Sex in Fungi: Molecular Determination and Evolutionary Implications
Edited by Joseph Heitman et al.

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Analysis of Mating-Type Locus Organization and Synteny in Mushroom Fungi:
Beyond Model Species

The ability of a fungal individual to mate or outcross with another individual is dependent on its mating type. The mating type of an individual is determined by the phenotypic expression of its mating-type locus (*MAT*) or loci, and only individuals with different mating types are compatible. Species that have two *MAT* loci are termed tetrapolar, and those that have only a single *MAT* locus are bipolar. *MAT* loci correspond with regions of the genome that may be as small as a single gene (e.g., *Cochliobolus heterostrophus* [81]) or as large as 0.5 Mbp (e.g., *Ustilago hordei* [50]). As discussed in detail in this volume, the genes that function in determining mating type in both Ascomycota and Basidiomycota encode either transcription factors or pheromone receptors and their pheromone ligands (12, 32). *MAT* loci encode both genes whose expression directly determines mating-type specificity as well as those that do not determine the mating type of a cell but are nonetheless mating-type specific. For example, the gene *mtA-2* in *Neurospora crassa* is specific to the *A* mating-type allele of the *MAT* locus, functions in ascospore development, but is not utilized in mating-type determination (21). Likewise, the *a2* allele of the *Ustilago maydis MAT-a* locus harbors two genes (*lga2* and *rga2*) that are specific to a single mating type but are involved in mitochondrial fusion rather than mating-type control (8, 83). In this review, genes that are in or near the *MAT* locus but do not function in determining mating-type specificity are referred to as non-mating-type *MAT*-linked genes.

The Basidiomycota appear to be divided into three major lineages: rusts (Urediniomycetes), smuts (Ustilaginomycetes), and mushroom-like fungi (Hymenomycetes, including homobasidiomycetes and jelly fungi; Fig. 19.1). Most of the described basidiomycete species are homobasidiomycetes (42), and these species are common components of soil ecosystems (33, 61). The basidiomycetes are unique among fungi in having species with tetrapolar mating systems. Mushroom fungi are further distinct from other basidiomycete species because there may be numerous (up to hundreds) mating-type alleles at both of the *MAT* loci. Most of the proliferation of mating types is due to the manner in which the mushroom *MAT* locus is composed of multiple, redundant subloci that can display recombination distances as high as 16 centimorgans (cM) (66). There is a great body of literature on the mating systems and mating-type number and distribution in mushrooms, in part because of the

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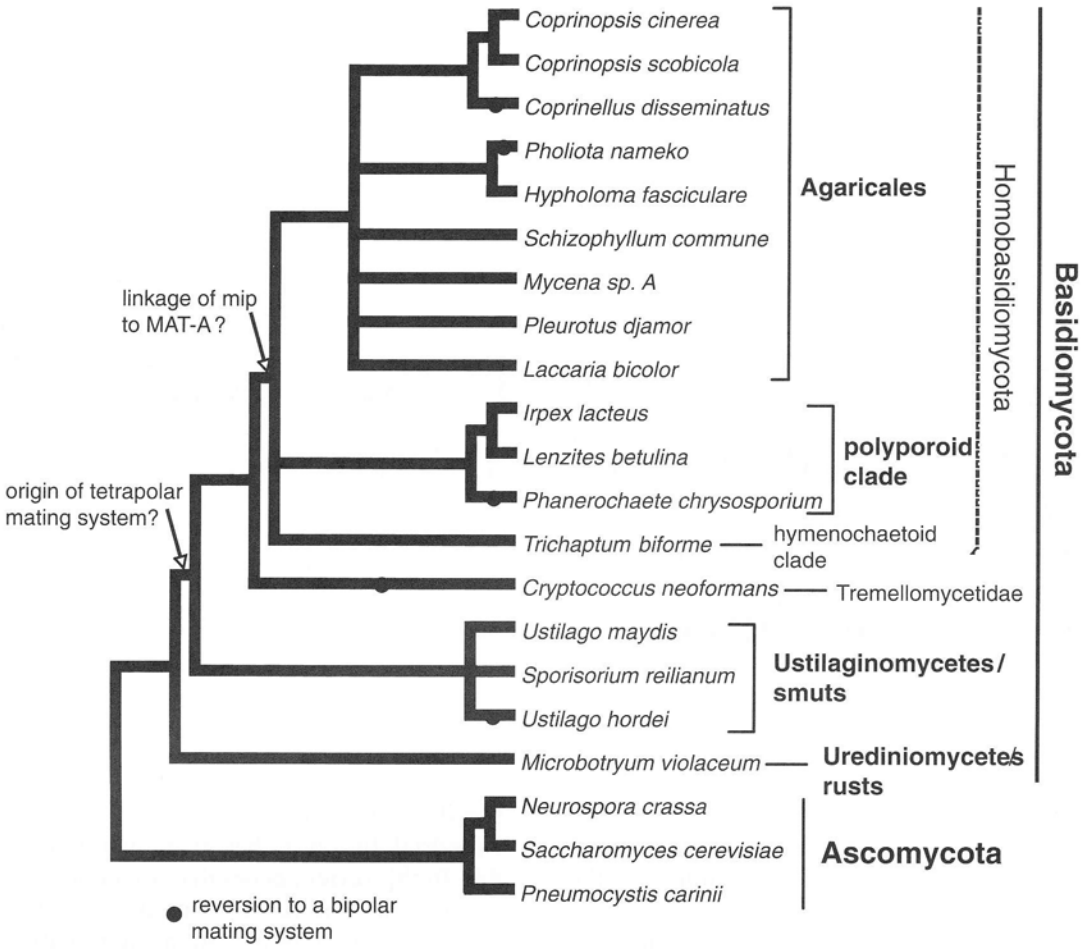

Figure 19.1 Phylogeny of the Basidiomycota. The cladogram depicts the current knowledge of the relationships among the fungi discussed in the text. Data are derived from references 30, 59, and 79, the Tree of Life Web project (http://tolweb.org/tree/phylogeny.html), and the *mor* Web project (http://mor.clarku.edu/).

utility of the mating-type locus to serve as a highly polymorphic marker (60, 82). Among homobasidiomycetes, an estimated 10% are homothallic (non-outcrossing), 25 to 35% are bipolar, and 55 to 65% are tetrapolar (68, 88). From an evolutionary perspective, mating-system switches in fungi are fascinating and none more so than the former genus *Coprinus*, in which homothallic, bipolar, and tetrapolar species interdigitate along the species phylogeny (7, 34). The molecular genetic bases for these mating-system switches have been less tractable than that observed in Ascomycetes (91), in part due to the complexity of the homobasidiomycete mating genes (41).

Coprinopsis cinerea (= *Coprinus cinereus*) and *Schizophyllum commune* are both model systems for studying mating genetics in mushrooms (10, 66). *C. cinerea* and *S. commune* are excellent model systems because they mate readily and can fruit directly on a petri dish of simple nutrient agar. *C. cinerea* has the added advantages of producing asexual propagules (oidia) and meiotic tetrads that can be isolated (44). Both species have been advanced as model systems by the development of auxotrophic mutants, *MAT* mutants, and genetic transformation (85).

During the 1990s the *MAT-A* and *MAT-B* loci of *C. cinerea* and *S. commune* were cloned. Cloning of the *MAT* loci was accomplished by various means (see chapters 16 and 17 by Stankis and Specht and by Casselton and Kües), but the determination that the cloned region actually carried *MAT* genes relied on transformation of the genes into a suitable strain followed by screening for a mating-compatible phenotype (27, 64, 76). The mushroom *MAT-A* locus encodes two types of dissimilar homeodomain proteins (HD1 and HD2 [48]). Dimerization between heteroallelic HD1 and HD2 proteins forms

a transcription factor that activates a *MAT-A*-specific developmental pathway. The mushroom *MAT-B* locus encodes pheromone receptors and their lipopeptide pheromone ligands (64, 87). As with *MAT-A*, heteroallelic combinations of pheromones and pheromone receptors in mated cells cause the activation of the *MAT-B*-specific developmental pathway via a putative mitogen-activated protein kinase signaling cascade triggered by the G-protein-coupled pheromone receptors (10).

Cloning of these *MAT* loci revealed not only that they encode a large number of alleles but also that the alleles were highly dissimilar in sequence. The alleles were so different that they generally failed to cross-hybridize in Southern hybridizations (64, 77). This also holds at the amino acid level; for example, the *MAT-A* homeodomain proteins of *S. commune* are only 42 to 54% identical between alleles (78).

When trying to study the *MAT* genes of mushroom fungi other than *C. cinerea* and *S. commune*, there are three major obstacles. First, the loci are likely to be complex and may comprise several genes. Second, the loci display high sequence variation among *MAT* alleles and cannot be cloned by heterologous hybridization using probes from *C. cinerea* or *S. commune*. Lastly, the absence of a transformation system in which to test the cloned fragments prevents definitive proof that an isolated DNA region carries *MAT*. One way of getting around these obstacles is to target not the mating-type genes themselves but the more slowly evolving genes that they are tightly linked to, provided that synteny (or conserved gene order) has been maintained among the species of interest. This approach allows the investigator to isolate *MAT* loci and to determine homology of the isolated genes using syntenic arguments rather than by genetic transformation. This review focuses on how *MAT* genes may be cloned in nonmodel mushroom species.

SYNTENY AND *MAT-A* IN MUSHROOMS

In order that non-mating-type *MAT*-linked genes can be targeted as a proxy for *MAT* genes, it is essential that the linkage of non-mating-type *MAT*-linked genes to *MAT* be conserved among species. Because of the high amounts of genome rearrangements among closely related species (80), there was no reason, a priori, to assume that conserved gene order would hold near the *MAT* loci. Classical genetic studies, however, indicated early on that some markers might display highly conserved linkage to the *MAT-A* locus (66). Specifically, the earliest mapping studies of the *MAT-A* chromosomal regions in *C. cinerea* and *S. commune* indicated that they were syntenic and very tightly linked to loci conferring para-aminobenzoic acid (*pab*) and adenine (*ade*) auxotrophy in mutants (66). The *pab* locus mapped to <1 cM from *MAT-A* in both species, and the *MAT-A* genes in *S. commune* were first cloned by a chromosomal walk from *pab*, ultimately found to be ~50 kbp from *MAT-A* (27). After *MAT-A* loci from *C. cinerea* and *S. commune* had been cloned, it was further apparent that they shared the presence of a metalloendopeptidase encoded immediately adjacent to *MAT-A* (13, 78). The metalloendopeptidase gene (*mip*) was not part of *MAT-A* as delimited by transformation assays (27). Mip is a mitochondrial matrix-localized enzyme that functions in the cleavage of the leader peptides of precursor proteins targeted to the mitochondrial matrix or inner membrane (37). The first use of non-mating-type *MAT*-linked genes in positional cloning of *MAT* genes was accomplished when Kües et al. (47) used a heterologous *mip* probe from *C. cinerea* to isolate the *MAT-A* locus from the related *Coprinopsis scobicola* (= *Coprinus bilanatus*). After recovering cosmid clones containing the *C. scobicola mip* gene, Kües et al. were able to delimit the *MAT-A* locus to an ~15-kbp region adjacent to *mip* through the use of genetic transformation in *C. cinerea* and *C. scobicola* host strains.

In order to develop *mip* as a marker for *MAT-A* in mushrooms, conservation of the genetic linkage between the two loci was explored. Because the *mip* gene of *C. cinerea* did not appear to hybridize to genomic DNA of other mushroom genera (U. Kües, personal communication), an approach based on PCR amplification of *mip* was attempted (39). The *mip* gene was successfully amplified from over 30 species of mushrooms throughout the diversity of homobasidiomycetes. The linkage relationships in several species were analyzed by studying cosegregation of *mip* and *MAT-A* in progeny arrays of single spore isolates obtained from single fruiting bodies (Table 19.1). The result of the cosegregation studies, as well as information derived from the sequencing of complete genomes, is that linkage of *mip* to *MAT-A* is completely conserved throughout homobasidiomycetes. Furthermore, in all known cases, the *mip* gene is directly adjacent and less than 1 kbp from the 3′ end of one of the mating-type genes.

How deep into evolutionary history was linkage between *MAT-A* and *mip* established? More data on heterobasidiomycetes, including rust and smut fungi, would be useful to address this question. The *b* mating-type locus (*MAT-b*) of *U. maydis*, which is homologous to *MAT-A* in mushrooms, displays no linkage to *mip*, nor does the single *MAT* locus of the pathogenic yeast *Cryptococcus neoformans* (25, 51) (Table 19.1). Synteny between *mip*

Table 19.1 Linkage of *mip* and *cla4* to *MAT-A* and *MAT-B* in basidiomycetes[a]

Genus and species	Clade[b]	Mating system	Ecology	*mip* linked to *MAT-A*?	*cla4* linked to *MAT-B*?	Method of inference	Reference(s)
Coprinellus disseminatus	Homobasidiomycetes: euagarics clade	Bipolar	Wood decay	+	?	Cosegregation/partial genome sequence	39, 41
Coprinopsis cinerea	Homobasidiomycetes: euagarics clade	Tetrapolar	Coprophilic	+	+	Genome sequence	Broad Institute website[c]
Coprinopsis scobicola	Homobasidiomycetes: euagarics clade	Secondarily homothallic	Coprophilic	+	?	Partial genome sequence	47
Cryptococcus neoformans	Heterobasidiomycetes: Tremellales	Bipolar	Saprophyte/ pathogen	−	+	Genome sequence	52, 86; TIGR website[d]
Hypholoma fasciculare	Homobasidiomycetes: euagarics clade	Tetrapolar	Wood decay	+	?	Cosegregation	39
Irpex lacteus	Homobasidiomycetes: polyporoid clade	Tetrapolar	Wood decay	+	−	Cosegregation	39
Laccaria bicolor	Homobasidiomycetes: euagarics clade	Tetrapolar	Ectomycorrhizal	+	?	Genome sequence	JGI database[e]
Lenzites betulina	Homobasidiomycetes: polyporoid clade	Tetrapolar	Wood decay	+	?	Cosegregation	39
Mycena sp. A	Homobasidiomycetes: euagarics clade	Tetrapolar	Litter decay?	+	?	Cosegregation	39
Phanerochaete chrysosporium	Homobasidiomycetes: polyporoid clade	Bipolar	Wood decay	+	+	Genome sequence	JGI database[f]
Pleurotus djamor	Homobasidiomycetes: euagarics clade	Tetrapolar	Wood decay	+	+	Cosegregation	39, 40
Schizophyllum commune	Homobasidiomycetes: euagarics clade	Tetrapolar	Wood decay	+	+	Cosegregation/partial genome sequence	78 and Fig. 19.3
Trichaptum biforme	Homobasidiomycetes: hymenochaetoid clade	Tetrapolar	Wood decay	+	?	Cosegregation	39
Ustilago maydis	Ustilaginomycetes: Ustilaginales	Tetrapolar	Plant pathogen	−	+	Genome sequence	Broad Institute website[g]

[a]In this table, *MAT-A* refers to the homeodomain-encoding *MAT* region, and *MAT-B* refers to the pheromone/receptor gene locus. In smut fungi (e.g., *Ustilago maydis*) *MAT-A* (encoding the homeodomain genes) is actually referred to as the *b* mating-type locus and *MAT-B* is referred to as the *a* mating-type locus. +, linkage conserved; −, genes unlinked; ?, linkage relationship uncertain.
[b]Clade follows nomenclature of Hibbett and Binder (30) or GenBank.
[c]http://www.broad.mit.edu/annotation/genome/coprinus_cinereus/Home.html (Broad Institute).
[d]http://www.tigr.org/tdb/e2k1/cna1/ (The Institute for Genomic Research).
[e]http://genome.jgi-psf.org/Lacbi1/Lacbi1.home.html (Joint Genome Institute).
[f]http://genome.jgi-psf.org/Phchr1/Phchr1.home.html (Joint Genome Institute).
[g]http://www.broad.mit.edu/annotation/genome/ustilago_maydis/Home.html (Broad Institute).

and *MAT* is generally absent in Ascomycota but exists in the genome of *N. crassa*. Linkage in this species is over 100 kbp and quite possibly a statistical artifact. Further studies of Tremellales, Auriculariales, and other heterobasidiomycete genomes will be necessary to determine when the linkage relationship was established. Interestingly, the genome sequence of *U. maydis* shows that *mip* is syntenic (at a distance of ~150 kbp) with the *MAT-a* locus that encodes the pheromone and pheromone receptors (homologous to *MAT-B* of mushroom fungi).

The tight linkage between *mip* and *MAT-A* was also used to study the *MAT* loci of *Coprinellus disseminatus* and *Pleurotus djamor* (40, 41). The entire *MAT-A* loci and surrounding DNA regions were sequenced, thus allowing an assessment of conservation of gene order for the genomic region (Fig. 19.2). These gene order comparisons reveal synteny to be widespread at the *MAT-A* locus. Besides the *mip* gene, 11 additional genes have conserved linkage to *MAT-A* in *C. cinerea*, *C. disseminatus*, *Phanerochaete chrysosporium*, and *P. djamor*. The functions of 8 of the 11 additional conserved genes are uncertain (*chp1-6* [conserved hypothetical proteins], *ypl109*, and *β-fg* [*Aβ*-flanking gene]), but the function of three of them (*sec61*, *glgen*, and *glydh*) can be speculated upon based on homology to genes in other organisms. *sec61* encodes the gamma subunit of a translocase involved in moving proteins from the cytoplasm to endoplasmic reticulum. *glgen* encodes an enzyme putatively functioning in lipopolysaccharide and glycogen synthesis. *glydh* encodes a putative glycine decarboxylating enzyme.

The *MAT-A* loci of *C. cinerea* and *S. commune* have both been divided into two subloci (*Aα* and *Aβ*) by fine mapping studies. A major difference between these two species was that the *Aα* and *Aβ* subloci are very close in *C. cinerea* (~7 kbp) whereas in *S. commune* the *Aα* and *Aβ* subloci are far apart. There are no non-mating-type *MAT*-linked genes harbored in the intervening region between the *Aα* and *Aβ* subunits of *C. cinerea*—hence the previous name of "homologous hole" for this region. In *C. cinerea*, a gene termed the *β-fg* was identified as a gene of unknown function on the other border of the *MAT-A* locus (48). This gene is among the 12 genes that show conserved linkage to *MAT-A* among the four homobasidiomycetes studied to date (Fig. 19.2).

In contrast to all other mushroom species for which data are available, in *S. commune* *MAT*-linked genes are housed between *Aα* and *Aβ* subloci, including *mip* and *pab1* (66). This arrangement of *Aα* and *Aβ* subloci could be explained by two large inversions moving the

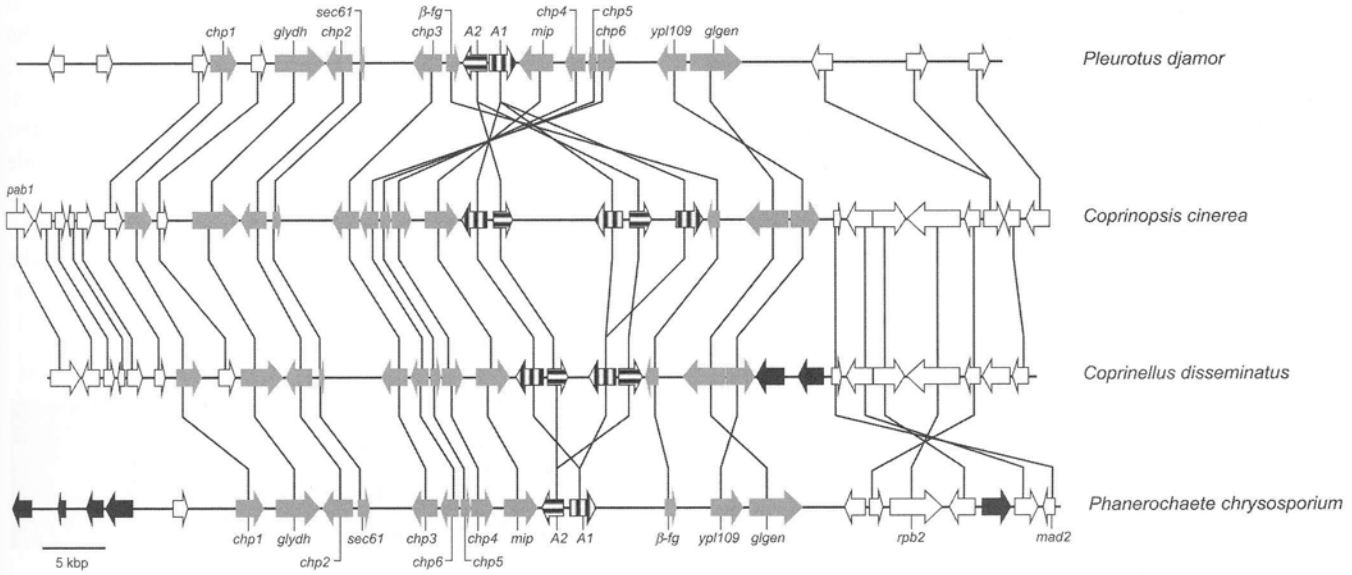

Figure 19.2 Schematic showing conserved gene order of the chromosomal region surrounding *MAT-A* in mushroom fungi. Arrows indicate genes and their direction of transcription. Vertical lines connect homologous genes between species. Genes with vertical stripes or horizontal stripes represent *MAT-A* homeodomain-type-1 (*HD1*) genes and homeodomain-type-2 (*HD2*) genes, respectively (14). Genes in gray are found in all four species, and genes in black are restricted to a single species. This map includes a number of additional genes for *P. djamor* that were missed in the previous annotation of the *MAT-A* region (40). *chp*, gene encoding a conserved hypothetical protein.

genes normally flanking the subloci into the region between the two subloci. The general rarity of non-mating-type *MAT*-linked genes between *MAT* subloci of mushrooms may relate to the theoretical prediction that increases in recombination between *MAT* subloci (thus generating recombinant *MAT* alleles at a higher frequency) will increase the chances of mating occurring among siblings and thereby reduce a species' outbreeding potential (74). In *S. commune*, a gene termed *X* was identified as the other *MAT-Aα* flanking gene (56). Disruption of *X* caused no obvious phenotype in development (56). Gene *X* may be specific to *S. commune*, as there are no clear homologues of *X* detectable in other mushroom genomes.

MAT-B ALSO DISPLAYS CONSERVED GENE ORDER

The *MAT-B* locus of mushroom fungi encodes small lipopeptide pheromones and G-protein-coupled pheromone receptors that have seven transmembrane-spanning domains (homologues of yeast *STE3* a-factor receptor). In *C. cinerea*, the *MAT-B* locus comprises three subgroups with redundant function (29). Each subgroup encodes 1 to 3 pheromone genes and a homoallelic receptor; the whole *MAT-B* locus of *C. cinerea* spans ~25 kbp, and no non-mating-type *MAT*-linked genes are encoded in the regions between the subgroups (29).

The sequencing of the first genome of a homobasidiomycete fungus (*Phanerochaete chrysosporium*) revealed homologues of the *MAT* proteins of mushrooms (57) and allowed an exploration of synteny at the *MAT-B* locus. Five genes encoding pheromone receptors (homologues of *STE3*) were detected in the *P. chrysosporium* genome. Three of these were found clustered into an ~12-kbp region (57), similar to the organization of *MAT-B* in *C. cinerea*. Further investigation of the *MAT-B*-like genomic region in *P. chrysosporium* demonstrated very close linkage to the gene *ste20* (38). Linkage to *ste20* was striking, as it is also linked to the *STE3* receptor homologues of both *Cryptococcus neoformans* (51) and *Pneumocystis carinii* (75). Thus, a conserved linkage between *MAT-B* and *ste20* was demonstrated for a wide diversity of organisms including both Ascomycota and Basidiomycota. Ste20 is a p21-activated kinase (PAK) required for mating in budding yeast (65). The closely related yeast protein Cla4 is also a PAK and is involved in budding and cytokinesis in yeast (15). Cla4 differs from Ste20 in possessing a pleckstrin homology domain, a region possibly involved in protein-protein interactions. The PAK genes linked to *STE3* in *Cryptococcus neoformans* and *Pneumocystis carinii* encode proteins with a pleckstrin homology domain and are phylogenetically more closely related to *cla4* of *Saccharomyces cerevisiae* and *U. maydis* (38, 53). Therefore, for the remainder of the chapter I refer to the *MAT-B* linked PAK as *cla4*.

The data from *Cryptococcus* and *Pneumocystis MAT* loci (or *STE3*-encoding regions) provided compelling evidence that these species have a cluster of genes all functioning in the process of mating (17, 75). Both *Cryptococcus* and *Pneumocystis MAT* loci also encode *ste12*, a key transcriptional activator of genes in the pheromone response pathway in *Saccharomyces* (3). The observation of pheromone receptors linked to genes that may be involved in the same developmental pathways suggested a possible coregulated cluster of genes that might be expected to show evolutionarily conserved gene order. Thus, the potential for cloning *MAT-B* from mushrooms by exploiting synteny with *cla4* was investigated. The linkage between *MAT-B* and *cla4* in additional mushroom species was explored by analyzing the cosegregation of the two loci among progeny of a fruited dikaryon. These data revealed linkage between the two loci in both of the agarics *Pleurotus djamor* (40) and *Schizophyllum commune* (Fig. 19.3), but the loci were unlinked in the polyporoid *Irpex lacteus* (Table 19.1).

By use of a positional cloning approach, the *MAT-B* locus of *Pleurotus djamor* was partially cloned by identifying cosmids from a genomic library that contained the *cla4* gene (40). *cla4* was determined to be ~29 kbp distant from a *STE3*-like pheromone receptor of the *P. djamor MAT-B* locus, as opposed to the ~6-kbp distance observed in the *Phanerochaete chrysosporium* genome. A search of the *Coprinopsis cinerea* genome demonstrates that *cla4* is ~95 kbp from *MAT-B*. In the heterobasidiomycetes *Cryptococcus neoformans* and *U. maydis*, *cla4* is ~5 and ~65 kbp, respectively, from the

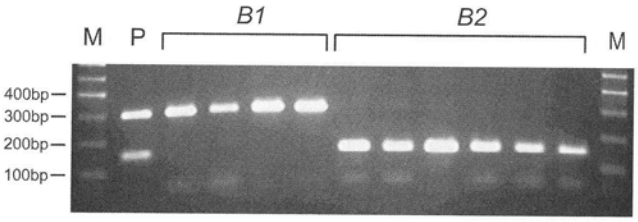

Figure 19.3 Cosegregation of *cla4* and *MAT-B* in *Schizophyllum commune*. Shown is an agarose gel (2%) of *cla4* amplicons digested with MseI. In lane P is the parental dikaryon (Guy.21.2) that is heterozygous at the *cla4* locus. The other lanes show the progeny of Guy.21.2. Lanes *B1* are of the *B1* mating type and possess a *cla4* allele that lacks an MseI cut site. Lanes *B2* are of the *B2* mating type and possess the MseI cut site that digests the 297-bp amplicon into two fragments of 148 and 149 bp. Lanes marked M contain DNA marker.

STE3 homologue in these species (53). These data demonstrate conserved synteny of *cla4* and *MAT-B* in the basidiomycetes, though this linkage is clearly looser than that of *mip* and *MAT-A* and there is at least one example in which synteny has been disrupted (*Irpex lacteus*). In ascomycetes other than *Pneumocystis carinii*, this tight linkage is not observed (based on a scan of several available genomes), though the genes are on the same chromosome in the ascomycetes *Magnaporthe grisea* and *Neurospora crassa* (at nearly 1 Mbp distance).

Comparisons of the genomic regions surrounding the *MAT-B* loci of the homobasidiomycetes (same species as that shown in Fig. 19.2) revealed no additional genes with conserved synteny to *MAT-B* shared by all four species. However, the *MAT-B* sequences available for *Pleurotus djamor* and *Coprinellus disseminatus* are much shorter than the available *MAT-A* sequences. Halsall and colleagues (29) identified a gene encoding a putative transporter of the major facilitator family (*mfs1*) directly flanking the *MAT-B* locus of *Coprinopsis cinerea*. Interestingly, alleles of *mfs1* differed by as much as 40% at the DNA level. A homologue of *C. cinerea mfs* is syntenic with the *MAT-B* locus of *Phanerochaete chrysosporium*, but the intervening distance is nearly 500 kbp. In general, comparative genomics of the *MAT-B* region suggests that rearrangements happen at a much greater frequency than those of the *MAT-A* region (data not shown).

APPROACHES TO CLONING *MAT* USING CONSERVED GENE ORDER

As opposed to the manner in which *MAT* genes were cloned in the model species, cloning *MAT* genes in nonmodel species lacks the advantage of directly testing the function of the cloned genes by transformation of the DNA into a mating-compatible host strain. This distinction means that care has to be given when assigning a cloned gene to the *MAT* locus. One strategy for cloning *MAT* using linked genes begins with amplifying the flanking genes (*mip* and *cla4*), using PCR with degenerate primers (39, 40). PCR amplification of these flanking genes is expected to be much easier than direct amplification of the *MAT* genes themselves. Primers for the *mip* gene have amplified a diversity of homobasidiomycetes, but this gene displays more sequence variation than *cla4*. Primers used successfully to amplify *cla4* are STE20-1F (5′-GTNATGGARTWYATGGARGG-3′) and STE20-2R (5′-ACNACTTCAGGNGCCATCCA-3′). These primers work readily on homobasidiomycetes but often amplify both PAKs (*cla4* and *ste20*).

After sequences of *mip* or *cla4* are obtained in the organism of interest, the fragment can be used to probe a genomic library. If genomic libraries are constructed using vectors capable of replicating a large DNA fragment (e.g., cosmids, bacterial artificial chromosomes, or phages), clones hybridizing to *mip* are very likely to contain all or part of the *MAT-A* locus. Alternatively, PCR-based approaches such as inverse PCR (62) or thermal asymmetric interlaced PCR (54) may be used to amplify and sequence the DNA regions immediately adjacent to *mip*. In the case of *cla4*, a strategy such as inverse PCR is unlikely to be fruitful given the larger physical distances typically observed between the gene and *MAT-B*.

Given that sequences homologous to *MAT* genes can be obtained for the species of interest, further effort may be required to demonstrate that the sequenced region actually harbors *MAT*. Ideally, it is desirable to show that the *MAT* homologues in nonmodel species cosegregate with *MAT* as determined by interstrain matings. For some species, such as ectomycorrhizal taxa for which single spore isolates cannot be obtained due to lack of spore germination (e.g., *Russula* and *Boletus* spp.), this may not be possible. Since the *MAT* genes are highly polymorphic in DNA sequence in all species for which they have been investigated (58, 69, 78), demonstration that the putative *MAT* genes are highly polymorphic in the species of interest can also provide evidence that they are indeed *MAT* genes. Finally, heterologous expression of the cloned *MAT* homologues, when transformed into *C. cinerea* or *S. commune*, may be used to confirm their function (41, 47). Although *MAT* genes of most mushroom species are unlikely to interact with those of the model host species, by transformation of two alleles into a single host strain or through mating of strains transformed with different alleles, the interactions between the gene products may be tested. This assay relies on common *MAT-A* and *MAT-B* downstream targets between the host and nonmodel species, which may not be valid for all mushroom species.

CLONING *MAT* USING DIRECT PCR AMPLIFICATION

Although most of this review has focused on how conserved gene order can be used to clone *MAT* from nonmodel species, a more direct option is through PCR amplification of the *MAT* homologues themselves. The *STE3 MAT-B* homologues display some relatively conserved amino acids among the seven transmembrane alpha-helices (amino terminus) that can be targeted by degenerate primers (1, 40, 41). These primers have successfully amplified *STE3* homologues from a wide range of

mushrooms, from the wood ear fungus *Auricularia polytricha* (GenBank accession number AY226009) to the ash bolete *Gyrodon merulioides* (GenBank accession number AY226018). Phylogenetic analyses of the *MAT-B* receptors suggest two divergent groups of *STE3*-like pheromone receptors in homobasidiomycetes (1, 40, 69), and the PCR-amplified receptors group among the ones that have been demonstrated to have true *MAT*-determining function (data not shown). However, data suggest that *STE3*-like genes are plentiful in mushroom fungi, and non-mating-type-specific pheromone receptor-encoding genes have been detected in *Coprinellus disseminatus* (41), *Phanerochaete chrysosporium* (57), and even *Coprinopsis cinerea* (T. Y. James, unpublished observations). For example, at least four *STE3* homologues have been detected in *C. disseminatus*, mapping to at least two separate locations distinct from the *MAT* locus. Similarly, in *P. chrysosporium* five *STE3* homologues are present and found in three separate regions of the genome (57). Understanding the function of these non-mating-type-specific pheromone receptors is an exciting prospect that may reveal developmental pathways specific to mushroom fungi.

PCR amplification of the homeodomain transcription factors of *MAT-A* has been heroically accomplished with the mushroom *Pholiota nameko* (1). Here the authors targeted the few conserved amino acids of the homeodomain region (HD1) by degenerate PCR and isolated the entire HD1 gene from this species by genome walking using cassette-mediated PCR. A similar approach was also used to amplify and sequence a pheromone receptor homologue of the *MAT-B* locus. Aimi et al. (1) went further and amplified homologues of both *pab1* and *ade5* in *P. nameko* and used cosegregation analyses to show that the homeodomain-encoding gene (*hox1*) was linked to both *pab1* and *ade5* and, moreover, segregated 1:1 with the *MAT* locus in this bipolar mushroom species. The isolated pheromone receptor (*rcb1*), however, was not linked to *MAT*. Attempts to amplify homeodomain-encoding genes in other mushrooms have been unsuccessful (James, unpublished).

THE ORGANIZATION OF *MAT* IN HOMOBASIDIOMYCETES

The ancestor of the homobasidiomycetes is inferred to be tetrapolar (31). In tetrapolar species, the homeodomain proteins encoded by the *MAT-A* locus in a monokaryon are unable to heterodimerize to form an active transcription regulator. In compatible matings bringing together nuclei with different *A* mating types into a single dikaryotic cell, the protein products of the two heteroallelic *MAT-A* loci are able to form the active heterodimer. It is unclear how *MAT* loci are organized in homothallic species, but the structure of *MAT* in heterothallic species suggests that recombination events between different *MAT* alleles could bring together compatible gene products into a single *MAT* allele capable of turning on dikaryotic development without the need for a mating partner. Recombination within a single *MAT-A* haplotype has been demonstrated to create a self-compatible *MAT* allele by the fusion of *HD1* and *HD2* genes from different subunits of the *Coprinopsis cinerea MAT-A* locus (46). This manner by which one of the two *MAT* loci can mutate to a self-compatible allele led Raper (66) to predict that this process led to the formation of bipolar species from tetrapolar species. He also suggested that homothallic species should originate from bipolar ancestors more readily than tetrapolar ancestors (i.e., bipolarity is a transition state from tetrapolar to homothallic). Data on a few species (*Coprinellus disseminatus* [41], *Pholiota nameko* [1], and *Phanerochaete chrysosporium* [38]) now suggest that bipolar mushroom species originate through the loss of mating-type-determining function of the pheromones or receptors of *MAT-B* (Fig. 19.1). These data agree with the facts that all previous attempts to mutate the *MAT* loci of mushrooms resulted only in self-compatible *MAT* alleles, rather than novel alleles (66), and that the self-compatible mutations observed at the *MAT-B* loci of *C. cinerea* and *Schizophyllum commune* occurred through single point mutations (24, 63).

Classical genetic studies determined that the *MAT* loci are composed of multiple, tightly linked, and redundant subloci (18, 67) and that recombination between these subloci, in part, generates the huge number of mating types. Molecular genetic studies have shown that the different subloci harbor the same genes; therefore, the diversity of mushroom mating types can also be explained by the hypervariability of the genes, particularly in their specificity-determining regions (2, 58). The number of subloci thus far observed at *MAT* loci ranges from one (e.g., *MAT-A* of *P. chrysosporium*) to three (e.g., *MAT-B* of *C. cinerea*); it is likely that species having four or more subloci will be found (45). These subloci can be close together (Fig. 19.2) or further apart (1 to 16 cM for *S. commune MAT-A*), though the close arrangement seems to be more common. Although previous observations suggested that the bipolar *MAT* locus may be indivisible into subloci (66), it is now apparent that this is not universally the case (1, 41). Conversely, tetrapolar species such as *Pleurotus djamor* may have only a single *MAT-A* sublocus. The *S. commune MAT-A* locus appears to be different from *MAT-A*

loci of other mushroom species because genes are encoded between the *MAT-Aα* and *MAT-Aβ* subloci, presumably due to large chromosomal inversions. There is good evidence in *S. commune* that the physical distance between subloci at *MAT-B* may be much closer than the mapping distance suggests and that the products of the subloci may actually be able to activate each other (23). Future research will be needed to explain the observation that different *MAT* alleles of *S. commune* generate recombinant mating types at different frequencies.

WHY HAS LINKAGE BEEN CONSERVED BETWEEN *MAT* AND OTHER GENES?

Conserved gene order is generally observed to disappear or decay as organisms diverge (80). Nonetheless, some regions of the genome, such as the X chromosome of vertebrates, display extensive synteny, even among puffer fish and humans, which diverged over 400 million years ago (28). Other regions of the genome have undergone rapid and extensive rearrangements, in eukaryotes often as small inversions (20). Why have some genes, but not others, remained syntenic for such an extended period of time?

There are at least five hypotheses for the observation of conserved gene order near the *MAT* loci of basidiomycetes. The first and most obvious hypothesis is that the gene linkages are just due to chance or historical accident. This postulates that the observed gene order is due to a historical genome rearrangement that was unrelated to the functional coding ability of the genes. Since then, the maintenance of the gene order between species is a probabilistic function related to time of species divergence and distance between genes. There are a few arguments against this simple explanation, however. One is that the degree of synteny or at the least frequency of genome rearrangement at *MAT-A* differs from that at *MAT-B*, suggesting that the process is not random. A counterargument to this point is that different regions of the genome experience different levels of gene rearrangement, i.e., telomeric and centromeric portions of a chromosome appear to be more dynamic than other portions (20). A second argument against the "historical accident" hypothesis is that the linkage between *mip* and *MAT-A* has been maintained despite a small inversion near the *MAT* locus that switched whether the *HD1* or *HD2 MAT* gene was proximate to *mip* (Fig. 19.2). Lastly, the observed synteny between *cla4* and *MAT-B* has been conserved for a very long stretch of time (~400-million-year estimated divergence between *Pneumocystis* and *Phanerochaete* [5]), despite the rapid rate at which synteny has been observed to decline in fungi (22).

Another possible hypothesis for the conserved synteny between *MAT* and other genes is that the genes form a coregulated cluster. Groups of bacterial and eukaryotic genes that display conserved gene order have been demonstrated to physically interact or be coexpressed (16, 36). The clustering of genes that interact with each other suggests that their transcription factors may be distributed heterogenously over the genome/nucleus or that the genomic region may be coregulated by general mechanisms such as methylation or histone modification (36). Genes such as *mip* and the homeodomain *MAT-A* genes may be so tightly linked that they share regulatory regions. Severing these gene linkages by inversions or translocations could disrupt proper expression of one or both genes.

A third hypothesis is that the flanking non-mating-type *MAT*-linked genes are undergoing coevolution with the *MAT* genes. In order for coevolution to occur, the genome region would have to experience recombination suppression such that alleles at one gene could be correlated with the alleles at the linked genes. In this scenario, cophylogeny of alleles at the non-mating-type *MAT*-linked genes and the *MAT* genes is expected; in other words, the gene tree for the two genes should show an identical branching order among alleles. This is observed at sex-determining loci where recombination is suppressed over large regions. For example, at the self-incompatibility locus of the angiosperm *Brassica*, the dispensable gene *SLG* shows coevolution, cophylogeny, and even gene conversion with the self-incompatibility specificity-determining gene *SRK* (71). Similarly, in the large *MAT* locus of *Cryptococcus neoformans*, cophylogeny is observed between the specificity-determining genes (e.g., *STE3*) and the genes more recently recruited to the *MAT* locus (e.g., *ZNF1* [25]). In contrast, in homobasidiomycetes, recombination appears to occur very frequently outside the actual specificity-determining genes of the *MAT* locus (41, 55). Recombination between *mip* and *MAT-Aα* in *Schizophyllum commune* is also observed such that the two loci do not show strict cophylogeny (James, unpublished). This recombination disrupts the linkage disequilibrium needed to create coevolved complexes of alleles at physically linked genes.

A fourth hypothesis is that recombination is suppressed at the *MAT* region and this has a depressing effect on the frequency of genome rearrangements as well. This does not seem to be the case, however, as recombination at the *MAT* loci appears to be normal relative to other regions of the genome (23, 49, 55). In addition, the high number of small inversions, gene duplications, and deletions observed (Fig. 19.2) (51) suggests that rearrangements are frequent near the *MAT* loci.

A final hypothesis for linkage between *MAT* and other genes is related to how the *MAT* loci originate. Hurst and Hamilton (35) hypothesized that *MAT* loci originate as a mechanism to minimize cytoplasmic gene warfare between fusing gametes. They envisioned a scenario in which the fusion of isogamous gametes creates a conflict between mates for the control of mitochondrial genome inheritance. In the first step of this three-step model, a mutant mitochondrial gene arises that can destroy the mitochondrion of a mating partner. These destroyer mitochondrial genotypes can reduce the fitness of zygotes, particularly when two destroyer genotypes fuse. But, nonetheless, the destroyer phenotype is very likely to reach fixation. In the second step, a nuclear gene arises that suppresses the destroyer phenotype of its mitochondrion, thus partially alleviating the fitness loss due to destroyer mitochondria. This "suppressor" gene can be shown to result in a stable polymorphism of suppressor and nonsuppressor alleles. In the final step, a "choosy" gene arises which allows the cell to preferentially mate with a cell of the opposite suppressor phenotype. These suppressor/nonsuppressor matings have the highest fitness if the destroyer mitochondrial genotype is at fixation. The choosy gene may show a preference for mating with a suppressor or nonsuppressor genotype, and this scenario is mechanistically the easiest to imagine. Selection will then favor tight linkage between the choosy gene and the suppressor gene and lead to the formation of a *MAT* locus and uniparental mitochondrial inheritance.

Hurst and Hamilton (35) envisioned this origin of sexes to occur when both parental nuclear and cytoplasmic genotypes were combined in a zygote, such as the fusion of mating yeast cells. The case of the *MAT-a* locus of the basidiomycete yeast *Ustilago maydis* (homologous to *MAT-B* in mushrooms and encoding pheromones and pheromone receptor genes) provides an interesting situation in which the Hurst and Hamilton model may be tested. In this species, genes that appear to be involved in mitochondrial morphology and fusion (*lga2* and *rga2*) are found in only one of the two *MAT* alleles (*a2*) of the *MAT-a* locus (8). When overexpressed, *lga2* causes both mitochondrial fragmentation and mitochondrial DNA degradation (8). In this scenario, the *lga2* and *rga2* genes are suppressors of selfish mitochondria, and the pheromone/receptor genes are the choosy genes that help cells choose the proper partner by signaling with lipopeptide pheromones. This observation provides a compelling case for how a *MAT* locus can maintain genes that do not control mating-type specificity but actually control other processes such as mitochondrial fusion and inheritance. In this example, selection to mediate nuclear/cytoplasmic conflict could have been the reason the pheromone/receptors became a *MAT* locus or could have merely tightened the linkage between the pheromone/receptors controlling mate preference and the *lga2/rga2* genes controlling mitochondrial morphology.

Additional support for an interaction between *MAT* and mitochondrial inheritance in basidiomycetous yeasts comes from the data on *Cryptococcus neoformans*. In this species with two mating types (**a** and α), laboratory crosses demonstrated that progeny almost exclusively inherit mitochondria from the *MATa* parent (90). Furthermore, Yan et al. (89) demonstrated that the homeodomain protein SXI1α encoded by *Cryptococcus MAT* controls the process of mitochondrial inheritance by demonstrating biparental inheritance of mitochondria in *SXI1*α deletion mutants. Since mitochondria from the *MAT*α parent are rarely inherited, the *MAT*α locus may encode a "suppressor" allele or gene that is missing from *MATa*.

In mushrooms, the non-mating-type *MAT*-linked gene most consistently linked to *MAT* is *mip*, a gene that also functions in the mitochondrion. In fact, a large number of genes that function in the mitochondrion are also linked to one or the other *MAT* loci in basidiomycetes (Table 19.2). One hypothesis for why this could occur is that the control of the sexual cycle (by *MAT*) is coregulated with the control of mitochondrial inheritance. In *Saccharomyces cerevisiae*, deletion of *mip* causes loss of functional mitochondrial genomes as well as severe defects in respiration (9). These data lead to the speculation that *mip* could be a suppressor locus of selfish mitochondrial genomes (70) and could control inheritance in heteroplasmic cells that result from mating. Hurst and Hamilton have argued that their theory does not apply to basidiomycetes since these fungi exchange nuclei but not cytoplasm following cell fusion. It now appears that mitochondria in mushroom species may, on occasion, be biparentally inherited, and recombinant mitochondria have now been detected (4, 72). Furthermore, reconciliation of their theory and the observation of mitochondrion-targeted genes linked to *MAT* loci in mushrooms is straightforward since the tetrapolar *MAT* loci of mushrooms and basidiomycetous yeasts are homologous and likely derived from a common origin.

Conservation of linkage between *MAT* and non-mating-type *MAT*-linked genes has also been observed in Ascomycetes. The two genes *sla2* (encoding an actin-binding protein involved in cytoskeleton assembly) and *apn2* (encoding a DNA lyase) flank the *MAT* locus in a large number of species including both hemiascomycetes and euascomycetes (11, 26, 84). Remarkably, the gene *sla2* also shows tight linkage to the *U. maydis*

Table 19.2 Non-mating-type *MAT*-linked genes in basidiomycetes that encode proteins targeted to mitochondria[a]

Gene	Taxon	Linked to *MAT-A* or *MAT-B*?	Function	References(s)
ETF1	*Cryptococcus neoformans*	*A* + *B*[b]	Mitochondrial electron transport	51
Glycine dehydrogenase (*glydh*)	Homobasidiomycetes	*A*	Amino acid transport and metabolism	—[c]
lga2	*Ustilago maydis*; *Sporisorium reilianum*	*B*	Mitochondrial morphology and fusion	8, 73
Mitochondrial intermediate peptidase (*mip*)	Homobasidiomycetes	*A*	N-terminal processing of nuclear encoded proteins targeted to the mitochondrial matrix or inner membrane	37
Mitochondrial ribosome small subunit component (*rps19*)	*Pleurotus djamor*	*B*	Protein translation	—[c]
rga2	*Ustilago maydis*; *Sporisorium reilianum*	*B*	Mitochondrial morphology and fusion	8, 73
RP041	*Cryptococcus neoformans*	*A* + *B*	Mitochondrial RNA polymerase	51
ypl109	Homobasidiomycetes	*A*	Possible role in ubiquinone biosynthesis	—[c]

[a]In this table, *MAT-A* refers to the homeodomain protein encoding *MAT* region, and *MAT-B* refers to the pheromone/receptor gene locus.
[b]The *MAT* locus in this bipolar species contains homologues of both *MAT-A* and *MAT-B* genes of homobasidiomycetes.
[c]—based on subcellular predictions using the software WoLF PSORT (http://wolfpsort.seq.cbrc.jp/).

MAT-b locus encoding homeodomain proteins. A functional link between *sla2* and mating is suggested by these data but yet to be established. It is worth noting that *sla2* and *cla4* have been demonstrated to interact in yeast based on yeast two-hybrid interactions (19).

CONCLUSIONS

Genomics tries to find higher-order meaning in the organization of genes within genomes. In this review, I discussed how mating-type genes can be cloned from non-model species and attempted to synthesize what is known about the organization of the *MAT* loci and neighboring genomic regions in mushroom fungi. Synteny appears to be more conserved at *MAT-A* than *MAT-B*. *MAT* genes in mushrooms can be cloned by both direct PCR amplification and positional cloning using non-mating-type *MAT*-linked genes by probing a large insert genomic library. Proper determination of whether a cloned gene is a mating-type gene is nontrivial but can utilize syntenic arguments and population genetics, as well as transformation into heterologous hosts. *MAT* loci show high gene order conservation, but the significance of this observation is unclear. Among the leading candidates for the conserved gene order are coregulation and remediation of nuclear/cytoplasmic genome conflict.

Model species are indispensable for understanding *MAT* at the molecular level and provide all of the information on how *MAT* genes function. However, in order to fully unravel the marvelous mysteries of mushroom mating we will need to explore *MAT* across the phylogenetic diversity of mushrooms. Observations, such as mushroom species having whorled (multiple) clamp connections per septum in both homokaryotic and heterokaryotic mycelium (e.g., *Stereum hirsutum*) and heterothallic species lacking clamp connections altogether (e.g., *Phanerochaete chrysosporium*), give an indication that a simplified mushroom mating system with *MAT-A/B*-controlled developmental pathways may not hold for the whole group. Further, the organization of *MAT* loci in homothallic species of mushrooms is unknown. As only a fraction of the edible and medicinal mushroom species can be readily fruited, access to *MAT* loci may provide information useful for breeding, production, or genetic engineering (43). Despite the previous notion that *Schizophyllum commune* was a member of the Aphyllophorales, molecular systematics suggests that it is related to fleshy mushrooms (Agaricales), the order to which *Coprinus* sensu lato also belongs (6). Thus, the molecular knowledge of *MAT* in mushrooms is primarily limited to one order. As we move deeper towards the base of the mushroom phylogeny, it is

expected that the same genes will be used in *MAT* determination; however, novel gene arrangements and functions are likely to be uncovered.

I am indebted to Ursula Kües for her countless discussions on mating type in mushrooms. I thank her and Greg Bonito for comments on the manuscript.

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