

## Primer

## Fungal sexual reproduction and mating-type loci

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Sexual reproduction is a hallmark of eukaryotes, generating diversity and variation through recombination and allele segregation, thereby facilitating natural selection. Unlike animals and plants, fungi do not have conventional male and female sexes but instead rely on mating types, which are determined by allelic differences at a specialized chromosomal region called the mating-type locus (*MAT*). The *MAT* locus typically exhibits great diversity in both organization and gene content, partially driven by reduced meiotic recombination in these regions during sexual reproduction. While ascomycetes predominantly have a bipolar mating system with a single *MAT* locus determining the mating type of the cell, there are also species that possess silent *MAT* cassettes that enable mating-type switching during vegetative growth. This process generates cells with compatible mating types, allowing mating between mother and daughter cells and resulting in inbreeding. In basidiomycetes, the ancestral mating system is tetrapolar, with two independent *MAT* loci (*P/R* and *HD*) collectively determining the mating type of the cell. Some species, however, have a bipolar mating system in which the *P/R* and *HD* loci have become completely linked genetically or even fused together, while others exhibit a so-called pseudobipolar mating system in which only partial genetic linkage has been established between the *P/R* and *HD* loci. Additionally, fungi employ a vast array of sexual reproductive strategies, including classical mating between cells of opposite mating types, as well as noncanonical modes such as unisexual, pseudosexual, and parasexual reproduction. In this Primer, we aim to introduce this

fascinating diversity in mating-type determination and modes of sexual reproduction in fungi, with a focus on *Cryptococcus* species as a model. We then discuss how recent advances in genomics research have facilitated studies on fungal *MAT* loci and mating systems, highlighting key outstanding questions in the field and potential ways to address them.

**The mating-type locus and mating-type determination in fungi**

In most ascomycetous species, there is a single *MAT* locus in the genome that encodes mating-type-specific transcription factors of various types, such as alpha-box, homeodomain, high mobility group (HMG), and HMG-like (Figure 1A). By contrast, most basidiomycetes have two unlinked *MAT* loci in their genomes, one encoding mating-type-specific homeodomain transcription factors (i.e. the *HD* locus) and the other encoding mating pheromones and the pheromone receptor (i.e. the *P/R* locus) (Figure 1B). It should be noted that while genes encoding pheromones and pheromone receptors are also present in the genomes of ascomycetes, they are not involved in determining mating compatibility and, thus, are not part of the *MAT* locus. Just like most other biological systems, there is amazing diversity in the *MAT* locus, even among closely related species, manifested as differences in size, gene content, configuration, as well as how they define mating type of the cell. Here we provide a broad overview of the diversity of *MAT* in fungi and then discuss in more detail two types of transitions between different *MAT* systems that have attracted considerable research interest, one in ascomycetes and the other in basidiomycetes.

**Diversity in *MAT* gene content**

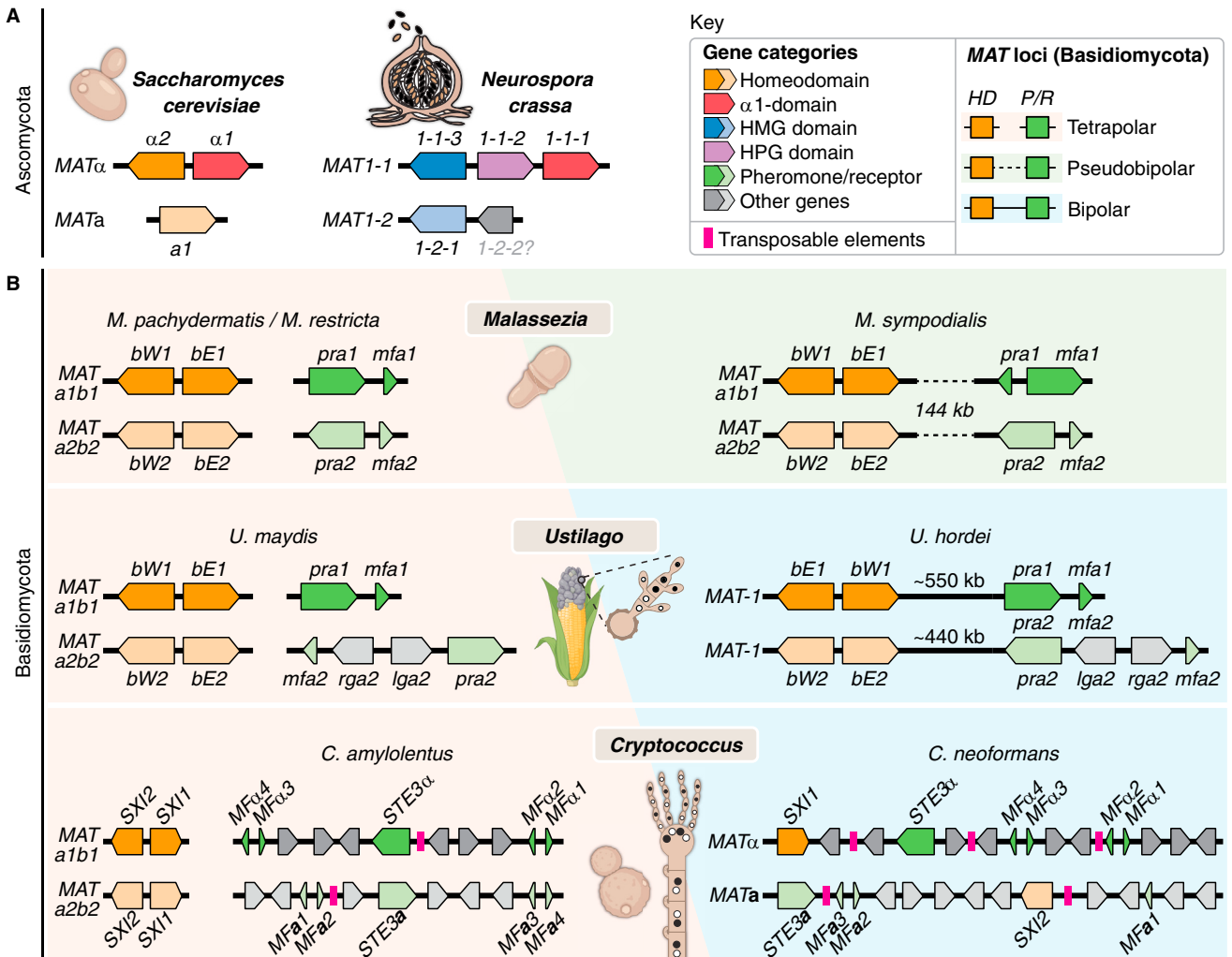
While in most cases the genes within the *MAT* loci are mating-type-specific transcription factors and mating pheromones and their receptors, there is considerable variation in the gene content. In ascomycetes, the number of transcription factors located within the *MAT* locus can vary even between closely related species. In basidiomycetes, genes

other than mating pheromone and pheromone receptors have been found within the *MAT* locus. For example, in the bipolar pathogenic *Cryptococcus* species, the *MAT* locus is unusually large (~120 kb) and contains more than 20 genes. Many of these genes are not necessarily required for sexual reproduction, as strains carrying deletions of those genes are able to undergo mating even in bilateral mutant crosses (i.e. the gene is deleted in both of the strains in the cross). Interestingly, similar genes are also located in the *P/R* locus of the tetrapolar species closely related to the pathogenic *Cryptococcus* species complex, such as *Cryptococcus amyloletus* and *Cryptococcus wingfieldii*, supporting the hypothesis that these genes have been gradually 'recruited' into the *P/R* locus, resulting in the expansion of the non-recombining *MAT*. Recent studies in *Cryptococcus neoformans* confirmed that at least four genes (*PRT1*, *RPL22*, *RPL39*, and *RPO41*) within the *MAT* locus are essential for cell viability and are involved in cellular processes such as translation and mitochondrial transcription. Studies have shown that the *MAT* locus is also involved in mitochondrial uniparental inheritance (mito-UPI) during sexual reproduction. In *Ustilago*, the *P/R* locus-encoded *LGA2* and *RG2* genes have been implicated in mito-UPI. Similarly, studies of *C. amyloletus* also provide evidence that the *P/R* locus, not the *HD* locus, controls mito-UPI. In the bipolar pathogenic *Cryptococcus* species, none of the non-essential genes within the *MAT* locus have been shown to affect mito-UPI based on classical genetic analyses of gene deletion strains. One possibility could be that the essential genes, or the genes required for mating (e.g. pheromone receptors), are involved, and additional functional analyses of these genes in pathogenic *Cryptococcus* species could help shed light on the molecular basis of mito-UPI.

**Mating type switching in ascomycetes**

While many ascomycetous species are heterothallic, with the mating type of each cell defined by a single allele at the *MAT* locus, there are also





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**Figure 1. Diverse MAT organizations and mating type determination systems.**

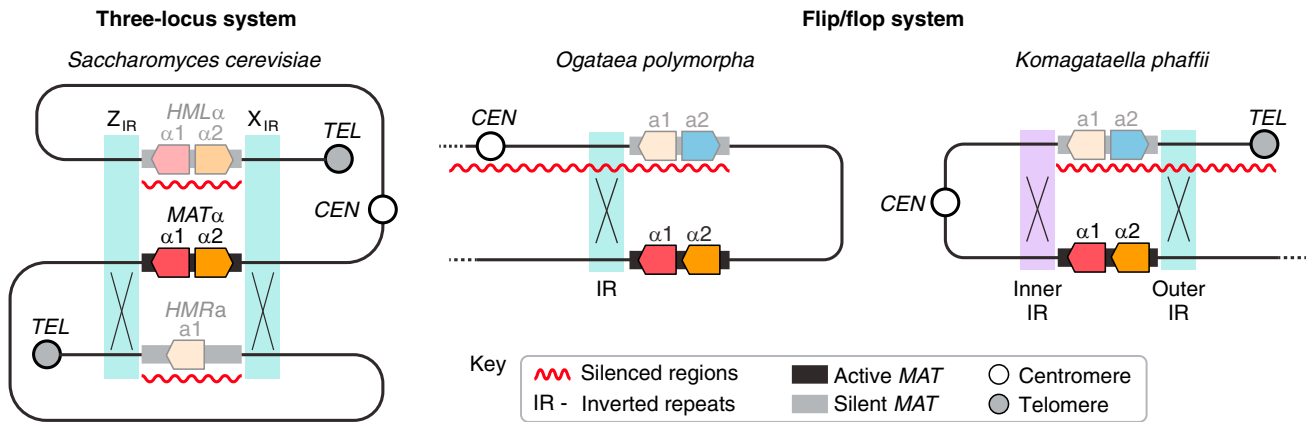
(A) Representative bipolar MAT loci in the ascomycetes *S. cerevisiae* and *N. crassa*, which primarily contain mating-type-specific transcription factors. (B) The MAT loci in basidiomycetes typically comprise an HD locus encoding mating-specific transcription factors and a P/R locus encoding mating pheromones and pheromone receptors. The HD and P/R loci can be unlinked (i.e. tetrapolar), physically linked but wide apart, with occasional recombination in between (i.e. pseudobipolar), or fully linked genetically (i.e. bipolar). Closely related species can differ in their MAT configurations, as illustrated here with examples of tetrapolar (left) and pseudobipolar/bipolar (right) MAT loci in three basidiomycete genera *Malassezia*, *Ustilago*, and *Cryptococcus*.

homothallic species where the cell possesses compatible MAT alleles, either via a fusion of the two MAT alleles or by having them integrated at distinct genomic locations. Mating-type switching is considered to be a special case of homothallism as it gives rise to cells with compatible mating types within the population propagated from a single cell. Two types of mating-type switching have been characterized so far: the three-cassette model and the flip/flop model (Figure 2). Remarkably, both models were first proposed almost 50 years

ago, and later confirmed in extant ascomycetous species.

In the budding yeast *Saccharomyces cerevisiae*, each cell possesses an active MAT locus that defines the mating type, as well as two silent MAT cassettes (HML and HMR) containing opposite mating-type alleles. HML and HMR are transcriptionally silent due to the formation of heterochromatin at these loci, regulated by the Sir protein complex that includes a histone deacetylase (Sir2). During mitosis, a DNA double-strand break

at the active MAT locus is introduced by the HO endonuclease, initiating a switching process that replaces the active MAT allele with that from either HML or HMR through gene conversion (Figure 2). A similar, but independently evolved, three-cassette mating-type switching system has been characterized in the fission yeast *Schizosaccharomyces pombe*, which compared to that in *S. cerevisiae*, differs in both the enzymes that incite mating-type switching and the mechanisms involved in maintaining the silenced cassettes.



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**Figure 2. Mechanisms of mating type switching.**

Illustrated here are three examples of mating type switching in *S. cerevisiae*, *O. polymorpha*, and *K. phaffii*, respectively. In each system, the active *MAT* is highlighted in black, and the silent *MAT* cassettes are shaded in grey. All three systems are shown in the configuration during which switching takes place, with alignments of the *MAT*-flanking inverted repeats, in which homologous recombination occurs, highlighted with light-blue and light-purple blocks.

A simpler flip/flop switching model was first characterized in *Komagataella phaffii* (formerly *Pichia pastoris*) and *Ogataea polymorpha* (formerly *Hansenula polymorpha*) (Figure 2). In this system, each cell contains two physically linked *MAT* cassettes that encode opposite mating types. One of the cassettes is transcriptionally active and defines the mating type of the cell, while the other is silenced due to its proximity to a heterochromatic region, either a centromere (as in *O. polymorpha*) or a telomere (as in *K. phaffii*). The chromosomal region encompassing the two *MAT* cassettes is flanked by inverted repeats, and recombination within these repeats leads to inversion of the entire region, switching the active and silent status of the two *MAT* cassettes (Figure 2). Interestingly, comparative genomics and phylogenomics studies of species in the budding yeast subphylum revealed multiple independent transitions from heterothallism to homothallism, primarily via the flip/flop system, suggesting the presence of selective advantages that drive the evolution of homothallism in budding yeasts, such as spore formation assurance.

**Tetrapolar and bipolar mating systems in basidiomycetes**

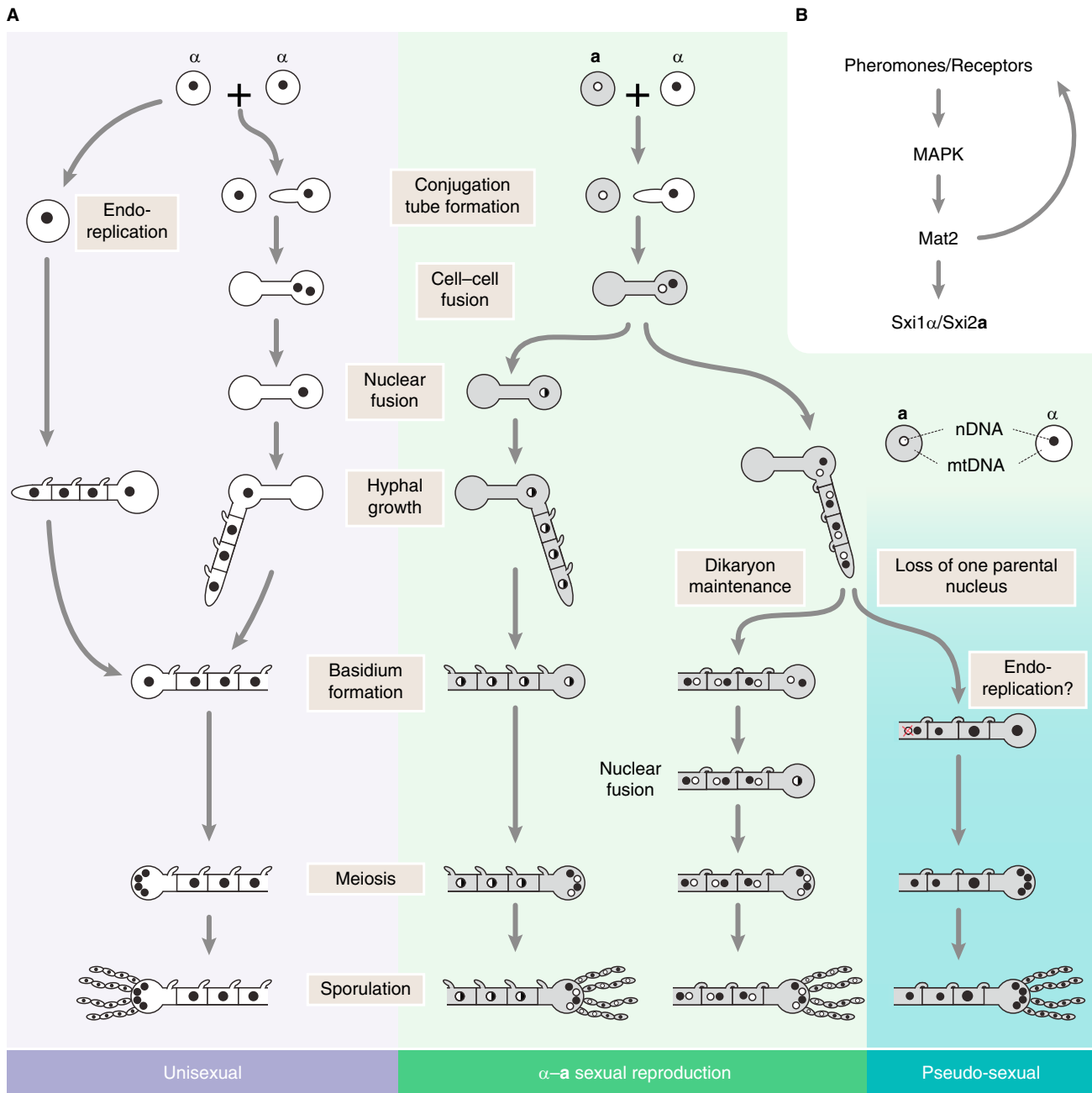
The tetrapolar mating system first evolved in the last common ancestor of basidiomycetes, where

in addition to the *HD* locus encoding the mating-specific transcription factors, a physically unlinked (often on a different chromosome) *P/R* locus encoding mating pheromones and pheromone receptors also became a component of the mating-type determination system (Figure 1B). The tetrapolar mating system is the most common *MAT* locus organization in basidiomycetes and is found in species such as the smut fungus *Ustilago maydis*, as well as mushrooms including *Coprinopsis cinerea*, *Schizophyllum commune*, and *Laccaria bicolor*. For species with a tetrapolar mating system, two cells are mating compatible if they possess different alleles at both the *P/R* and the *HD* loci. Thus, in species that are multi-allelic at both *P/R* and *HD*, the number of possible mating types can be enormous. For example, in *S. commune*, the numbers of different *P/R* and *HD* alleles identified in natural populations suggest there could be theoretically more than 20,000 different mating types. A similarly large number of mating types have also been suggested to be present in the mushroom *C. cinerea*, which could facilitate efficient outcrossing.

While the tetrapolar mating system is the most common *MAT* locus organization in basidiomycetes, there are also many species that have a bipolar system where mating type is controlled by a single, bi- or

multi-allelic *MAT* locus (Figure 1B). The wide distribution of bipolar species across the basidiomycete phylogeny suggests that multiple independent transitions from tetrapolar to bipolar mating systems have occurred, and examples of such transitions can be found in closely related lineages, including the human fungal pathogen *Cryptococcus* species (bipolar) and their non-pathogenic sister species such as *C. amyloletus* (tetrapolar), the smut fungi *U. maydis* (tetrapolar) and *U. hordei* (bipolar), as well as among species in the genera *Coprinus* (*sensu lato*) and *Malassezia* (Figure 1B).

How can bipolar species evolve from tetrapolar ones? Nearly 60 years ago, the pioneering mycologist John Raper put forward three hypotheses: firstly, mutations in one of the *MAT* loci render it self-compatible; secondly, chromosomal rearrangements (translocations and inversions) bring the two *MAT* loci into close genetic linkage, eventually forming a single non-recombining locus; and, thirdly, the function of one of the two *MAT* loci is gradually assumed by the other. Since then, evidence supporting the first two hypotheses has been found in many different fungal species including the pathogenic *Cryptococcus* species complex, the smut fungus *U. hordei*, and the mushroom *Coprinellus disseminatus*. Evidence has also been found for a transitional stage



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**Figure 3. Diverse modes of sexual reproduction in the pathogenic *Cryptococcus* species complex.**

(A) The  $\alpha$ - $a$  sexual (center panel, shaded in light green), unisexual (left panel, shaded in light blue), and pseudosexual (right panel, shaded in light teal) reproduction modes in the human fungal pathogens *Cryptococcus* species are depicted. The  $\alpha$ - $a$  sexual reproduction starts with conjugation and then cell-cell fusion (i.e. plasmogamy) between *MAT* $\alpha$  and *MAT* $a$  cells that form *MAT* $\alpha/a$  zygotes. The zygotes then initiate sexual development and grow as dikaryotic hyphae with fused clamp cells (right branch). Eventually, the tips of the hyphae enlarge and form basidia, in which the two parental nuclei fuse (i.e. karyogamy) and undergo meiosis, producing four meiotic products that go through rounds of mitosis to produce four chains of basidiospores deposited onto the surface of the basidium. Alternatively, the two parental nuclei can fuse soon after plasmogamy, in which case the zygotes grow as diploid uninucleate hyphae with unfused clamp cells, and then undergo basidium formation, meiosis, and sporulation. Pseudosexual reproduction is similar to the dikaryotic  $\alpha$ - $a$  sexual reproduction, except that one of the two parental nuclei is lost during hyphal growth (e.g. due to aberrant nuclear migration at the hyphal branching sites). The remaining nucleus then undergoes diploidization (e.g. through endoreplication), meiosis, and sporulation. In this case, the basidiospores from the same basidium all have identical nuclear genomes that are clonal to that of the parental nucleus in the basidium. In pseudosexual reproduction, the nucleus that is eliminated from the dikaryotic hyphae can be that from either the *MAT* $\alpha$  or *MAT* $a$  parent. Unisexual reproduction also mirrors  $\alpha$ - $a$  sexual development, except that in this case two cells of the same mating type fuse and undergo karyogamy (or a cell undergoes diploidization through endoreplication), and the resulting diploid zygote grows as diploid uninucleate hyphae with unfused clamp cells. It should be noted that unisexual reproduction also occurs in *MAT* $a$  cells. (B) A simplified example of a signal transduction pathway that is involved in regulating sexual reproduction in *Cryptococcus* species.

of the second hypothesis, where the two *MAT* loci are distantly located on the same chromosome. In this case, recombination can still occur between the two loci during meiosis, giving rise to progeny with *P/R–HD* allele combinations (i.e. mating types) that are different from their parents. Such *MAT* configurations where the *P/R* and *HD* loci are physically linked, but without complete genetic linkage, is called pseudobipolar. An example is the human skin commensal *Malassezia sympodialis*, where both the *P/R* and *HD* loci are located on chromosome 1 but remain greater than 100 kb apart. Analyses of the natural population identified *MAT* allele combinations that are indicative of recombination between the two loci, suggesting that *M. sympodialis* has a pseudobipolar mating system (Figure 1B).

### Fungal life cycles and sexual reproduction

Many fungi exhibit a haploid-dominant life cycle, with cells primarily existing in a haploid state, containing a single set of chromosomes. In these species the diploid phase, resulting from fusion of the two parental nuclei (i.e. karyogamy), is typically brief, with haploidy quickly restored by meiosis. However, fungal life cycles vary significantly across lineages. For example, in many basidiomycetes, mating between cells of opposite mating-type leads to cell fusion and the formation of a dikaryotic stage, where the haploid nuclei of both partners remain separate within the same cytoplasm but divide synchronously during hyphal growth. This phase can persist for extended periods until karyogamy occurs in the basidium, a specialized structure where meiosis produces recombinant basidiospores. The predominance of haploidy may facilitate more efficient natural selection, as mutations are immediately exposed to selection.

The key consequence of sexual reproduction in fungi, as in many other organisms, is the production of progeny through meiosis, which possess genomes that are recombinants of those from the two parents. Consequently, sex can bring together both beneficial, as well as deleterious, spontaneous mutations that arose independently, increasing

genetic diversity in the population and facilitating natural selection. Additionally, mobile genetic elements such as transposons are often hyper-activated during sexual reproduction, potentially introducing novel genetic changes. To counter this, fungi have evolved mechanisms such as RNA interference (RNAi) to prevent rampant transposon movement that can be highly deleterious. Sex can also break apart co-adapted allele combinations favorable under certain conditions.

Given that most fungi undergo facultative sex, they can take advantage of both reproductive modes: sexual reproduction through meiosis to produce recombinant progeny that are better suited when the environment changes, and clonal expansion through mitosis by individuals with genotypes that are well-suited to the current environment. This flexibility is of particular importance for fungi, mostly unicellular and simple organisms, to quickly adapt to shifting external environmental factors, including temperature, nutrients, antifungal compounds, and interactions with other species. Additionally, fungal sexual reproduction produces spores that tend to be more resistant to harsh environments and better suited for long distance dispersal, allowing them to escape unfavorable conditions. It has been hypothesized that in fungal species such as the budding yeast *S. cerevisiae*, mating-type switching has evolved to allow individual cells to produce mating-compatible cells through minimal rounds of mitosis under conditions unfavorable for vegetative growth, facilitating entry into sexual reproduction and production of spores that can better withstand the harsh conditions. These meiotic spores can then germinate and resume mitotic growth when favorable conditions arise. In some fungal species, such as *U. maydis*, sexual reproduction is required to produce dikaryotic hyphae that are crucial for infection of the plant host.

### Homothallism and heterothallism

Homothallism and heterothallism represent two fundamental mating strategies in fungi, and both can profoundly impact genetic diversity and adaptation. Heterothallic species

require different mating-type partners for sexual reproduction, thereby promoting outcrossing and often increasing the probability of generating novel genotypic combinations. In contrast, homothallic fungi can self-fertilize through mechanisms such as possessing both mating types within a single genome (primary homothallism), mating-type switching, or engaging in unisexual reproduction (please see below). Although this intrinsic inbreeding can bypass the need for a compatible partner, homothallism can also support outcrossing in certain scenarios. For instance, during outcrossing unisexual reproduction, cells of the same mating type but differing genetic backgrounds may still fuse and undergo recombination, thereby infusing populations with new variants.

A compelling example is the basidiomycete human pathogenic fungus *Cryptococcus deneoformans*. While classically described as heterothallic (i.e. requiring opposite mating types: *MAT $\alpha$*  and *MAT $\mathbf{a}$* ), this species has also evolved a robust unisexual cycle (Figure 3A; also see below). Most natural isolates of *C. deneoformans* are *MAT $\alpha$*  (>99%), yet under nutrient-limiting conditions, they can complete the sexual cycle in the absence of *MAT $\mathbf{a}$*  partners, forming diploid intermediates through processes such as same-clone-mating, mating between genetically distinct strains of the same mating type, or even endoreplication of a haploid cell's genome. Research has shown that unisexual reproduction in *C. deneoformans* generates phenotypic (e.g. drug resistance) and genotypic (e.g. aneuploidy) variation and confers an ecological advantage by promoting colony 'foraging' for nutrients or mating partners over long distances. Despite the costs potentially associated with inbreeding (such as limited genetic diversity and increased accumulation of deleterious mutations), unisexual reproduction can serve as an alternative strategy when compatible partners are scarce, ensuring the production of both hyphae that can scavenge for more distant nutrients and mating partners and meiotic spores that bolster environmental resilience and pathogen survival.

On the broader evolutionary landscape, homothallism appears repeatedly in multiple fungal lineages, suggesting that self-fertility may be selectively advantageous under specific conditions. Some species, such as *C. deneoformans* or the ascomycete *Podospora anserina*, can alternate between outcrossing and inbreeding depending on environmental constraints and partner availability. This underscores the flexible nature of fungal sexual reproduction, with some lineages maintaining the ability to undergo a homothallic sexual cycle — allowing access to benefits such as ploidy variation and yeast-to-hyphae transitions even in the absence of a compatible mating partner — as well as a heterothallic sexual cycle when a compatible mating partner is available.

### Diverse reproduction modes in human fungal pathogen *Cryptococcus* species

The basidiomycete pathogens *C. neoformans* and *C. deneoformans* exemplify fungal reproductive diversity, employing  $\alpha$ -a sexual, unisexual, and pseudosexual reproduction through both shared and distinct molecular mechanisms (Figure 3A).

$\alpha$ -a sexual reproduction occurs between *MAT* $\alpha$  and *MAT* $\alpha$  cells and begins with the secretion of mating pheromones (MF $\alpha$  and MF $\alpha$ ), which bind to their cognate G-protein-coupled pheromone receptors (Ste3 $\alpha$  and Ste3 $\alpha$ ), both encoded by the *MAT* locus. This triggers a MAPK cascade involving Ste20, Ste11, Ste7, and Cpk1, culminating in activation of the master regulator Mat2, which drives cell fusion and sexual differentiation (Figure 3B). Key downstream effectors include the transcription factor Znf2, controlling the yeast-to-hypha transition, and the homeodomain proteins Sxi1 $\alpha$  and Sxi2 $\alpha$ , which heterodimerize upon fusion to promote karyogamy and progression through the sexual cycle (Figure 3B). Karyogamy can occur at different stages: either late, within the basidia that form on dikaryotic hyphae containing two separate haploid nuclei, or early, resulting in monokaryotic hyphae

with a single migrating diploid nucleus (Figure 3A). In both cases, the resulting hyphae differentiate into basidia, where the diploid nuclei undergo meiosis, producing genetically diverse basidiospores for dispersal and infection. Environmental cues, including nutrient deprivation and plant-derived compounds such as myo-inositol, stimulate  $\alpha$ -a sexual reproduction.

Unisexual reproduction, predominantly observed in *C. deneoformans* *MAT* $\alpha$  cells, bypasses the need for an opposite mating partner. It shares developmental and some of the molecular processes with  $\alpha$ -a sexual reproduction but produces monokaryotic hyphae, with clamp cells (which aid in nuclear segregation in  $\alpha$ -a sexual reproduction) remaining unfused. Diploidization occurs either through fusion of same-mating-type cells or endoreplication, where a single nucleus duplicates its genome without prior cell fusion (Figure 3A). Unlike  $\alpha$ -a sexual reproduction, the pheromones, pheromone receptors, and homeodomain proteins appear to be largely dispensable. Instead, the Qsp1–Cqs2–Pum1 cascade regulates unisexual reproduction, where Qsp1 (a quorum-sensing peptide) signals through Cqs2, an atypical zinc finger regulator, to activate Pum1, a Pumilio-family RNA-binding protein essential for auto-diploidization, hyphal development, and sporulation. The spindle pole body-associated protein Cua1 is also essential for ploidy doubling and pre-meiotic diploidization.

Pseudosexual reproduction is an unconventional reproductive strategy in *C. neoformans* where progeny inherit nuclear DNA from only one parent despite typical  $\alpha$ -a cell fusion. Here, one parental nucleus is excluded during hyphal branching, while the retained nucleus undergoes meiosis in the basidium, producing uniparental progeny. Deletion of the meiotic recombinase *DMC1* significantly impairs pseudosexual reproduction, reducing sporulation and germination, indicating that meiosis and recombination remain essential. Evidence also suggests that endoreplication may precede meiosis to ensure proper ploidy

before gametogenesis. It is possible that pseudosexual reproduction may have evolved as a rescue mechanism to resolve genomic incompatibilities between highly divergent or chromosomally rearranged strains, enabling the formation of viable progeny where traditional sexual reproduction would fail.

Overall, this remarkable reproductive plasticity not only enables *Cryptococcus* pathogenic species to thrive in diverse environments but also drives genetic innovation, fostering the emergence of hypervirulent and drug-resistant lineages.

### Biological consequences of different modes of sexual reproduction

While different modes of sexual reproduction (e.g.  $\alpha$ -a sexual versus unisexual) have similar consequences regarding production of meiotic progeny, there are also differences. First, mitochondria are inherited uniparentally (mitochondrial uniparental inheritance, mito-UPI) in many fungal species. For example, in the pathogenic *Cryptococcus* species and their closely related non-pathogen *C. amyloletus*, mitochondria are almost exclusively inherited from only one of the two parents during heterothallic sexual reproduction — the *MAT* $\alpha$  parent in pathogenic *Cryptococcus* and the parent carrying the A1 allele at the *P/R* locus in *C. amyloletus*. In contrast, mitochondria are inherited bi-parentally during unisexual reproduction in pathogenic *Cryptococcus*, supporting the hypothesis that elements linked to *MAT*, and more specifically, those associated with the *P/R* locus, are involved in mito-UPI. Second, while meiotic genes are required and meiosis occurs in different modes of sexual reproduction, recombination frequencies and biological consequences can differ. For example, in pathogenic *Cryptococcus* species, global recombination frequencies have been found to be higher during  $\alpha$ -a sexual reproduction than in unisexual reproduction. Additionally, studies have shown that while no ‘recombinant’ genome can be produced through meiosis,

a considerable amount of progeny with aneuploidy are generated during solo unisexual reproduction, allowing *de novo* genomic changes that lead to phenotypic variation such as antifungal resistance. Furthermore, chromosomal regions such as the *MAT* locus in the pathogenic *Cryptococcus* species tend to be highly divergent between cells of different mating types, due to elevated sequence polymorphism and structural variation (e.g. inversions and translocations). While meiotic recombination within such regions is highly repressed during  $\alpha$ -a sexual reproduction, these regions could, and have been found to, undergo normal meiotic crossovers during unisexual reproduction, which could have consequences for the maintenance and evolution of these unique genomic regions.

### Study of fungal *MAT* and sex in the 'omics' era

Advances in technologies for analyzing genomes and transcripts in the past decades have brought great insights into the regulation of sexual reproduction and the evolution of the mating-type loci in fungi and opened doors for future studies. Chromosome-level reference genome assemblies are now available for many fungal species, which was made possible by long-read sequencing technologies (e.g. PacBio and Nanopore), improved assembly algorithms, polishing with short-read sequencing (e.g. Illumina), and sometimes further validation with Hi-C sequencing data. These advances also allowed independent assembly of haplotypes in dikaryons or diploids. Long-read sequencing also enabled better assembly and characterization of the mating-type loci in many species, which are often chromosomal regions enriched with repetitive sequences that have been challenging to assemble with only short-read sequences.

Having highly accurate genome assemblies is critical for better analysis of genome-wide polymorphisms and construction of robust phylogenies. This is important for more accurate interpretation of evolutionary events, such as transitions between tetrapolar and

bipolar mating systems in both *Cryptococcus* and *Malassezia* species complexes. Additionally, it is essential for improved analysis of polymorphisms present in natural populations. For example, studies of 387 natural isolates of *C. neoformans* showed that there are four cryptic lineages within the species (VNI, VNBI, VNBII, VNII), each having unique profiles of sequence diversity and signals of meiotic recombination, providing insights into the ecology, reproduction modes in nature, population genetics, and evolution of this prominent human fungal pathogen.

Additionally, Hi-C analyses allow studies on genome-wide chromatin architecture and dynamics, as well as temporal and spatial control of genome organization and gene functions, which could have implications in mating type determination and sexual reproduction. Furthermore, new sequencing technologies such as Nanopore can provide information on DNA and RNA modifications (e.g. methylation), as well as the detection and characterization of non-coding RNAs and novel RNA elements such as RNA viruses, allowing studies on possible non-genetic mechanisms that might be involved in mating type determination and regulation of sexual reproduction. Finally, the application of single-cell transcriptome analyses could offer a high-resolution view of mating-type gene expression at the individual cell level, providing insights into cellular heterogeneity in mating responses and reproductive processes.

### Future directions

What can we look forward to learning and discovering with respect to fungal sexual reproduction and mating-type loci over the next decade?

The mating-type locus has been extensively characterized in several fungal phyla, including the Ascomycota, the Basidiomycota, and the Mucoromycotina, whereas in other fungi such as the Blastocladiomycota and the Chytridiomycota the *MAT* locus has not as yet been defined. We may find that these less-studied phyla have variants of known *MAT* components, such as homeodomain transcription factors that define

cell-type identity. Or we may find that they have entirely novel types of *MAT* loci that establish cell-type identity in new ways. Most functional studies of *MAT*-encoded genes have focused on protein products of the *MAT* locus. Given that RNA biology is turning out to be increasingly complex in fungi, including the discovery of small interfering RNAs (siRNAs) and long non-coding RNAs (lncRNAs), we may find entirely new aspects of *MAT* locus function are mediated by a complex interplay between protein and RNA products. Several *MAT* loci are now known to encode essential genes, and how these genes influence the evolution and integrity of the *MAT* locus remains to be explored.

Studies of fungal sexual reproduction have revealed novel modes of sexual reproduction (including parasexual, unisexual, and pseudosexual) that may turn out to be generalizable to other eukaryotes across the tree of life. A breakthrough in fungal sex could be the publication of definitive proof of a fungal species being truly asexual. One candidate is *Candida auris*, among others. Studies on fungal sexual reproduction have revealed general principles of signal transduction (GPCRs, heterotrimeric G proteins, MAPK signaling cascades, scaffolds, etc.) and thus should continue to reveal the molecular blueprints for how cells are wired and organized. Many studies on fungal sexual reproduction focus on observations under lab conditions. Further studies on fungal sexual reproduction in the wilds of nature has great potential to reveal environmental signals and possibly also microbe-microbe or animal-microbe interactions that might promote or enhance sexual reproduction. Finally, with the advent of whole genome sequencing at unprecedented scales, we have the opportunity to understand how species evolve, and how species boundaries form and are strengthened. Given how challenging it is to establish species through the biological species concept, which necessitates being able to conduct genetic crosses in the lab, finding populations that are isolated at the whole genome level holds great promise to show operationally when isolates belong to different species in nature.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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Primer

Fungal secondary metabolism

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Fungi are prolific producers of secondary metabolites, a diverse group of low-molecular-weight compounds that are not directly involved in growth, development, or reproduction but play critical roles when fungi interact with their environment. Secondary metabolites serve a wide array of ecological functions, including defense from abiotic stresses, inhibition of microbial competitors, or predator deterrence. When cultured with the necessary

environmental cue(s), transcriptional and epigenetic regulatory networks lead to expression of the biosynthetic genes required for secondary metabolite production, which are often co-localized into biosynthetic gene clusters.

Bioinformatic efforts have revealed that great biosynthetic potential exists across fungal genomes and is awaiting discovery. The challenge is that many biosynthetic gene clusters remain cryptic or silent under standard laboratory conditions. Fungal secondary metabolites have provided important chemistry for the medical, agricultural, and food sectors. Unlocking the hidden potential of silent biosynthetic gene clusters is the exciting future of research, which will lead to even more natural products to aid humanity. In this primer, we discuss

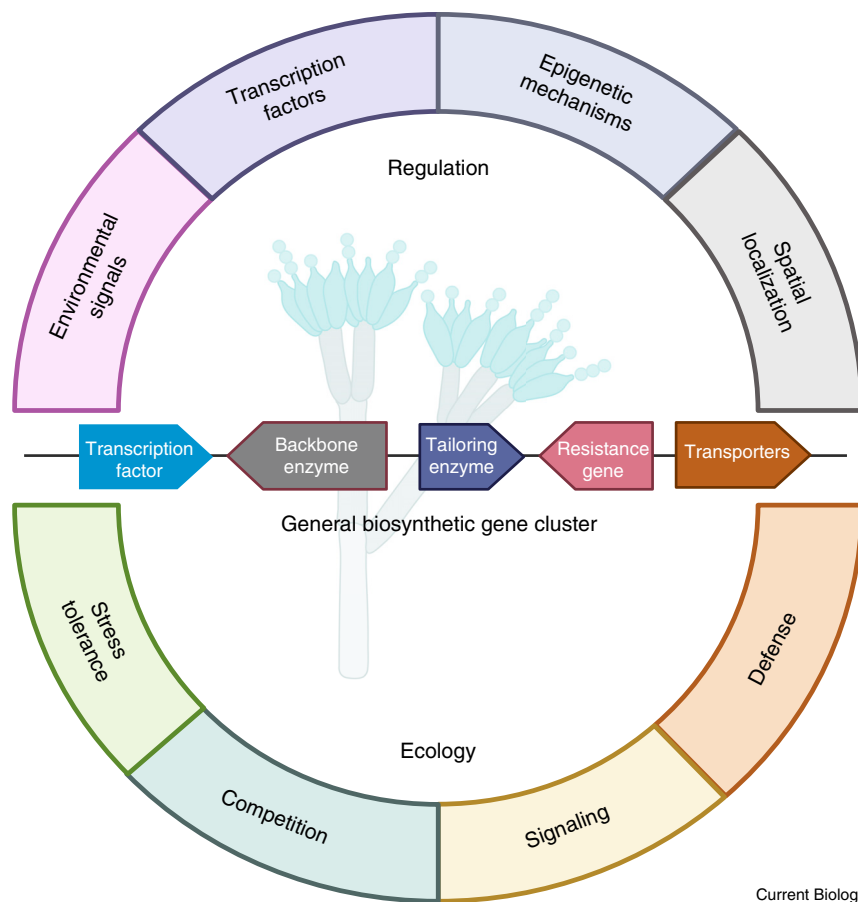


Figure 1. Elements of fungal secondary metabolism.

Fungal secondary metabolites originate from biosynthetic gene clusters. The genes encoding their biosynthesis, as well as the metabolites themselves, are regulated by the environment, transcriptional factors, epigenetic mechanisms, and spatial localization. The metabolites often serve beneficial roles in the producing fungus, including stress tolerance, competitive advantages, acting as signaling molecules, and providing defense. Created in BioRender. Nadig, N. (2025) <https://BioRender.com/qjly7c6>.

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