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Fungal Evolution: *Mucor* and *Phycomyces* See Double

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Newly available genome sequences of two Mucoralean fungi, *Phycomyces blakesleeanus* and *Mucor circinelloides*, provide evidence for an ancient whole genome duplication that contributed to the generation of expanded gene families. These fungi have robust responses to light that can be correlated with the expansion of gene networks involved in light sensing and signaling.

Zygomycete fungi are distinctive, early-diverging lineages of the fungal tree of life that inhabit a broad range of ecosystems and include the order Mucorales – a commonly found group of molds [1]. Almost 200 years ago, the *Phycomyces* were first described as species with large aerial hyphae, named sporangia, with what look like small round balls attached to the end of a stalk (Figure 1) [2]. Since that time, *Phycomyces blakesleeanus* has served as a useful model for the study of perception in fungi and of how fungi process environmental signals. *P. blakesleeanus* is a (mostly) innocuous mold commonly isolated from dung and general organic matter [3], and early work explored its metabolic regulation and the incredible sensitivity of the stalk-like sporangiophores to sense and orient towards light sources as faint as starlight [4]. *P. blakesleeanus* continues to be studied for its ability to respond rapidly to light and other environmental cues, and has inspired a variety of researchers including Max Delbrück to study metabolism and phototropism responses [4, 5]. Methods for mutagenesis and performing sexual crosses have been developed for *P. blakesleeanus,* but gene deletions via replacement are not yet possible [6]. *Mucor* species and the related *Rhizopus* fungi are some of the fastest-growing fungi known and are common soil dwellers. *M. circinelloides,* in particular, is found on woody surfaces and soils, but if introduced to humans, especially through a skin wound, is a dangerous and difficult-to-treat pathogen that can lead to an often-severe, flesh-eating disease [7]. The role of specific genes in the biology and pathogenesis of *M. circinelloides* has been investigated with forward genetics via RNAi knockdown screens [8]. Isolation of recessive mutants in Mucormycotina fungi has low success rates because the coenocytic mycelium and spores have multiple nuclei in the same cell, but gene deletion and RNAi have been successful in Mucor species. Although the linking of genes to function remains a challenge for fungi in these clades, genomic and transcriptomic approaches are feasible and can assist in generating hypotheses about gene functions.

In a recent study published in *Current Biology*, Corrochano and colleagues [9] have now reported on the genome sequences and analyses of *M. circinelloides* and *P. blakesleeanus,* and have identified an extensive expansion of genes involved in transferring information within fungal cells. Comparison of these species to their relatives

has also provided additional perspective on recent evolution of zygomycete fungi. The generation of a genome sequence is as much about what can be learned from the initial sequencing as what will be enabled in the future. Comparative genomics of many of these species has already provided some perspective on which genes are necessary for high-temperature growth and animal-associated lifestyles [10]. Studies of the fungal kingdom are benefiting from the increased application of genomic technology [11]. The early diverging fungi, especially, are seeing focused genomic sampling through projects like 1KFG (http://1000.fungalgenomes.org) and Zygolife (http://zygolife.org). Population genomic studies of fungi are enabling additional ways to match genes to functions; natural genetic variation and inferences of selection can be matched to indicate ecological contexts where different gene functions are preferred [12, 13].

Genomic comparisons of *P. blakesleeanus*, *M. circinelloides* and related species pointed to several major changes during their evolutionary history [8]. One observation was that many gene families had large copy numbers within the Mucoralean species. Whole genome duplication (WGD) has been observed multiple times in plants [14] and also in some microbial eukaryotes [15], but is a relatively rare phenomenon in the Fungi. WGD has been documented in only a few Ascomycete fungi lineages (*Saccharomyces* and *Hortaea werneckii*) [16, 17], and was previously found in the Mucorales (*Rhizopus delemar*) [18]. The analysis presented by Corrochano *et al.* [9] confirmed that there was likely at least one WGD in the Mucorales fungi, but that it occurred early in the evolution of the Mucoromycotina group. A WGD generates effectively twice as many copies of every gene; however, not all duplications are equally retained because degeneration and loss will occur, especially in redundant copies. Notably, the *P. blakesleeanus* and *M. circinelloides* genomes showed enrichment for regulators of signaling and light responses amongst the duplicated genes. For example, four times as many copies of photoreceptor genes are present in *P. blakesleeanus* compared with Dikarya fungi. Genes encoding signaling proteins, such as kinases and G proteins, were also found in higher abundance, with 30–100% more in *P. blakesleeanus* than in Dikarya fungi.

Upon duplication, gene copies can easily diverge. The expansion of genes coding for putative light-sensing and signaling proteins provides a useful opportunity to explore whether there are changes in the regulation of these genes. The transcriptional response to blue-light induction was assayed in both *P. blakesleeanus* and *M. circinelloide* as a kind of replicated evolutionary experiment to see if the functions or the regulation of sets of duplicated genes changed or stayed the same [9]. Although many of the genes encoding the master photoreceptor — White Collar Complex — and protein kinase A had similar transcriptional responses to light induction in both fungi, genes encoding a variety of other kinases or the photoreceptor WcoB had opposite patterns of expression between the two species. These species differences in gene regulation in response to light may have been driven by adaptation to the different ecological niches these fungi occupy, a hypothesis that can be tested by comparing light response gene expression among additional related species.

Light is likely an important signal for orientation of growth and for synchronizing the timing of spore release. Mutagenesis experiments in *P. blakesleeanus,* performed by Max Delbrück in the 1960s, identified mutants that appeared blind and failed to orient towards a light source. The genes in which these mutation occur were later named *mad* in honor of Delbrück, and some have now recently been cloned and identified as

photoreceptor genes responsible for light sensing. One of these, *madA*, was found to encode a White Collar photoreceptor [19]. Gene expression profiling of wild-type and *madA/madB*-deficient mutant strains revealed that even in the mutants, a significant number of genes still showed expression changes in response to light, indicating that additional photoreceptors remain to be identified [9]. The highly sensitive light perception of *P. blakesleeanus* is likely a result of the expanded photoreceptor repertoire, which can now be better explored with the newly available genomic resources.

In addition to WGD, some gene-family expansions also appear to have been driven by local or segmental duplications. The chitin deacetylases are one family with a large number of recent duplications, which could be responsible for the observed changes in cell-wall architecture in Mucorales fungi [20]. The Gα proteins, which are involved in G protein-coupled receptor signaling, are also expanded in copy number driven by local duplications that could play a role in overall changes in GPCR signaling in these fungi.

New research directions are possible using the newly sequenced genomes from *M. circinelloides* and *P. blakesleeanus*. These include the continued development of molecular tools for genetic manipulation and targeted gene knockdowns, as well as more efficient identification of mutations through genome resequencing. A comparison of the variation in clinical or environmental isolates of *M. circinelloides* will be facilitated by the genome sequence, and may identify underlying pathogenesis factors. The combination of gene expression, comparative genomics, and genetics has identified important gene families in signaling and light response. Future work should use these resources to further explore the molecular basis of the regulation and architecture of networks that control the fungal response to light and other stimuli.

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Figure 1: Characteristic appearance of *Phycomyces.*
Shown are *Phycomyces blakesleeanus* sporangiophores — the asexual fruiting structures that each contain tens of thousands of mitotic spores. The panel on the right shows sporangiophores in various stages of maturity as evidenced by the variation in color range of the sporangia staring with light orange color that darkens to a deep brown or black in many species. Image on the left by María del Mar Gil Sánchez. Image on the right by Carmen Ruger Herreros.

In Brief:
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