pathogen interactions at the alveolar interface, mimicking critical aspects of invasive pulmonary disease. These models are crucial for elucidating the mechanisms of angioinvasion, immune evasion, and tissue damage, thereby accelerating therapeutic discovery. Together, they bridge the gap between traditional *in vitro* assays and complex mammalian studies, enhancing our understanding of *Lichtheimia* pathogenicity and informing strategies to combat this deadly infection. The pathogenicity of the entire *Lichtheimia* species complex was assessed through invertebrate infection models, and the invasive mechanisms of *Lichtheimia corymbifera* were further elucidated using exemplar lung-on-chip models.

741C

Botryozyma yeast , a sexually transmitted infection of Panagrellus redivivus nematodes

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Climate change, habitat disruption, and global travel are increasing the risk of the emergence of novel infectious diseases. A sizable literature has studied the origin of human pathogens that have emerged after a host jump from an alternate animal reservoir, such as COVID-19 and HIV. How microbes specialize to pathogenic lifestyles in the first place is much less well understood. A subset of pathogens includes microbes transmitted through sexual behaviors—so-called sexually transmitted infections (STIs). Here, we characterize a little-studied fungus, Botryozyma yeast, as a model system to answer such questions. Botryozyma attached to the reproductive organs of the dioecious nematode Panagrellus and could be transmitted between individuals via sexual behavior. Severe Botryozyma infections led to host immobilization and a decrease in host fecundity, which mimics the consequences of other STIs. These characters are unique to Botryozyma and not observed anywhere else across the fungal kingdom. Botryozyma thus holds great promise for studies of the evolution of STI pathogens, including molecular mechanisms and population dynamics.

742C

Mucoromycotina ‘Fine Root Endophytes’: a distinct mycorrhizal strategy in early-diverging fungal lineages

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Mucoromycotina ‘Fine Root Endophytes’ (MFRE) are an early-diverging fungal lineage that has recently emerged as a widespread and ecologically important group of plant symbionts, capable of alternating between saprotrophic growth and root-associated lifestyles. Physiological and microscopic studies have shown that MFRE colonise roots intracellularly and engage in nutrient-for-carbon exchange, yet the molecular basis of this nutritional flexibility remains poorly understood. To address this, we combined genomic and transcriptomic approaches to investigate nutrient acquisition strategies and host interaction.

We assembled two high-quality MFRE genomes from genetically distinct isolates collected at the same field site and used comparative genomics to identify conserved signatures across both isolates, including a distinctive repertoire of nutrient transporters and CAZymes that characterise saprotrophic competence alongside root-associated capacities. For one isolate, we conducted transcriptomic profiling across contrasting nutrient conditions and during plant colonisation, revealing transcriptional transitions that differentiate free-living and host-associated states.

Our key findings demonstrate that both genomes retain a complete lipid biosynthetic pathway, and that interaction with MFRE did not induce lipid-transfer mechanisms in the plant host. In contrast to the obligate lipid-dependent strategy of arbuscular mycorrhizal fungi, these features support a non-obligate

biotrophic endomycorrhizal lifestyle. In one of the genomes, we also detected previously reported Mollicute-like endobacteria, supporting the view that bacterial endosymbionts are recurrent associates of early-diverging fungal lineages.

Together, our findings show that MFRE combine hallmarks of mycorrhizal symbiosis with autonomous saprotrophic capacity, refining the concept of facultative mycorrhizal behaviour in this lineage. These results establish the first molecular framework for understanding this distinctive mycorrhizal lifestyle in early-diverging fungi.

743C

Bacterial partner protects *Aspergillus calidoustus* from Voriconazole

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Microbial communities are made of interacting and competing microorganisms that play a critical role in human, plant, and environmental health. These communities are partially shaped by the production of natural antimicrobial compounds (AMCs) that exclude the growth of susceptible bacteria and fungi. Phenazines are one major AMC that most fungi are susceptible to. Our lab uses a co-isolated bacterial-fungal pairing where the bacterium, *Paraburkholderia edwinii* can protect its partner fungus, *Aspergillus calidoustus*, from the oxidative stress of phenazine-1-carboxylic acid. To determine if the same protection occurs in the presence of synthetic antifungal compounds, we have conducted a co-colony experiment using our model organisms in the presence of voriconazole. Voriconazole is a frontline clinical antifungal drug used to treat serious *Aspergillus* and *Candida* infections. The co-colonies confirmed the protection of *Aspergillus calidoustus* by *Paraburkholderia edwinii* in the presence of Voriconazole. Bacterial genes previously associated with protection of *A. calidoustus* from phenazines were found to be unassociated with protection from voriconazole, suggesting a different mode of protection. We are working to identify the bacterial genes responsible for voriconazole protection by conducting mariner transposon mutagenesis of the bacterium, and screening co-colonies for a lack of fungal growth in the presence of Voriconazole. Preliminary analyses will focus on the pathways that may implicate the sharing of sterol-like lipids or their precursors with the fungus due to their physical association, secreted proteins, and surface-exposed carbohydrates on the bacterial surface. Understanding how bacteria protect fungi from synthetic antifungal assault can provide insight on overcoming both clinical and agricultural concerns such as antifungal resistance in both humans and crops.

744C

Identifying Antigen Targets for mRNA-LNP Vaccine Development Against *Cryptococcus neoformans*

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Fungal infections are one of the emerging public health concerns in the 21st century. Around 300 fungal species are responsible for causing approximately 3.8 million invasive diseases annually. Yet there are no licensed vaccines available to prevent any human fungal infections. One of the major pathogenic fungi is *Cryptococcus neoformans*, responsible for killing 112,000 people annually. Our lab recently developed the first mRNA-LNP vaccine against a fungal pathogen. We have shown that a molecular adjuvant in combination with cryptococcal capsule can provide strong protection against lethal cryptococcosis in a mouse model. Remarkably, treatment of the capsule with proteinase K completely abolished protection, supporting the idea that capsular proteins are immunogenic components. We have tested the serological reactivity of different capsular proteins to serum from vaccinated and protected mice to identify potential immunogens. These antigens will be employed to develop a multi-valent vaccine against *Cryptococcus neoformans*. Additionally, we have also tested the addition of different molecular adjuvants that can augment the protective immune response elicited by our current mRNA-LNP vaccine. We propose to optimize the mRNA-LNP formulation with strong host-protective antigens in pairing with effective adjuvant against cryptococcosis.

745C

Analyzing differences in codon usage patterns in *Rhizopus micosporus*, its bacterial endosymbiont *Mycetohabitans* sp., and the novel prophages residing in the bacterial genome

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Mycetohabitans spp. are gram negative bacterial endosymbionts of *Rhizopus microsporus,* a known plant pathogen and opportunistic human pathogen. Though related to free-living *Burkholderia spp.*, *Mycetohabitans* strains have smaller genomes and are considered a separate genus undergoing genome contraction. Despite their reduced genome, many of the individual *Mycetohabitans* strains appear to have intact bacteriophage within their genomes. We identified 36 novel prophages with the computational tool GeNomad across 28 strains of *Mycetohabitans*. Preliminary results indicate that UV light can induce the prophage to produce viral-like particles. Computational analysis revealed two separate phage families based on average nucleotide identity, with one prophage falling in neither category. In order to better understand the pathogenicity and the symbiotic relationship of the fungus, bacterium, and bacteriophage, we investigated the differences in codon usage patterns between the various organisms compared to the tRNA pools. Uncovering these differences and how it relates to available tRNAs could pave the way for future fungal control mechanisms targeting codons exclusive to the bacterium and prophage.

746C

Uncovering cell-specific responses of barley during *Magnaporthe oryzae* infection

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Plants rely on the defense responses of individual cells to counter pathogen attack. To overcome host resistance, pathogens secrete specialized molecules, known as effectors, that enable them to evade, suppress, or manipulate the immune responses of penetrated cells. However, from microscopic analyses it is known that even when a pathogen successfully suppresses immunity in a single cell, adjacent cells may still resist invasion. Thus, to obtain a comprehensive understanding of the transcriptomic and metabolic differences between susceptible and resistant plant cells, and of the mechanisms by which pathogens manipulate host immunity, analyses at the whole-tissue level are insufficient, instead investigations at the single-cell level are essential.

In this study, we aimed to establish a strategy to selectively identify barley cells colonized by the fungal pathogen *Magnaporthe oryzae* and to characterize their transcriptomic profiles in comparison to uninfected cells within the same tissue. Therefore, protoplasts were generated from infected barley leaves, followed by barcoding of RNA derived from individual cells and subsequent RNA sequencing. Cells exhibiting similar gene expression profiles were grouped into distinct clusters. These clusters were then analyzed for infection-associated marker genes and for the presence of *M. oryzae*- specific transcripts. Through this approach, we will identify plant and fungal genes that play crucial roles in immune response and virulence, respectively.

747C

Identification and characterization of core *Cercospora beticola* biotrophic effectors through pan-genomic analysis.

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Cercospora beticola, causal agent of Cercospora leaf spot (CLS), is the most distructive foliar disease affecting sugar beet production. The infection caused by *C. beticola* can significantly deteriorate sugar beet foliar tissue leading to severe yield losses. Despite being a pathogen of high economic importance, very little is known regarding the molecular mechanisms deployed by C*. beticola* to manipulate the sugar beet host. Recently, we sequenced a large globally diverse population of *C. beticola* to identify effector genes that are conserved among the species and leveraged RNAseq data to select potential effectors that were transcriptionally active during the biotrophic phase of *C. beticola* infection, resulting in 26 candidate effectors. Effectors candidates were subjected to structural prediction via Alphafold2 and structural homologs were identified using a combination of Foldseek and Dali, revealing homology to diverse enzymes such as hydrolases, metalloproteases, superoxide dismutase, cellulases, ribotoxins, as well as uncharacterized functions. Candidate effectors are currently being characterized via targeted gene knockouts and transient expression in in *Nicotiana benthamiana* to screen for cell death suppression. Further, i*n planta* localization studies of fluorescently tagged candidates is underway to identify the cellular location of these effector genes.

748C

Identification of pathogenicity-related genes in *Fusarium oxysporum* f. sp. *lycopersici* using an accessory chromosome-deletion library constructed by CRISPR/Cas9 genome editing

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Fusarium oxysporum is a soilborne plant-pathogenic ascomycete fungus that invades plants through their roots and causes wilt symptoms by blocking the vascular system. This fungus carries multiple accessory chromosomes (ACs) in its genome that are not essential for growth, and some of these are known to be involved in pathogenicity (Ma *et al*., 2010). However, due to technical difficulties, the identification of pathogenicity-related genes on the ACs has remained limited, and the specific AC responsible for pathogenicity has not been fully elucidated. In this study, we established a method for large-scale chromosomal deletion (up to 800 kbp) by optimizing the CRISPR/Cas9 system for *F. oxysporum*. Using this method, we performed a comprehensive analysis of ACs in the tomato-infecting pathogen *F. oxysporum* f. sp. *lycopersici* (Fol) to assess their contribution to pathogenicity. The genome of the Fol isolate MAFF 103036 (race 1) is assembled into 19 contigs. Comparative analysis with the Fol 4287 (race 2) genome as a reference estimated contig 5 (partial), contigs 6, 11, 14, 15, and 16 in the MAFF 103036 as ACs. We constructed a deletion mutant library covering 99% (37 out of the 39 regions) of the ACs and evaluated the pathogenicity of each deletion mutant on tomato (cv. Ponderosa). This analysis identified six regions (55–230 kbp) among the 37 regions contributing to pathogenicity. Further detailed deletion analyses revealed two key subregions (25 kbp and 15 kbp) involved in pathogenicity. The 25 kbp subregion contained one copy of the transcription factor *FTF1* (Niño-Sánchez *et al*., 2016), which is present in six copies across the genome. Mutation of the *FTF1* gene within the 25 kbp subregion reduced virulence on tomato, whereas deletion of the copies outside this subregion did not affect pathogenicity. Furthermore, we uncovered previously uncharacterized pathogenicity-related genes whose deletion significantly reduced virulence.

749C

Dissecting the *Candida albicans* and *Pseudomonas aeruginosa* relationship through diverse isolates.

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Candida albicans is a major model yeast species and the target of decades of genetic research. And yet, its genome consists of a great number of genes of unknown function. One consideration is that growth is often limited to standard lab conditions and in isolation. *Pseudomonas aeruginosa*, a gram-negative bacterium, is well-documented for co-association with *C.a. in the clinic*. *In vitro* co-cultures studies further highlight a complex interaction, where both organisms exhibit large scale transcriptomic changes, including many unstudied pathways in both species, presenting a system with the potential to study microbial genes specific to this interaction.

To dissect this multi-faceted interaction, we took advantage of natural genetic diversity in clinical isolates. Utilizing a collection of over 70 *P. a.* isolates, we screened for inhibition of *C. a.* growth in a high throughput plate assay and identified 4 isolates that showed significant loss of inhibition. This loss was repeated in a liquid killing assay. To identify pathways of interest, we performed genetic and transcriptomic profiling of the isolates and identified massive strain-to-strain variations where we filtered for shared pathways that are lost or down-regulated. We focused on one pathway down-regulated in all 4 isolates – denitrification, the process where nitrates are reduced in the absence of oxygen and generate toxic nitric oxide (NO) as intermediates. Our interest was driven by evidence that *C.a.* experience NO stress during infection by innate immune cells. Through this work, we add denitrifying bacteria as an additional source of toxic NO. Using transcriptomic comparisons, we show that *C.a.* NO-response genes are highly induced in co-culture. We further validate experimentally by generating and testing *C.a.* NO-stress mutants and *P.a.* denitrification mutants in liquid killing assays. Additionally, we questioned the selective advantage for these *P.a.* isolates in the clinic and, through evidence that NO challenges *P.a.* biofilm stability, discovered that despite losing denitrification abilities, these isolates gained significant folds in biofilm formation.

At the intersection of our analyses, we also gained functional insight into an uncharacterized ABC transporter in *C.a.*, which is among the most induced genes in both *P.a.* co-culture and *C.a.* NO stress response. We discovered that this transporter complements the function of sulfite transporter SSU1 to transport nitrates, the detoxified version of NO. This is a rare case of such substrates for yeast ABC transporters and, interestingly, no sequence or

structural homology is shared between the two. We hypothesize that the massive induction of this transporter benefits *P.a.* in a cycle of nitrate feeding and NO-production which *C.a.* ultimately succumbs to, and is actively investigating the consequences of a transporter mutant in co-culture.

750C

Among Us: Cheaters and transposons drive adaptations in experimental evolution of *Botrytis cinerea*

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We conducted experimental evolution to enhance the tolerance of the fungus *Botrytis cinerea* to the plant defense compound α-tomatine. While our initial goal was to select for physiological tolerance to α-tomatine, the evolutionary outcomes revealed complex trade-offs between survival, reproduction, and social behaviour. One out of 5 α-tomatine evolved lines exhibited a specific adaptive response: a three-fold increase in spore production only when only exposed to α-tomatine. To determine the genetic basis of this hyper-sporulation phenotype, we performed RNA-seq and whole-genome sequencing. We identified insertion of flipper transposable element (Fot-1) in the promoter region of a transcription factor that was significantly down-regulated in the evolved line. We are currently analysing the knock-out mutant of this transcription factor to confirm if its suppression is responsible for the adaption toward hyper-sporulation. Beyond individual adaptation, we observed a breakdown in social cooperation. In 6 out of 10 independent lines, "cheater" phenotypes emerged. These strains lost the ability to produce sufficient spores themselves but thrived by exploiting the resources provided by sporulating "cooperator" strains in mixed cultures. We were able to successfully separate the cheater and cooperator pairs from all six lines and sequenced their genomes to pinpoint the mutations driving this social exploitation. Later we investigated the consequences of this cheating on pathogenicity. In tomato infection assays, cheater strains were significantly less virulent than wild-type strains. Cooperator strains retained full pathogenicity even after 30 transfers. However, in mixed inoculations their virulence was reduced in the presence of cheaters. These findings suggest that cheaters act as a social burden, effectively dragging down the population's overall ability to cause disease. Overall, in our work we highlight laboratory experimental evolution can often see the emergence of cheater-like lines that can drive the outcome away from the anticipated traits and towards social diversification within populations.

751C

Characterization of compounds that inhibit filamentation in the human fungal pathogen *Candida albicans*

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Candida albicans, both a commensal of the healthy human microbiota and a leading opportunistic fungal pathogen, represents the most prevalent etiological cause of candidiasis, with a mortality rate approaching 40% despite therapeutic intervention. The high disease burden is due in part to the limited arsenal of antifungal agents for systemic infections—a challenge further compounded by the growing prevalence of drug resistance. In light of this, an alternative strategy to combat fungal infections is to target virulence traits implicated in pathogenicity without impairing growth. Such an approach can potentially expand the therapeutic target space while minimizing disruption to the host microbiota and reducing selective pressure on the pathogen to develop resistance. In *C. albicans*, the ability to transition between yeast and filamentous forms represents a particularly promising target for anti-virulence therapy, as most mutants locked in either state show virulence defects in mouse models of systemic candidiasis. This work explores this approach by performing a high-throughput screen of ~50,000 compounds to identify those that inhibit *C. albicans* filamentation without impacting growth. Out of 16 prioritized compounds, we followed up on two that inhibit the yeast-to-filament transition through distinct mechanisms. Compound 1 inhibits filamentation in response to multiple inducing cues by targeting *C. albicans* mitochondria, leading to mitochondrial dysfunction and a reduction in Efg1 protein levels, a core transcription factor that activates the filamentation programme. In contrast, compound 2 inhibits filamentation by acting as a cationic amphiphilic drug, disrupting vacuolar integrity and lipid homeostasis in a pH-dependent manner. Future work will focus on further elucidating the compounds' mechanism of action and assessing their therapeutic potential as an anti-virulence strategy.

752C

Molecular Diagnostics for Rice Brown Spot: qPCR and LAMP Markers Targeting *Bipolaris oryzae* and *Nigrospora oryzae*

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Rice brown spot is caused mainly by *Bipolaris oryzae* and *Nigrospora oryzae*, yet these species are often difficult to distinguish morphologically. To enable rapid and accurate detection, we developed species-specific molecular markers using quantitative real-time PCR and LAMP assays. Eight isolates of *B. oryzae* and eight of *N. oryzae* collected in 2023 were analyzed, and ITS, *β-tubulin*, *EF1α*, *TEF1*, and *GAPDH* sequences were obtained for primer design. Three primer sets were generated for each species, and PCR screening identified the *EF1α*-based set as most specific for *B. oryzae* and the *TEF1*-based set as most specific for *N. oryzae*. All primers showed strong species specificity in both PCR and qPCR assays. LAMP primers designed from ITS sequences also reliably amplified genomic DNA of both pathogens across two platforms (SimpliAmp Thermal Cycler and Iso Quark M4), producing clear SYBR Green I fluorescence and gel-visible products, while negative controls remained amplification-free. These results provide robust qPCR and LAMP tools for rapid differentiation of the two major brown spot pathogens and offer a foundation for field-ready diagnostics and epidemiological studies. (This work was supported by the Rural Development Administration of Korea (RS-2024-00400211))

754A

Genomic Insights into the Emergence of *Colletotrichum chrysophilum*: the Major Causal Agent of Apple Bitter Rot and Glomerella Leaf Spot

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Apple Bitter Rot (ABR) and Glomerella Leaf Spot (GLS), caused by fungi of the *Colletotrichum* genus, represent an increasing threat to apple production in Europe and worldwide. Recent outbreaks have revealed a more complex *Colletotrichum* community than previously recognized, with species belonging to the *Acutatum*, *Gloeosporioides*, and *Orchidearum* complexes, including *C. fioriniae*, *C. siamense*, *C. grossum*, *C. chrysophilum*, and *C. sojae*. In Europe, the population structure closely mirrors global patterns and is dominated by *C. chrysophilum*, which represents about 60% of isolates and is commonly reported from both North and South America. This species has recently emerged as a relevant pathogen in Mediterranean orchards, raising questions about its introduction routes, ecological plasticity, and capacity for rapid host adaptation.

Despite improved species delimitation, major gaps remain concerning the pathogenicity range, infection mechanisms, and population dynamics of *Colletotrichum* species affecting apple. Here, we highlight the need for an international collaborative framework to coordinate sampling, genome sequencing, and population genomics analyses of apple-associated *Colletotrichum*. Such efforts would enable comparative studies across continents and the development of shared genomic resources.

Our work aims to integrate comparative genomics and population genetics to identify genomic determinants of virulence, trace introduction pathways, and uncover signals of host specialization in *C. chrysophilum* and related species.

Part of this project was funded by the CLARITY (CoLletotrichum Assessment and Re-evaluation to Improve the knowledge on plant pathogenic species recently reviewed in their TaxonomY) project funded by the European Food Safety Authority (EFSA - GP/EFSA/PLANTS/2023/06 – "Experimental and observational evidence to reduceknowledge gaps for risk assessment of new and emerging plant pests" Lot 1: Improving the knowledge on the European and global distribution of plant pathogenic species of the genus Colletotrichum, recently subject to taxonomical changes).

755A

What makes a fungal species? Investigating speciation concepts in the polymorphic tiger sawgill Letninus tigrinus.

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Fungal species are notoriously complex, as morphology is often plastic, many lineages exhibit extensive convergent evolution, and gene flow between even transcontinental populations is not uncommon. The revolution in genomics data has enabled us to investigate fungal speciation in unprecedented detail. However, the delineation of what constitutes a species in Fungi is complex and may not fit cleanly into any single species recognition criterion. Here, we use *Lentinus tigrinus* (Bull.) Fr., the tiger sawgill mushroom, as a model system to discuss the myriad ways to investigate species recognition criteria in Fungi. *Lentinus tigrinus* is a saprotrophic fungus that inhabits the dead wood of hardwoods along riverbanks across North America and Eurasia. *Lentinus tigrinus* is a member of *Polyporales* (e.g, pored mushrooms) that produces gills and presents a unique system for studying fungal morphological development. They are polymorphic in North America, including an "agaricoid" form with exposed gills and a "secotioid" form with enclosed spore-bearing structures. However, the genus *Lentinus* has long been a systematic quagmire with many cryptic relationships, hindering our ability to ask more profound questions about their evolution. In particular, a seemingly simple question about the conspecificity of North American and Eurasian populations of *L. tigrinus* remains unanswered. Transcontinental species distributions are not uncommon in Fungi. However, previous molecular work has shown that *L. tigrinus forms* a complex of four cryptic clades that cannot be resolved using conventional DNA barcoding. We sequenced over 200 *Lentinus* genomes worldwide, including populations of *L. tigrinus* from North America and Eurasia, including historical and type specimens from museum collections. We investigated multiple species recognition concepts as they relate to fungi, including morphological differences, phylogenomic analysis (across the genus *Lentinus*), gene flow between populations (within North America and intercontinental with Eurasia), hybridization and hybrid vigor of these significant populations, and how historical DNA sequencing (museomics) of type specimens can help us delineate fungal species and resolve taxonomic issues in the modern era. With these results, we present a comprehensive case for splitting *L. tigrinus* into two species, *Lentinus squamulosus* (Morgan) Bradshaw, Roehl, Tabima, and Hibbett **comb. nov**. (North America) and *Lentinus tigrinus* (Bull.) Fr. (Eurasia), and provide a framework for discussing how multiple species recognition criteria should be considered when delineating fungal species.

756A

Whole-genome sequencing reveals high gene flow between native and non-native Myrtales hosts of the *Eucalyptus* pathogen *Chrysoporthe austroafricana*

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Chrysoporthe species are important fungal pathogens of plantation-grown *Eucalyptus*. These fungi reside in the Cryphonectriaceae, which also includes *Cryphonectria parasitica*, the causal agent of chestnut blight that devastated the American chestnut in its native range. Chr*ysoporthe austroafricana* was first identified (initially as *Cryphonectria cubensis*) in South Africa's KwaZulu-Natal province, where it causes a severe girdling canker disease on non-native *Eucalyptus* and *Corymbia* (Myrtaceae) hosts. After its initial discovery, the pathogen was also found on native *Syzygium* (Myrtaceae) and on non-native ornamental *Pleroma* (Melastomataceae), which like *Eucalyptus*, both reside in the Myrtales. The occurrence of Chr. *austroafricana* on indigenous hosts led to the hypothesis that the pathogen is native to southern Africa. While this view was supported by earlier studies, it could not be tested with the rigorous tools now available.

In this study, we analysed whole-genome sequence data for 48 isolates of Chr. *austroafricana*, collected between 1989 and 2005 from *Eucalyptus*, *Corymbia*, *Pleroma*, and *Syzygium* in KwaZulu-Natal. Sequencing was performed using the Illumina NovaSeq platform, and genome-wide variant calling using GATK, generated a high-confidence SNP dataset. These SNPs were used to investigate genetic diversity, population structure, and potential recombination. We also examined the distribution of mating types among the isolates. Distinct structure was observed across the population, exhibiting high levels of genetic diversity between clusters, with varying levels of diversity within them. No strong association was found between the different clusters and their host, geographic location, or time of sampling. However, of these, the areas where isolates were collected had the greatest impact on the population structure. Furthermore, both mating types of the pathogen were detected frequently across the population. But evidence of recombination was limited, which could reflect the existence of multiple clonal or inbreeding lineages.

The results suggest high levels of gene flow and movement of Chr. *austroafricana* among the different Myrtales hosts with the potential for recombination. The implication is that plants in the Myrtales may act as alternative hosts for the pathogen. This has important implications for breeding disease-resistant *Eucalyptus and Corymbia* species, necessary for sustainable management of commercially important plantations in the region.

757A

Environmental Selection and Population Genomics of *Fusarium graminearum* Across Diverse Climatic Regions

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Fusarium graminearum is a major fungal pathogen of cereals, responsible for Fusarium Head Blight and associated mycotoxin contamination. While genomic variation among *F. graminearum* populations has been documented, the influence of climatic factors on pathogen diversity remains poorly understood. In this study, we will examine a broad collection of *F. graminearum* isolates (N > 300) sampled from multiple regions across North America and South America, integrating whole-genome SNP datasets with long term climatic data, including average temperature, precipitation, and humidity. Using genome-environment association approaches such as Bayenv2 and related statistical frameworks, we aim to identify loci showing signatures of climate-associated selection. We hypothesize that climate driven selective pressures contribute to allele frequency shifts and regional differentiation across these continental populations. The anticipated findings will improve our understanding of climate linked evolutionary dynamics in fungal pathogens and help inform long term strategies for managing FHB risk under variable environmental conditions.

758A

Haplotype-Resolved Genomes Reveal Diversity at the Homeodomain Mating-Type Locus in *Rhizoctonia solani* AG1-IB

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Rhizoctonia solani is a genetically diverse fungal species complex. Within this species complex, *R. solani* Anastomosis Group 1-IB (AG1-IB) causes brown patch disease on cool-season grasses and other leaf, root, and stem diseases of bean, lettuce, and rice. To assess the evolutionary potential and population structure of AG1-IB on tall fescue (*Festuca arundinacea*), we used ddRAD sequencing to generate over 22,000 single-nucleotide polymorphisms from 55 isolates collected across six North Carolina field plots. Population-genetic analyses identified four populations: three primarily clonal, including one smaller group showing internal subdivision, and one recombining population with evidence of admixture. Comparative genome analyses showed conservation of homology and local synteny at homeodomain (HD) mating-related loci among AG1-IB isolates, the rice sheath blight pathogen *R. solani* AG1-IA, and *Coprinopsis cinerea*, a saprobic mushroom-forming fungus with a well-characterized mating system in which HD genes regulate nuclear pairing and compatibility. Within AG1-IB, isolates encoded one to four HD genes and displayed allelic variation, including a putative allelic pair at the E-HD2 locus, consistent with patterns observed for basidiomycete mating-type genes. Clonal populations showed low within-population diversity, fixed HD profiles, and strong linkage disequilibrium, all indicative of long-term clonal reproduction. In contrast, the admixed population exhibited heterogeneous HD-allele combinations and reduced linkage disequilibrium, consistent with historical or ongoing recombination mediated by mating-type HD genes. In the consensus assemblies, one E-HD2 allele is recovered. However, in the primary and alternate haplotype assemblies, clear heterozygosity is detected at this locus: two distinct E-HD2 alleles, each unique to one haplotype. This shows that both alleles are present in this heterokaryotic isolate. Comparisons of primary and alternate haplotypes demonstrate that rapidly evolving loci, such as mating-type genes, can be obscured when allelic variation is collapsed into a consensus genome. Reference-based approaches that rely on a single consensus assembly can therefore miss haplotype diversity. Together, these results show that haplotype-based assemblies can uncover hidden heterokaryotic nuclear heterogeneity in mating-type loci that govern compatibility and influence genetic exchange and recombination across populations in *R. solani* AG1-IB.

759A

Polygenetic Determinants of Azole Resistance, Tolerance, and Heteroresistance in *Candida albicans*

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Azole antifungals are widely used to treat *Candida* infections, yet therapeutic failures are common. In addition to resistance, fungal populations can exhibit tolerance and heteroresistance, subpopulation-driven drug responses that can contribute to treatment failure. Here, we performed a genome-wide association study (GWAS) on 557 genetically diverse *C. albicans* isolates to identify genetic loci associated with fluconazole susceptibility, tolerance, and heteroresistance. We uncovered a complex, polygenic architecture underlying all three traits, involving novel loci linked to stress responses, cell cycle, and genome integrity. Notably, canonical resistance genes were absent from our top associations, reflecting either their low frequency or lineage specificity. Functional validation of several candidate genes confirmed distinct genetic determinants for each drug response. Heritability estimates and conditional analyses revealed that susceptibility and heteroresistance are governed by partially overlapping but largely independent loci, with some genetic variants

displaying opposing effects on different traits. This limited overlap suggests that each phenotype represents a distinct evolutionary strategy for drug evasion, which may not be simultaneously addressed by a single therapeutic approach. Our findings underscore the polygenic nature of adaptation to antifungal drugs and expand our understanding of drug response mechanisms in *C. albicans*.

760A

Population genomic diversity of VNI lineage of *Cryptococcus neoformans* from North Carolina

Erick Figueroa-Ildefonso, Kayla T Wilhoit, Marhiah C Montoya, John R Perfect, Paul M Magwene Duke University

The opportunistic fungal pathogen *Cryptococcus neoformans* causes ~180,000 deaths each year and is found in environmental niches where develops traits relevant for its pathogenicity. Among the *C. neoformans* lineages, VNI causes most of the human infections, is globally spread and structured in three major sublineages. However, most of the studies on *C. neoformans* have been conducted in global collections isolated from clinical patients, with a significant underrepresentation of environmental isolates and from regions like North America. This lack of diversity limits the understating of the population genetic variation locally and between environmental and clinical sub-populations. Given that most virulence genes are conserved across pathogenic and non-pathogenic species, a better understanding of the local population structure and the genetic diversity of *C. neoformans* is needed to explore the impact of population genetic variation on pathogenicity traits. In that regard, we conducted the population genomic characterization of a sample of more than 300 isolates collected in North Carolina across 30 years from clinical and environmental sources. A maximum likelihood phylogeny shows that the isolates from North Carolina fall within the VNIa and VNIb sublineages. The VNIa sublineage in North Carolina includes four of the seven subclades reported globally. We also identified a fifth undescribed VNIa sub-clade formed mainly by environmental isolates. Population genetic analyses suggests that the North Carolina VNIa and VNIb sublineages are predominantly clonal but there is evidence of recombination occurring between sublineages. Our findings highlight a high diversity of subclades in the isolates from North Carolina; despite being restricted to a single geographic area. The presence of an undescribed VNIa subclade highlights the importance of including environmental isolates to understand the genetic diversity of *C. neoformans* in nature. The population structure and clonal characteristics identified in this study pose a challenge to consider when conducting a genomic analysis aimed at discovering the genetic factors underlying differences in virulence traits in the subpopulations of *C. neoformans.*

761B

Genome-Scale Insights into Diversity and Fungicide Resistance Potential of *Botrytis cinerea* Populations in California Nurseries

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Botrytis cinerea is a well-known widespread necrotrophic pathogen of ornamentals and vegetables, yet its genetic diversity and evolutionary dynamics within nursery production systems in California remain largely unexplored. Nurseries provide environments where pathogen lineages can mix, diversify, and move across host species, making them key systems for understanding pathogen emergence and fungicide resistance evolution. We analyzed whole-genome sequencing data from 94 *B. cinerea* isolates and two additional *Botrytis* species collected from California nurseries between 2020–2024. Reference-guided SNP calling generated a dense genomic dataset that we integrated with detailed metadata on host, county, nursery and year. Population genomic analyses including PCA, DAPC, revealed multiple coexisting lineages with substantial genetic diversity, including one highly divergent cluster that may represent a unique evolutionary lineage within the nursery environment. Despite this genomic structure, we did not detect clear associations between genetic clusters and known fungicide resistance metabolism genes. Resistance-associated loci such as those in SDH subunits, *erg27*, *mfsM2*, *Mrr1*, and other efflux transporters appeared distributed across lineages without lineage-specific enrichment. These patterns suggest either widespread movement of alleles, complex resistance mechanisms, or strong environmental mixing across nurseries. Comparisons between whole-genome variation, fungicide resistance genotypes, and phenotypic assays will help resolve whether resistance traits align with specific genomic backgrounds or arise independently across populations. Together, these data illustrate nursery systems as dynamic reservoirs of *Botrytis* diversity, shaped by the genotypes observed over the years, nursery locations and hosts. This work provides a foundation for future surveillance efforts aimed at limiting the spread of resistant or highly divergent *Botrytis* lineages in plant production systems.

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Genetic basis of variation in the level of mycotoxin production in *Fusarium graminearum* NA2 isolates

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Wheat Fusarium Head Blight (FHB), caused by the fungal pathogen *Fusarium graminearum*, is a significant constraint on wheat production in the United States due to reduced yields and mycotoxin contamination. The pathogen disrupts grain development and produces trichothecene mycotoxins, such as deoxynivalenol (DON), which contaminate kernels and pose significant health risks to humans and livestock. DON also functions as a pathogenicity factor, facilitating the spread of the fungus from the initially infected spikelet to adjacent spikelets within the wheat head. Although *F. graminearum* populations have often been treated as homogeneous, considerable variation exists in pathogenic traits, including mycotoxin (DON) production. Identifying the genetic determinants of this phenotypic diversity is essential for improving disease management. We hypothesized that specific genomic variants underlie these differences and can be mapped to loci associated with key pathogenic traits, including mycotoxin production. Using genome-wide association studies (GWAS), we aimed to identify genetic variants associated with variability in deoxynivalenol (DON) production levels. A total of 158 *Fusarium graminearum* NA2 isolates were cultured on rice media in triplicate, and the resulting biomass was processed for mycotoxin extraction and gas chromatography–mass spectrometry (GC–MS) quantification. We used standard statistical methods to detect associations between genetic variants derived from whole-genome sequencing and mycotoxin phenotypes. To broaden the scope of our findings across populations, we will compare DON production patterns and candidate loci identified in this NA2 study with previously reported DON-associated variants from the NA1 population. We anticipate that our results will aid in developing strategies to manage FHB outbreaks and identify targets for pathogen control.

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Defining the *Candidozyma auris* pan-genome

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Candida auris is an emerging fungal pathogen that exhibits a high degree of phenotypic variability both within and between the five defined clades, but the genetic basis of this variability has not been studied on a population level. A pangenome analysis of *C. auris* that incorporates structural variants could give considerable insight into the genetic changes driving these significant differences in phenotype. We selected 694 isolates of *C. auris* from clades I, III, and IV, representing a mix of publicly available data and samples collected from Michigan and Chicago. All isolates underwent *de novo* assembly and annotation via a custom-built pipeline optimized for *C. auris*. Hybrid assembly was performed for several isolates from each clade, generating high-quality chromosome-level assemblies that served as clade-specific reference sequences. After taking introns into account, we found that pangenome analysis with Panaroo outperformed Orthofinder in this highly clonal dataset, identifying 6,183 gene families and reducing the proportion of the accessory and singleton families to roughly 11%. A total of 93 gene families were fully clade-specific, exhibiting only between-clade variation and no within-clade variation. Ancestral reconstruction was used to further distinguish accessory gene families that were lost or gained multiple times from those derived from a single event. To connect this genetic variation to structural changes, we performed structural variant calling with perSVade on both the species and clade level, with high-confidence structural variants identified by calculating variant allele frequencies at each breakend. Many of these high-confidence variants were supported by the presence and absence of corresponding genes from the pangenome, with deletions being the most common variant type identified. Clade III exhibited notably fewer structural variants at the clade level than Clades I and IV. Both the pangenome and the structural variants were consistent with known relationships between clades, with Clade IV being more diverged among the gene families and carrying more structural variants. We aim to use this information to further characterize the gene families and structural variants specific to these three clades of *C. auris*, and how this variation contributes to phenotypic variation among the clades.

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Genetic mechanisms and fitness consequences of rapid evolution in severe and complex heavy-metal environments with *Saccharomyces cerevisiae*

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When there is a large, rapid change to the environment, a stable population will likely go extinct unless beneficial alleles are introduced via random mutation or migration. The rare individuals bearing these alleles may survive, reproduce, and rescue the population from extinction. Theory predicts this phenomenon, “evolutionary rescue”, will favour first-step mutations with large effects (e.g., LOF, GOF). However, this theory typically considers only single-stressor scenarios, despite natural environments imposing multiple simultaneous selection pressures. Our models of evolutionary rescue may be insufficient when combined stressors display non-additive interactions (i.e., synergism/antagonism), and these interactions may favour different genetic mechanisms and result in different fitness outcomes. To investigate this, I evolved *Saccharomyces cerevisiae* in various concentrations and combinations of six metals (Cd, Co, Cu, Mn, Ni, and Zn). First, I performed evolutionary rescue experiments in individual metals and measured cross-tolerance of evolved lines to each of the other metals. Whole-genome sequencing revealed that lines with the broadest cross-tolerance often carried mutations impacting phosphate metabolism, likely associated with loss of Pho84p. Next, I quantified the combined effects of each of the 15 metal pairs using two metrics: a previously established interaction value, a, describing deviation from a null model of additivity, and a new metric, δ, describing how harmful a combination is relative to the average of its components. I observed a wide range of interactions, with manganese driving antagonistic toxicity (less toxic than expected) and copper driving synergistic toxicity (more toxic than expected). Finally, I performed evolutionary rescue experiments in the metal pairs, generating evolved lines in eight of the 15 combinations. Genotypes evolved in antagonistic metal combinations tended to gain fitness in each individual metal in addition to the combination, while synergistic combinations generated mutations that only increased fitness in the metal combination. By connecting evolved fitness outcomes to observed mutations in highly replicated selection regimes, this work deepens our understanding of evolutionary rescue under multiple stressors and improves our ability to predict and manage extinction risk in environments contaminated by heavy metals.

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Dosage-Dependent Genetic Architecture of Specialized Metabolite Resistance in *Botrytis cinerea*

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Plants produce a rich diversity of specialized metabolites that shape evolutionary interactions between hosts and their pathogens. These metabolites can act directly and indirectly on pathogens, influencing survival, growth, and competitive outcomes. Yet pathogens such as Botrytis cinerea remain remarkably successful despite encountering a chemically heterogeneous world in which metabolite identities and concentrations vary widely across host tissues and environments. This raises the question of how a generalist pathogen maintains fitness across the dosage gradients it experiences within and between hosts.

To address this, we exposed a population of B. cinerea to increasing doses of the green leaf volatiles cis-3-hexanol and trans-2-hexanal to quantify phenotypic variation in dose-response and identify the underlying genetic architecture of resistance. Genome-wide association analyses revealed that resistance to both GLVs exhibits a polygenic architecture across doses, indicating that tolerance relies on many loci whose contributions shift depending on concentration. Notably, the strength, direction, and effect sizes of associated loci changed across inhibitory dose levels, suggesting that different genomic regions contribute to resistance at low versus high exposure.

These dosage-dependent patterns indicate that B. cinerea does not deploy a single uniform strategy to tolerate plant volatiles. Instead, the pathogen uses flexible, concentration-specific resistance mechanisms that allow it to respond dynamically as chemical intensity changes. Our work advances our understanding of how B. cinerea persists across the diverse and fluctuating chemical landscapes

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Quantitative and evolutionary genetics of titan cell formation in *Cryptococcus neoformans*

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The fungal pathogen *Cryptococcus neoformans* can undergo a morphogenetic transition referred to as titan cell formation, which is characterized by the production of enlarged, polyploid cells. The large size of titan cells, and cellular and physiological changes that are associated with this morphogenetic switch, play an important role in persistence and dissemination of this pathogenic fungus within animal hosts. In order to identify segregating genetic variation that underlies differences in titan cell production, we undertook a series of evolutionary and population genomic analyses coupled with quantitative trait locus (QTL) mapping. Employing a genetically diverse panel of strains, we demonstrate that there is significant variability among strain backgrounds in the propensity to form titan cells in vitro. Using a mapping population constructed from a cross of two strains that exhibit extreme differences in titan cell formation, we carried out QTL mapping and discovered multiple genetic loci that contribute to variation in this trait. For the largest effect QTL in our cross, we identified PDE2 as a candidate gene; PDE2 encodes a phosphodiesterase that acts as a negative regulator of cAMP-signaling. Using gene knockouts, allele swaps, and chemical genetics, we demonstrate that PDE2 loss-of-function alleles are correlated with strong titan cell formation, while functional alleles of PDE2 reduce the frequency of titan cell. These in vitro results are supported by parallel analyses of titan formation in segregants from this cross, analyzed in mouse models of infection. Our findings suggest a model in which *C. neoformans* strains that have alleles that promote high levels of cAMP-PKA signaling readily produce titan cells, while strains genotypes with suppressed cAMP-signaling may fail to undergo this morphological transition.

767B

Host-specific isocyanide natural product diversification in the pathogenic mold *Fusarium sporotrichioides*

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Fungi are renowned for their staggering array of natural products, ranging from harmful mycotoxins to life-saving antibiotics. Isocyanide natural products mediate competition with other microorganisms by chelating metals and binding to other metabolites, sometimes causing dangerous diseases in livestock that consume infected wild or cultivated grasses. Most Ascomycete genomes have recently been shown to encode two to three unique isocyanide synthase (ICS) genes; however, we have revealed that many plant-pathogenic lineages have expanded repertoires of these compounds. For example, the reference genome for *Fusarium sporotrichioides*, a common endophyte of wild grasses and a causal agent of Fusarium head blight on wheat and barley, encodes seven ICS genes. To understand how ICSs in *F. sporotrichioides* have genetically differentiated between hosts, we analyzed the pangenome of 151 *F. sporotrichioides sensu stricto* isolates collected from wild and domesticated grasses worldwide. We compared ICSs from our pangenome with 34 closely related isolates from the Sporotrichioides species complex and other closely related Sordariomycetes and found that the expansion in ICSs began prior to the diversification of the Sporotrichioides species complex. Most *F. sporotrichioides* isolates retain three ICS families that are widespread across genera, two Fusarium-specific ICSs, and up to two species-specific ICSs. One of these species-specific ICS families shows signatures of overrepresentation from wild-grass isolates, suggesting a potential role of its associated biosynthetic gene cluster in host-range specificity. This expanded cluster is restricted to our sampling of North America, where our sampling suggests two geographically isolated North American lineages and three populations with global distributions. Together, our work suggests that isocyanide expansion may contribute to host-range in an agriculturally important pathogen.

768B

Genomic insights into the reproductive biology and population diversity of *Xanthoporthe myrticola* (Cryphonectriaceae), an emerging tree pathogen in South Africa

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Xanthoporthe myrticola (Cryphonectriaceae, Diaporthales) is an emerging pathogen causing a stem canker disease on planted eucalypts in South Africa. The disease causes infections of the cambial tissues that can result in tree mortality. To better understand the biology and epidemiology of this pathogen, we generated two draft genomes using Illumina sequencing. These resources were used to develop mating-type and microsatellite markers to characterize the reproductive biology and population structure of isolates from five locations in KwaZulu-Natal (KZN). A number of older collections from eucalypts in South Africa and Eastern Australia were also included. Analysis of the mating type loci revealed a typical heterothallic system, with isolates having either the *MAT1-1*idiomorph (containing *MAT1-1-1*, *MAT1-1-2*, and *MAT1-1-3*), or *MAT1-2* (*MAT1-2-1*). Both idiomorphs occurred at roughly equal proportion in the KZN populations, suggesting the occurrence of sexual outcrossing. Analyses using the 18 polymorphic microsatellite markers revealed high genotypic diversity and gene flow across all five sampled locations in KZN, with no detectable geographic or host-associated structure. Despite the balanced mating-type ratios, significant linkage disequilibrium suggested either limited sexual reproduction in nature, or a recent population expansion following a genetic bottleneck. The isolates from earlier collections showed that *X. myrticola* may have been present, but overlooked in South Africa since the early 2000's. Furthermore, some isolates from Eastern Australia, where most *Eucalyptus* species are native, were also found to be of *X. myrticola*. This suggests that the pathogen may have originated in Australia and was subsequently introduced into South Africa.

769C

Identifying the consequences of genomic content changes in *Cryptococcus neoformans*.

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Cryptococcus neoformans causes an estimated 147,000 deaths per year worldwide, primarily in immunocompromised patients. *C. neoformans* consists of 4 different molecular types: VNI, VNII, VNBI, and VNBII. While we understand sequence variation separating these groups, we have a limited understanding of the differences in genetic content that differentiate each isolate. We have de novo assembled a pangenome using existing Illumina sequences of 384 diverse *C. neoformans* isolates. We have curated a set of *C. neoformans* orthologs that are either: core (present in >95% of genomes), accessory (present in >=95% and <1 genomes), or singletons (present in 1 genome). We found a high conservation of orthologs within molecular types, indicating potential genetic isolation between molecular types. Additionally, we found most genetic content variation in *C. neoformans* is from accessory genes, not singletons. Our pangenome revealed that a large portion of accessory genes were located near subtelomeres in reference strain H99. We also found that hundreds of accessory genes were isolated to specific molecular types and that singletons shared close sequence similarity to closely related species of *Cryptococcus.*

770C

Genomics insights into the *Bipolaris gigantea* host range expansion from grasses to hemp

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The ascomycete *Bipolaris gigantea* has recently emerged as a significant pathogen of hemp (*Cannabis sativa* containing <0.3% THC), triggering severe outbreaks following the legalization and resurgence of hemp production. Since its first report in Kentucky in 2014, *B. gigantea* has caused leaf spot epidemics on hemp in 15 eastern states, with crop losses reaching up to 100%. Widespread epidemics on the invasive grass *Microstegium vimineum* in the same region led to the hypothesis that grass-infecting populations served as sources of inoculum driving emergence on hemp. To investigate gene flow and reproductive dynamics of *B. gigantea* among hosts, we conducted a field survey in September 2024, collecting over 250 samples from six locations across Kentucky. From these, we selected ten representative isolates from hemp and adjacent grass hosts for PacBio HiFi long-read sequencing and *de novo* genome assembly. Assembly sizes spanned 30–32 Mb, with scaffold N50 values of 2.8–3.7 Mb, L50 of 3–6, and GC content near 50–52%. Multiple scaffolds terminated in telomeric repeats, indicating near chromosome-scale contiguity. BUSCO completeness for Ascomycota database was consistently high (~98–99%), indicating nearly complete assemblies with low fragmentation. Average pairwise nucleotide identity between hemp- and grass-derived isolates exceeded 99%, indicating a shared population across hosts and supporting *Bipolaris gigantea*'s recent host range expansion from grasses to hemp. Mating-type searches identified both idiomorphs (MAT1-1 and MAT1-2), each present in isolates from hemp and *M. vimineum*, revealing the coexistence of complementary mating types in the landscape. Additionally, RNA-seq data from a hemp isolate (A4-1) were generated and used as transcript evidence to support structural gene prediction and functional annotation of a representative reference genome, with annotations subsequently transferred to the remaining assemblies. Comparative genomic analyses revealed high genome collinearity and shared gene content between isolates from hemp and grass, which together with ecological data, indicate ongoing cross-host gene flow. Overall, these genomic resources provide a valuable foundation for investigating the genomic basis of host expansion and have important implications for the management of *B. gigantea* in hemp production.

771C

Evidence of Mixed Clonal and Recombining Reproduction in *Colletotrichum graminicola* in Europe

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Maize anthracnose, caused by the ascomycete *Colletotrichum graminicola*, is an important crop disease worldwide. For many years, *Colletotrichum* species were regarded as predominantly clonal plant pathogens. However, recent genomic studies have revealed that recombination and introgression contribute substantially to the evolutionary history of several lineages, including *C. graminicola*. At a global scale, population genomic analyses showed that the species structure is shaped by geographic differentiation driven by long-distance migration and a long history of recombination (Rogério et al., 2023, 2025). These insights suggest that similar processes may also influence population structure at regional scales, where epidemics actually occur. To evaluate this, we analyzed a densely sampled local population from Croatia. We performed whole-genome sequencing of 95 *C. graminicola* isolates collected over two consecutive years (2021 and 2022) at the maize breeding station of the Bc Institute in Rugvica (Zagreb County). Across the dataset, we identified 428,271 high-quality SNPs. AMOVA revealed that 94% of genetic variance occurred within years and only 7% between years; although statistically significant, differentiation was low (FST = 0.04), indicating that yearly turnover did not generate strong population subdivision. After clone correction, 43 multilocus genotypes (MLGs) were retained, corresponding to a clonal fraction of 0.55. This pattern reflects substantial asexual reproduction or repeated sampling of identical genotypes within the field. Despite the high clonal fraction, several independent indicators supported ongoing recombination. The PHI test detected significant recombination, linkage disequilibrium (LD) decayed rapidly with physical distance, and the index of association indicated weak multilocus linkage. LD matrix analyses further revealed a heterogeneous genomic landscape: while some contigs harbored large high-LD haplotype blocks ($r^2 \approx 1$), others displayed diffuse, low-LD patterns consistent with frequent recombination. Overall, our findings show that regional *C. graminicola* populations exhibit a mixed reproductive system, combining clonality with measurable recombination. This mirrors global patterns and underscores the importance of incorporating both processes when predicting pathogen evolution and designing sustainable disease management strategies.

772C

Population genomics of the laminated root rot fungus *Coniferiporia* Lluvia Vargas[1], Shawn McMurtrey[2], Jared LeBoldus[1] [1]Botany and Plant Pathology, Oregon State University, [2]USDA-Agricultural Research Service

The wood-inhabiting fungi *Coniferiporia sulphurascens* and *Coniferiporia weirii* are classified as aggressive pathogenic basidiomycetes, causing laminated root rot (LRR) disease in economically important conifer species worldwide. While it has been suggested that these two pathogens were introduced to North America from Eurasia 7.5 million years ago, they are considered endemic to western North America's mixed conifer forests. *C. sulphurascens* and *C. weirii* differ in their host specificity, with the former being predominantly associated with *Pseudotsuga menziesii* and *Abies grandis*, and the latter being primarily associated with *Thuja plicata* and *Callitropsis nootkatensis*. Although much is known about the biology and spatial distribution of *Coniferiporia*, little is known about the processes that affect the population dynamics of the pathogen. The successful management of LRR requires a more comprehensive understanding of disease transmission dynamics and the formation of new infection centers. In order to gain a deeper insight into the demographic and population structure of *C. sulphurascens* and *C. weirii*, 85 whole genome sequences of *C. sulphurascens* and 5 whole genome sequences of *C. weirii* were produced using Illumina HiSeq 3000 technologies. These sequences were derived from 12 populations across Canada, Japan, Russia, and the United States. A population genomics approach was undertaken utilizing genome-wide single nucleotide polymorphism (SNP) data with the objective of estimating population structure, characterizing gene flow among populations, and to compare the evolutionary history of the two species.

773C

Investigating genetic and molecular mechanisms of amphotericin B resistance in *Candida auris* Dominique K Walker[1], Nick C. Cauldron[2], P. David Rogers[3], Christina A. Cuomo[2] [1]Molecular Microbiology and Immunology, Brown University, [2]Brown University, [3]St. Jude

Background: *Candida* auris is a multidrug resistant fungal pathogen that has caused worldwide hospital outbreaks, rising clinical cases, and high mortality rates. Among the three major antifungal drug classes, resistance mechanisms to the polyene, amphotericin B (AMB) are the least understood. Broadly, *Candida* species combat antifungal drugs through genetic mutations that either affect the drug target and/or transcriptional regulators. Therefore, analyzing clinical isolates that are resistant and susceptible to AMB may pinpoint key mechanisms of AMB resistance in *C. auris*.

Methods: To accomplish this, we used genome-wide association studies (GWAS) to target genes with genetic features and identify copy number variants (CNVs) associated with AMB resistance. Nonsynonymous mutations were screened for their impact on protein function, and broth microdilution and Etests were performed on mutant strains to determine AMB MICs.

Results: Our results reveal statistically significant variants in the protein-coding region of two *C. auris* genes predicted to encode the transcription factors *FLO8* and *TAC1B*. These genes have been previously implicated in fungal virulence and resistance. We also identified variants in *HSP70*, *RAD18*, and *CDR1*, genes involved in stress tolerance, DNA repair, and efflux pumps. We further identified CNVs linked to resistance genes from resistant clinical isolates in *ERG11* and a chromosome 5 aneuploidy, most commonly. The impact of mutations on *HSP70*, *CDR1*, and *TAC1B* identified in our GWAS were predicted to affect protein function. Antifungal susceptibility testing of Clade I strains with the A657V mutation showed a slight decrease in susceptibility and visibly displayed greater cell growth than controls.

Conclusions: Ultimately, these findings provide a strong framework for further characterization of AmB resistance in *C. auris*.

774C

New insights into genomic diversity and population structure of hybrid and rare lineages of *Cryptococcus neoformans* Kayla T Wilhoit, Erick Figueroa-Ildefonso, Marhiah C Montoya, Claudia Zirión-Martinez, Jason Mohabir, Debra Murray, John R Perfect, Paul M Magwene Duke University

As an opportunistic fungal pathogen, populations of *Cryptococcus neoformans* must adapt to radically different fitness landscapes as they transition from the external environment to the host niche. To date, most population genomic studies of *C. neoformans* and related species have focused on global collections of clinical isolates derived from human patients. Much less understood is the nature of genomic variation at local geographic scales or how genome variation differs between environmental and clinical sub-populations of *Cryptococcus*. To gain new insight into local population structure, we performed whole genome sequencing on a unique set of more than 700 clinical and environmental isolates of *C. neoformans* collected from a single geographic region in North Carolina, USA. We find an unexpectedly high local diversity of co-occurring genotypes, with representation across multiple *Cryptococcus* species and lineages in both clinical and environmental populations. These include a significant number of interspecies and intraspecies diploid hybrids likely derived from ancient crosses, as well as the largest number of the rare and understudied VNII lineage sequenced to date. Genomic analysis reveals large-scale genomic structural variants that are almost exclusively found in clinical isolates and are especially prevalent in in diploid hybrids. These structural variants, including aneuploidy, chromosome loss, loss-of-heterozygosity, and translocations, could represent a major source of adaptive genomic change during the transition *C. neoformans*. Phylogenetic and population genetics analysis of the North Carolina samples in the context of globally sequenced isolates reveals novel population structure not previously characterized, including a deep split in the newly expanded VNII clade. The conservation of genotypes and population structure across geographical distance has implications for the speciation and ecology of the *Cryptococcus* genus. Our results leverage a unique set of isolates to provide insights into the population genomics and global epidemiology of *Cryptococcus neoformans* and highlight the impact of diversifying selection on the genomes of fungal pathogens in the complex environment of human hosts.

775C

Uncovering in-field dynamics, persistence and adaptation of a clonal Ascochyta rabiei population of chickpea using a novel molecular workflow Hayley Wilson[1,2], Ido Bar[1], Kristy Hobson[2],

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Ascochyta rabiei is a major fungal disease of global chickpea (*Cicer arietinum*) production. The Australian *A. rabiei* population is clonal with low genetic diversity, and detection to date of a single mating type. Despite this, aggressive isolates have become more frequent, overcoming host resistance, however, their interaction with host cultivar, dynamics and persistence remain largely unstudied. Accordingly, a field-based experimental evolution study was conducted where an experimental population was released then re-captured after three years of continuous cropping, propagation via rain-splash and crop residue transmission. A workflow incorporating 30× whole genome sequencing and discriminant analysis of principal components (DAPC) was used to define the experimental population. This comprised of six *A. rabiei* genotypes with pathogenicity level ranging from moderately aggressive to highly aggressive. After three cropping seasons, DAPC of 304 re-captured isolates revealed nine distinct multi-locus haplotypes (MLH). Six MLH 'founder groups' aligned with the initially released experimental population in addition to three MLH wild-type introductions, demonstrating survival and relatively minor mutations away from the source genotypes. Recaptured isolates were then assessed for relative fitness measured by frequency of detection on a range of chickpea cultivars of varying resistance/susceptibility grown in a randomised block design independently over three years at the same cropping site. Patterns of differential fitness of some MLH on specific chickpea genotypes were identified, however, no correlation was found between pathogenicity level and host resistance rating. Implying adaptation may be driven by response to specific host genetic backgrounds and pathogen population exposure over time. These findings provide new insights into the field dynamics and persistence of *A. rabiei* and present a novel workflow for detecting adaptation in low diversity, clonal fungal populations where other genetic markers are unknown. This highlights the necessity of maintaining pathogen population surveillance and collaboration between chickpea breeders and pathologists to maintain durability in commercial cultivars.

776C

BRC-Analytics.org: a platform for free, high performance fungal genomics analysis

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The genomic landscapes of fungal pathogens and their hosts present unique analytical challenges that reflect the remarkable diversity of fungal biology. From the compact genomes of early-diverging fungi to the complex chromosomal architectures of pathogenic Candida and Aspergillus species, the polymorphic life cycles of dimorphic fungi like Histoplasma, and the programmed genome rearrangements observed in certain fungal lineages, this biological diversity demands correspondingly sophisticated analytical approaches. Fungal pathogens span a vast evolutionary range—from basal fungi to ascomycetes and basidiomycetes—each presenting distinct genomic features, repetitive element landscapes, and host interaction strategies. Navigating this complexity, particularly when integrating host genomic responses and comparing across diverse fungal clades, can be daunting. BRC-Analytics.org addresses this challenge by unifying fungal genomic data analysis across all pathogenic systems, whether conventional or highly atypical. It integrates comprehensive fungal reference datasets with hundreds of curated analytical workflows from iwc.galaxyproject.org, supporting tasks from fungal genome assembly and annotation to comparative genomics, variant analysis, and host-pathogen transcriptomics. Most importantly, it provides free, scalable, and unrestricted computational infrastructure via the Texas Advanced Computing Center (TACC), empowering researchers to analyze fungal datasets of any size and complexity—from small yeast genomes to large filamentous fungal genomes with extensive repeat content. Here we demonstrate the platform's capabilities through a variety of analyses using published fungal genomic datasets, illustrating reproducible workflows for common research questions in fungal pathogenesis. BRC-Analytics eliminates technical bottlenecks and democratizes access to sophisticated mycological bioinformatics, accelerating discovery across the fungal infectious disease research continuum.

777A

Exploring the biosynthetic potential of the leaf-cutter ant pathogen *Escovopsis weberi* using heterologous expression

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Escovopsis weberi is a specialised fungal pathogen that disrupts the mutualistic symbiosis between the leaf-cutter ants, their cultivated fungus *Leucoagaricus gongylophorus* and the protective bacteria *Pseudonocardia*.[1] In this highly competitive environment, *E. weberi* can establish itself by negatively impacting other biological elements within the symbiotic relationship.[2] One way these interactions are mediated is by producing bioactive secondary metabolites, and up to this day, only some compounds have been isolated from this fungus, and their ecological roles have been assessed. For example, indole-containing metabolites such as shearinines can impact the ants' behaviour, while the antimicrobial polyketides emodin and cycloarthropsone can suppress the growth of competing microbes.[1,3] Bioinformatics analysis revealed that the genome encodes 25 putative biosynthetic gene clusters (BGCs) that could be involved in synthesising various putative uncharacterised metabolites, with only three BGCs linked to previously isolated metabolites. However, the lack of established genetic tools for *E. weberi* has limited the functional investigation of its biosynthetic pathways.

This study explores the applicability of the well-established expression platform *Aspergillus oryzae* NSAR1 as a heterologous host for BGCs from the fungus *E. weberi* with the overarching goal of discovering new bioactive metabolites with potential antimicrobial properties and ecological roles. Two distinct BGCs, a putative standalone polyketide synthase (PKS) and a hybrid polyketide synthase-non ribosomal peptide synthetase (PKS-NRPS), were selected for heterologous expression based on predicted novelty. Modular plasmids with inducible promoters were constructed to dissect and express the biosynthetic pathways into the host *A. oryzae* NSAR1. Genetic and chemical analyses were applied to confirm the transformation process and gene integration.

Overall, this work establishes the first framework for exploring *E. weberi* secondary metabolites using heterologous expression and validates the first step towards reconstructing *E. weberi* biosynthetic pathways outside their native background. Thereby fulfilling the purpose of discovering new metabolites with potential ecological and pharmaceutical value.

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778A

Towards synthetic biology in edible mushrooms: characterization of endogenous regulatory elements in the mushroom *Coprinopsis cinerea*

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Agaricomycetes, a clade of mushroom-forming basidiomycetes, play major ecological roles in carbon storage and nutrient cycling and can grow on both natural and man-made waste streams. Engineering these species with synthetic biology could enhance and refine their native capacities, expanding their utility in basic research as well as in biotechnological applications for sustainability. Progress in mushroom synthetic biology is limited by the lack of precise control over heterologous gene expression. Existing genetic regulatory elements —primarily promoters and terminators from highly expressed constitutive genes—support simple overexpression but constrain more complex design. Re-using the same regulatory elements increases the risk of recombination and cassette instability, and their uniformly high expression levels prevent the construction of circuits with broader dynamic range. To expand the set of available genetic parts in the model mushroom *Coprinopsis cinerea*, we analyzed published RNA-seq datasets to identify constitutive genes across a spectrum of expression levels. We further filtered these candidates for conservation across mushroom species to increase the likelihood that resulting parts will function in other Agaricomycetes. To measure the effect of individual promoters and terminators on gene expression, we developed a dual-reporter vector encoding GFP and RFP in the same cassette. The GFP reporter is driven by a standard promoter–terminator pair, and the RFP by the part being tested. Quantifying the GFP:RFP ratio allows us to isolate each regulatory element's effect while controlling for transformation variability. We first validated this system by transforming *C. cinerea* protoplasts, isolating stable transformants, collecting asexual oidia spores, and measuring reporter ratios by flow cytometry. We then cloned candidate promoter and terminator sequences from *C. cinerea* genomic DNA into the dual-reporter vector and transformed them using the same workflow. Our next goal is to evaluate these genetic regulatory elements in additional mushroom species of laboratory and industrial interest, with the broader objective of establishing a generalizable toolkit for mushroom engineering.

779A

Assessing Agricultural Waste Utilization by the Basidiomycete Fungus Coprinopsis cinerea

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Basidiomycete fungi offer a promising biological route for decentralized waste valorization, with the capacity to convert agricultural residues into protein-rich biomass, bioactive metabolites and valuable chemical precursors. Yet, our understanding of how individual substrates influence fungal metabolism, growth, and the spectrum of native fungal compounds produced remains incomplete. Here, I present ongoing efforts to assess Coprinopsis cinerea as a tractable model for agricultural waste utilization. C. cinerea provides an experimentally consistent platform for probing substrate-dependent metabolism due to its well-defined life cycle, controlled cultivation conditions, increasing genetic tractability, and broad use in fundamental fungal biology.

In collaboration with producers in California's Central Valley, we are examining how differences in substrates and processing methods shape fungal growth and native metabolic outputs across both simple, defined substrates (e.g. glucose, cellulose, lignin monomers) and more complex, real-world residues (e.g. tomato pomace, spent brewer's grains). Comparative cultivation experiments, combined with basic compositional analyses and targeted metabolite profiling, will allow us to evaluate how substrate characteristics influence growth dynamics, substrate utilization, and native compound production. These data will provide a foundation for understanding how C. cinerea grows on heterogeneous agricultural residues and which substrate features influence metabolic programs and carbon utilization. Ultimately, this work aims to inform rational feedstock selection and evaluate the potential of basidiomycete mushrooms as regional waste-upcycling platforms.

780A

Nutrient-responsive metabolites and conserved biosynthetic capacity of *Hyaloscypha finlandica*, a prominent dark septate endophyte of poplar

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Dark septate endophytes (DSEs) have garnered attention for their contributions to plant growth and stress resilience. DSEs, a polyphyletic group of fungi, are globally distributed and symbiotically associated with numerous plant hosts, yet many aspects of their biology, including metabolomic profiles, remain poorly understood. *Hyaloscypha finlandica* is a prevalent DSE in *Populus* roots, pointing to a potential role within the poplar holobiont. We integrated genome mining with untargeted metabolomics to profile *H. finlandica* strain PMI 746. Comparative *in- silico* analyses across closely related taxa using antiSMASH, FunBGCeX, and BGC Prophet revealed a shared repertoire of biosynthetic gene clusters among DSEs, while highlighting tool specific differences in predicted cluster composition and putative products. Additionally, cultures were grown under two nutrient conditions and metabolites were recovered by untargeted metabolomics. Spectral matching against a standard reference library yielded putative annotations, including shared and condition dependent production of numerous candidate metabolites, of which some resembled genome mining predictions. Collectively, these results establish a reference dataset that underscores the unexplored metabolomic potential of root-associated DSEs and demonstrate how predictive genome-mining tools can guide targeted analyses of resource enrichment in non-model fungi.

781A

Illuminating brown-rot genetics: An eGFP-based molecular toolbox for *Gloeophyllum trabeum*

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Wood-decay fungi are the primary decomposers of lignocellulosic biomass. Fungi have evolved two main strategies to overcome the recalcitrant lignin barrier: white-rot and brown-rot. White-rot fungi secrete ligninolytic enzymes to break down lignin, leaving the wood as a white fibrous residue. Brown-rot fungi have evolved from white-rot fungi at multiple points in evolution by the shedding of the genes encoding for ligninolytic enzymes. Despite this genetic loss, brown-rot fungi remain efficient wood decomposers by relying on Fenton chemistry to produce radical oxygen species that disrupt the lignin matrix to gain access to polysaccharides. The preferential degradation of the sugars results a lignin-rich residue that appears brown with a cubical-checked pattern. This unique strategy of decomposition has potential for bioremediation of pollutants and biotechnological applications in biomass conversion. Yet, the genetic basis of brown-rot degradation mechanisms remains to be clarified due to a lack of genetic tools.

To address this challenge, we developed genetic tools for the brown-rot species, *Gloeophyllum trabeum* (*G. trabeum*). The enhanced Green Fluorescent Protein (eGFP) is used as a versatile "molecular flashlight" to develop and demonstrate the functionality of conventional tools in this non-model fungal organism. Each tool was assembled as a gene cassette through overlap extension PCR and delivered to *G. trabeum* protoplasts via a previously established PEG-Cl_2-mediated transformation protocol. Four specific tools were developed in this study, outlined below.

1. Selectable marker genes: Hygromycin- and neomycin-resistance genes were used for effective transformant screening and evaluated for high co-transformation rates.
2. eGFP reporting system: A constitutive promoter was used to express eGFP in the fungus for a reliable visual signal.
3. Localization of eGFP: A nuclear localization signal was tagged onto eGFP to guide the protein to the nucleus, and an eGFP-chitin-binding domain fusion was designed to anchor protein to the fungal cell wall.
4. Tunable expression of eGFP**:** An alcohol oxidase promoter, inducible by methanol and repressed by glucose, was employed to use eGFP as an indicator for lignocellulose degradation-related activity.

With a well assembled gene editing toolbox, we can illuminate the genetic pathways of brown-rot degradation and accelerate the engineering of this non-model organism for future biotechnological applications.

782B
Illuminating transcriptional dynamics underlying circadian clock regulation and photoentraiment.

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Circadian clocks rely on transcriptional-translational feedback loops coordinated by cis-regulatory elements. In Neurospora crassa, the frq promoter is key to circadian regulation, yet its complex structure obscures the functional contributions of individual elements. To address this, we engineered Minimal Synthetic Oscillators (MiSOs) composed of various configurations of the frq promoter's two core cis-elements: the c-box (clock-controlled) and the pLRE (light-responsive). Using luciferase reporters and frq-luc fusion constructs in an arrhythmic background lacking the endogenous promoter, we systematically evaluated how promoter architecture influences rhythmicity, phase, light responsiveness, and temperature compensation.

MiSOs containing only the c-box and pLRE, with or without a core promoter (CP), generated robust oscillations, though performance varied by element arrangement and dosage. Constructs with two c-boxes enhanced light responsiveness and phase tuning. Most MiSOs preserved key circadian properties, including temperature compensation and the ability to synchronize downstream output pathways. However, light-pulse experiments revealed that promoter design determines not only the direction (advance vs. delay) of phase shifts, but also the oscillator's sensitivity to light duration and timing.

Our results show that minimal sets of cis-elements are sufficient to reconstitute key features of circadian regulation, and that promoter architecture exerts fine control over phase, amplitude, and environmental responsiveness. MiSOs provide a powerful synthetic biology platform for dissecting transcriptional control within biological oscillators and may offer new strategies to reprogram clock function.

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783B
Development of a synthetic biology toolkit for heterologous gene expression in the oyster mushroom, *Pleurotus ostreatus*

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Basidiomycota fungi are prolific producers of high-value metabolites including terpenoids, alkaloids and sugar derivatives. Although many fungal-derived natural products are known today, the production of these compounds in the native hosts is limited primarily due to the low/cryptic expression level of biosynthetic genes under the laboratory conditions. In recent years, new genetic and cultivation-based strategies for engineering of Basidiomycota fungi have become available, yet these are often species-specific, and genetic engineering techniques require a sophisticated knowledge of the biology of the producing organisms. Heterologous expression of Basidiomycota biosynthetic pathways in Ascomycota fungi such as *Aspergillus* spp. is feasible, however, more often they are unable to process numerous introns found within Basidiomycota genes.

Here in, we aim to develop a robust synthetic biology toolkit for the heterologous expression of Basidiomycota genes in a tractable basidiomycete, *Pleurotus ostreatus* (oyster mushroom), for which several protocols for introducing exogenous DNA as well as genetic manipulation have been established.[1,2] To date, we have tested *P. ostreatus* strains N001 (dikaryon), PC9 and PC15 (monokaryons) for growth on selected culturing media and performed RNA-seq analyses to identify genes which are highly and constitutively expressed or expressed in selected growth conditions. We are currently testing their respective promoters and terminators to express reporter genes in *P. ostreatus*.

We hope that the toolkit would benefit both the synthetic biology community as well as fungal natural products researchers in order to exploit Basidiomycota genomes as the source for the production of high-value compounds and enzymes.

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784B

AI-driven optimization of growth factor manufacturing from waste by *Aspergillus oryzae*

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As natural decomposers, filamentous fungi hold tremendous potential for circular biomanufacturing, but this potential is limited by their relatively underdeveloped synthetic toolkit. Specifically, *Aspergillus oryzae* is a major workhorse in the enzyme industry thanks to its well-developed secretion mechanisms for potent endogenous amylases. However, it is rarely used to valorize starchy waste streams into heterologous proteins, which are often lost through endoplasmic reticulum-associated degradation after being recognized as misfolded. We aim to bridge this gap by optimizing the transcription and translation rates of bovine IGF, as a model protein, through AI-based machine learning of codon-usage patterns in the fungal host while it grows on waste sidestreams. Proteomic and transcriptomic data were collected from submerged cultures grown on broken rice, wheat bran, and apple pomace. Position-specific Chimera Map (PScMAP) and tRNA Adaptation Index Calculator models, trained on the collected data, predicted protein abundance from codon usage and tRNA index with a Pearson coefficient of up to 0.52 and a p value of $4.7 \cdot 10^{-104}$. Transcript variants generated by the trained models were tailored to ensure optimized transcription and translation when expressed under the top four promoters mined from the omics data. Constructs were integrated into the fungal genome using CRISPR Cas9-based transformation, and the resulting transformants were cultured on waste sidestreams and screened for product yield. Purified IGF was identified by LC-MS/MS following dual-protease digestion and quantitatively determined by ELISA. This AI-assisted optimization of heterologous protein production from complex substrates represents a significant step toward unlocking the full potential of *A. oryzae* in circular biomanufacturing.

785B

Deep homology and design of proteasome chaperone proteins in *Candida auris*

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A central tenet of biology is that protein structure mediates the sequence-function relationship. Recently, there has been excitement about the promise of advances in protein structure modeling to generate hypotheses about sequence-structure-function relationships based on successes with controlled benchmarks. Here, we leverage structural similarity to identify rapidly evolving proteasome assembly chaperones and characterize their function in the emerging fungal pathogen *Candida auris*. Despite the large sequence divergence, we demonstrate conservation of function across hundreds of millions of years of evolution, suggesting that the fold, and not the sequence, is required for function. This provides experimental evidence to support the theory that protein structures evolve more slowly than sequences, and demonstrates how structure can mediate the sequence-to-function relationship across evolutionary time. To reduce this theory to practice, we performed structure-informed protein design, mutating more than 40 residues in a single protein, and demonstrated that these artificial proteins can rescue complex biological processes in the context of the whole cell. Through computational design, we were able to explore sequence space that is not accessible via stepwise evolution. Through this sequence-structure-function relationship, we are able to expands our ability to use structure to identify deep evolutionary relationships between proteins and generate hypotheses about gene function in non-model organisms. Overall, this helps us understand functional constraints on protein evolution, with important implications for both future protein design and retrospective function prediction.

786B

Development of genetic transformation and imaging techniques to gain a better understanding of how filamentous fungi internalize, transport, and transform critical minerals

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Understanding how microorganisms interact with, utilize, and modify critical minerals is of interest to help improve their recovery and reduce the environmental impacts of traditional mining techniques. Furthermore, microbe-mineral interactions can have significant impacts on microbial community composition and function through their impacts on intra- and interkingdom interactions. However, current knowledge on the genetic mechanisms underlying microbial sensing, binding, recovery, transport, and transformation of these minerals is quite limited. This is particularly true in filamentous fungi, despite their numerous advantages for biomining applications. Our work is focused on developing new techniques to gain a better understanding of the genetic mechanisms that enable fungi to internalize minerals in order to assess how these mechanisms can be engineered to optimize the recovery of specific minerals. Towards this goal, we are developing and evaluating a new transformation technique based on interactions between fungi and nanoparticles. This technique will likely have benefits beyond understanding fungal-mineral interactions including facilitating genetic engineering in non-model filamentous fungi. Additionally, we are developing techniques which utilize coated quantum dots to enable the visualization of the internalization and transport of minerals within living mycelial networks. Advanced imaging techniques enabled by the use of quantum dots will improve our understanding of how minerals are transported and localized, as well as provide the means to accurately quantify internalization and transformation. The development of these techniques will help address critical knowledge gaps while simultaneously enhancing the use of synthetic biology to alter how fungi interact with their environment and other organisms.

787C

Monitoring DNA transmission across fungal communities using RNA barcoding

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Fungi transport resources across the soil environment by forming symbiotic relationships with diverse organisms. Identifying fungal partnerships is essential for understanding soil ecology, predicting impacts of climate change, and designing agricultural applications; however, mapping the fungal interactome with sufficient throughput and resolution for environmental communities remains a challenge. In particular, there are limited tools for tracking nucleic acid movement in fungal communities, such as the uptake of environmental DNA and inter-kingdom gene transfer. In this presentation, we will describe how synthetic biology can simplify studies of gene transfer in fungi using an approach called RNA addressable modification (RAM). This technique maps the host range of mobile DNA in a community. First, mobile DNA is encoded with a catalytic RNA. Upon transfer to a new host, the DNA is transcribed, and catalytic RNA is produced. The catalytic RNA binds to a target RNA and splices a barcode onto it. The target is chosen to contain both conserved and variable regions with respect to the community. Finally, the barcoded RNA is sequenced, and the variable regions are used to identify which members of the community participated in gene transfer. We report the first use of RAM in fungi. In proof-of-concept experiments, we have shown that fungal RNA can be barcoded when expressed in a bacterial host or in *Saccharomyces cerevisiae*. In ongoing experiments, we are testing catalytic RNA barcoders in a synthetic community of environmental yeast isolates to evaluate the taxonomic resolution that can be achieved with RAM in communities. This technology is expected to be useful for screening functional genetic parts in fungal communities and addressing ecological studies of gene transfer, including the uptake of environmental DNA, bacterial-fungal gene exchange, and mycovirus transmission.

788C

Investigating factors that govern heterologous gene expression in Agaricomycetes

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Mushroom-forming fungi belonging to the Agaricomycetes class can break down waste and produce a wide array of natural products. Synthetic biology has the potential to enhance these abilities and generate new sustainable biotechnologies including biomaterials, environmental remediation tools, and nutritionally-enhanced foods. However, expressing heterologous genes in these organisms - a key requirement for advanced engineering - is extremely challenging and has a low probability of success. For instance, in our experiments expressing twelve heterologous proteins in the model mushroom *Coprinopsis cinerea*, only two were successfully expressed. Previous studies have identified introns and AT content as independent factors governing gene expression, though systematic tests evaluating the impact of these variables are lacking. Here, we use *C. cinerea* as a model to interrogate how intron architecture and AT content influence heterologous gene expression in Agaricomycetes. Mining transcriptome and genome data, we explore the relationship between these variables and gene expression levels. We then use these findings to systematically design and test reporter gene constructs for *C. cinerea* by altering intron architecture and modifying codon usage of heterologous gene coding sequences. Taken together, this work will inform the design rules for expressing heterologous genes in Agaricomycetes, vastly expanding the metabolic engineering engineering potential in these organisms.

789C

Production of gentisyl alcohol in *Yarrowia lipolytica*

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Gentisyl alcohol is a secondary metabolite with redox properties, which function as antioxidant and has biological and pharmaceutical properties, including antibacterial and anticancer activities.

The compound has been identified in a wide range of diverse fungal genera, including *Epicoccum sorghinum*, *Phoma herbarum* and several species *Aspergillus* and *Penicillium*. In some of these species, gentisyl alcohol is the end-product of a biosynthetic pathway, while others have gentisyl alcohol as an intermediate of the well characterized pathway towards the mycotoxin patulin. The pathway is initiated by formation of 6-MSA by a partially reducing polyketide synthase (6-MSAS) based on one acetyl-CoA and three malonyl-CoA units. 6-MSA is decarboxylated in a reaction catalyzed by PatG, resulting in formation of m-cresol. The resulting m-cresol undergoes two oxygenation steps catalyzed by two cytochrome P450 monooxygenases (PatH and PatI), which first forms toluquinol and then gentisyl alcohol.

Given the promising features of gentisyl alcohol, we inserted codon optimized versions of the responsible genes into *Yarrowia lipolytica*, which has been shown to be a promising host for heterologous polyketide production [1, 2]. We further inserted a PPTase for functionalization of 6-MSAS and a cytochrome P450 reductase (CPR) for regenerating PatH and PatI. The resulting *Y. lipolytica* strain was able to produce >1.2 g/L gentisyl alcohol in liquid medium. The compound was isolated, and with subsequent cyclic voltammetry analyses we were able to determine the electrochemical potential.

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790C

Engineering *Trichoderma*-Based Biosentinels: Smart Systems to Detect, Remember, and Respond to Environmental Stressors

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Recent years have seen major advances in biological sensing in bacteria and plants, yet filamentous fungi remain largely unexplored in this space. This gap is striking given fungi's ecological importance, resilience across diverse environments, and intimate associations with plant hosts. Here, we are engineering the first synthetic fungal biosentinel system, using *Trichoderma* species as a model host. These systems are designed to sense soil stressors, record them through recombinase-mediated genetic memory, and produce interpretable outputs. By leveraging large-scale transcriptomic and proteomic datasets, we are identifying and characterizing native promoters in various *Trichoderma* species that are inducible under stress conditions such as drought and nitrogen limitation. These promoters are being employed to drive recombinase-based memory switches with luminescent reporters as proof-of-concept outputs, enabling rapid circuit optimization under controlled conditions. Future developments may integrate electrochemical outputs or functional stress-mitigation modules, transforming biosentinels from passive reporters into active agents of soil resilience. By positioning *Trichoderma* at the interface of synthetic biology and environmental monitoring, this platform opens new directions for dynamic, real-time, and biologically integrated assessment of soil and ecosystem health.

791C

Characterizing genetic parts in fungal mycelium using large particle flow cytometry

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Characterizing genetic parts in filamentous fungi is challenging as they form mycelial networks that complicate high-throughput single-cell analysis. Here, we design and characterize promoters within the mycelium using encapsulation and large-particle flow cytometry (COPAS). COPAS enabled the quantification of over 4,000 individual mycelial growths under each condition, enabling a detailed analysis of population-level dynamics. The promoters were introduced into nine fungal strains, including Fusarium oxysporum, Aspergillus awamori, Cladosporium cladosporioides, Trichoderma reesei, and Aureobasidium pullulans. The performance of two constitutive promoters and a doxycycline-inducible system was assessed. Flow cytometry of mycelium revealed that the fluorescence signal in transformants was 6-times higher under the control of gpdA promoter compared to wild-type strains in A. awamori and C. cladosporioides. COPAS enabled direct measurement of gene expression in encapsulated fungal mycelium providing data for cell-to-cell heterogeneity. Combining Agrobacterium-mediated delivery with large-particle flow cytometry could allow for engineering reliable genetic circuits in filamentous fungi, enabling advances in biomanufacturing, biomaterials, and synthetic biology applications.

792C

A tunable and strict gene expression system with a high dynamic range in filamentous fungi

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Filamentous fungi are widely used for the bioproduction of valuable compounds, owing to their strong secretory capacity, metabolic diversity, and low cultivation cost. Among them, *Aspergillus oryzae* is recognized as a safe and versatile host because of its long history of use in food industry. These features highlight the need for flexible gene expression systems applicable to synthetic biology in filamentous fungi. However, existing inducible expression systems rely highly on nutrient-dependent promoters, which limits flexibility of culture and induction conditions.

In this study, we developed a versatile drug-inducible gene expression system for filamentous fungi. We introduced an artificial transcription factor (LexA–ER–VP16; XEV) composed of the *Escherichia coli LexA* DNA-binding domain, the human estrogen receptor ligand-binding domain (ER), and the VP16 activation domain into *A. oryzae*. A GUS reporter gene (*uidA*), which encodes the enzyme *β*-glucuronidase, under the control of *lexO* and the *CYC1* core promoter derived from *Saccharomyces cerevisiae* showed strict *β*-estradiol–dependent induction with minimal basal expression. Notably, induction levels of this system reached those of the strong *amyB* promoter, while expression remained unaffected by the choice of major carbon sources. We further applied this system to control transcript level regulation of the endogenous transcription factor AmyR. *β*-Estradiol–dependent regulation of *amyR* expression led to dose-dependent increases in the production of multiple amylolytic enzymes, demonstrating that endogenous pathways can be precisely controlled with this system. We next optimized the system to achieve a broader dynamic range. Expressing XEV under a stronger promoter increased the overall induction level with fewer inducers. Furthermore, replacing the *CYC1* core promoter downstream of *lexO* with the *A. oryzae gpdA* (GAPDH) or *hhfA* (Histone H4) core promoters resulted in higher gene expression. The system was also functional in *Aspergillus nidulans*, demonstrating its versatility to *Aspergilli*.

Overall, we established a nutrient-independent inducible expression platform that offers low leakage, tunable induction, and a wide dynamic range, providing a highly useful tool for synthetic biology and industrial applications in filamentous fungi.

793C

Fungarium Sequencing Project - Large-Scale Museomics and Whole-Genome Recovery from Historical Type Material

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Large collections of preserved fungal material are available at many research institutions, but whole genome (or even single locus) sequences are yet to be retrieved from most specimens. The Fungarium Sequencing Project (FSP) aims to sequence the whole genomes of 7,000 type specimens from three major UK collections-based institutions: Royal Botanic Gardens, Kew (RBG Kew), Royal Botanic Garden Edinburgh (RBGE), and the Natural History Museum, London (NHM). Together, these invaluable collections contain over 1.8 million specimens and approximately 61,000 types. As the reference individual for each described species, type specimens are crucial to taxonomy and understanding evolutionary relationships. Genetic data derived from types will allow for greater stabilization of the fungal tree of life, and the FSP is a step towards integrating such genetic data with physical, preserved collections and their associated metadata. This work will explore genomic patterns across major taxonomic groups and will identify predictors of sequencing success from a breadth of diverse fungal groups. A detailed dive into Kew's type collection has necessitated a systematic approach to navigate taxonomic, curatorial, Access and Benefits Sharing (ABS), and sample quality challenges. Furthermore, laboratory techniques to maximize quality and quantity of DNA yield were tested to account for phenotypically and taxonomically diverse samples. A modular bioinformatic workflow for sequence quality control, whole genome assembly, and decontamination of fungal material is under development. As the first large-scale attempt to sequence whole fungal genomes from historical collections, the project is developing refined methodologies for three major processes: tissue sampling and taxonomic assessment, ancient DNA extraction and library preparation, and development of a dedicated ancient fungal genomics bioinformatics pipeline.