Fungal sex genes—searching for the ancestors

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Summary

The sex-determining genes of fungi reside at one or two specialised regions of the chromosome known as the mating type (*MAT*) loci. The genes are sufficient to determine haploid cell identity, enable compatible mating partners to attract each other, and prepare cells for sexual reproduction after fertilisation. How conserved are these genes in different fungal groups? New work[1] seeks an answer to this question by identifying the sex-determining regions of an early diverged fungus. These regions bear remarkable similarity to those described in other fungi, but the sex proteins they encode belong to only a single class of transcription factor, the high mobility group (HMG), indicating that these are likely to be ancestral to other proteins recruited for fungal sex.
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Introduction

It is over 100 years ago since Blakeslee,[2] studying the Zygomycete fungus *Phycomyces*, recognised that some of his strains were self-sterile and obligate maters whereas others were self-fertile. He introduced the terms heterothallic and homothallic, respectively, to describe these two different mating behaviours, terms that we still use in fungal biology. Despite this very early recognition of fungal self-sterility, it is only now that the molecular structure of the sex-determining region of *P. blakesleeanus*, the species named after him, has been described.[1] The authors of this recent paper raise interesting ideas on the evolution of sex-determining regions of the fungal genome and the conservation of sex gene function.

Our knowledge of the genes that confer heterothallism, the so-called mating type (*MAT*) genes, has previously been confined to members of a single monophyletic clade of fungi known as the Dikarya, comprising ascomycetes such as yeasts, and the mushrooms and smuts belonging to the basidiomycetes.[3] *P. blakesleeanus* belongs to an earlier diverged group of fungi, the Zygomycota,[4] and it thus of great interest to see how the *MAT* loci of this fungus compare with those of the Dikarya.

The authors took advantage of the recently published sequence of *P. blakesleeanus* and used a combination of bioinformatics and genetic mapping to identify the genes that determine sexual identity. They hypothesised that the genes at *MAT* were likely to be highly conserved throughout the fungal kingdom and, most relevantly, likely to be similar to those found in ascomycete fungi where, like zygomycetes, there are just two mating types.[5–7]

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MAT genes generally encode transcription factors

The *MAT* loci of many ascomycete fungi have been well characterised, some of these at both a structural and a functional level. Although we refer to the *MAT* locus as having different alleles, the DNA sequences of the alleles have been shown to be highly dissimilar. The alternative versions of *MAT* do not contain different allelic versions of the *MAT* genes, they contain completely different genes and these sex-determining regions are often termed idiomorphic to distinguish them from the allelomorphic nature of other loci.[8] The alternative ascomycete *MAT* loci encode a combination of transcription factors that permit mating type (sex)—specific expression of the many other genes required in mating, in particular the genes that encode sex pheromones and their receptors that are essential for cells of opposite mating type to attract each other and bring about fertilisation.[5,9] The characterised MAT transcription factors include two classes with homeodomains (HD1 and HD2) one with an alpha-box and one or more with HMG (high-mobility group) domains.[5,7,10]

A search by the authors[1] of the *Phycomyces* genome revealed no obvious homologues of genes encoding the homeodomain or alpha-box proteins, but it did identify 10 genes encoding potential MAT HMG proteins. Excitingly, attempts to use polymerase chain reaction (PCR) to amplify these genes from strains of both mating type (known as *MAT plus* and *MAT minus*) showed that one gene was specific to *minus* strains, and therefore a potential *MAT* gene. Amplification of the corresponding chromosome region from a *plus* strain showed that this also encoded an HMG protein and, significantly, each gene resided in a DNA sequence that was highly dissimilar, extending for 5,830 bp in the *plus* genome compared with 3,494 bp in the *minus* genome. Crosses between *plus* and *minus* strains confirmed that this polymorphic region of the chromosome segregated with mating type. The genes were consequently designated *sexM* (*sex minus*) and *sexP* (*sex plus*).

The standard functional test of sex gene function would be to show that heterozygosity in an otherwise haploid genome induces a mated cell phenotype. In ascomycete fungi, this is easily done by introducing a cloned *MAT* gene by transformation. As yet this technology has proved difficult for *Phycomyces*, but fortunately, abnormal chromosome segregations can generate partial diploid strains that provided the answers that the authors were looking for. The authors noted odd 'fluffy' progeny segregating in crosses and these not only failed to mate with either *plus* or *minus* strains, they were able to produce sexual spores, normally only produced after a successful mating. These odd progeny were behaving like mated cells and proved to have copies of both the *sexM* and *sexP* chromosomes.

Sex chromosomes and repetitive elements

The discovery that the *MAT plus* allele of *P. blakesleeanus* is larger than the *minus* allele is intriguing. Sequence comparisons identified a number of different small repetitive elements in the *plus* DNA region. These elements were also found outside the *MAT* locus on both chromosomes. There has been considerable interest recently in the sex-determining regions of several fungi and the role that repetitive elements may play in locus expansion and crossover suppression, even the generation of what might be called fungal sex chromosomes.[11] In a close relative of the bread mould *Neurospora crassa, N. tetrasperma*, some 75% of the chromosome on which *MAT* is located is subject to crossover suppression.[12] In the yeast-like basidiomycete, *Cryptococcus neoformans*, which causes meningitis in man, the two *MAT* alleles extend over 100 kb[13] and, in another basidiomycete, the barley smut, *Ustilago hordei*, the *MAT* alleles extend over 500kb.[14] In both cases, sequence analysis revealed that between or amongst the characteristic *MAT* genes, are many other genes, largely unrelated to mating. Gene inversions, sequence diversification and repetitive elements ensure that recombination over these long distances is suppressed. Similar chromosomal events have been implicated in Y chromosome evolution in man[15] and Idnurm et al.[1] are quick to point out the role of inversions triggered by repetitive elements in driving the expansion of sex-determining regions in diverse lineages. It is truly fascinating to discover that these events have already driven *MAT* evolution in *P. blakesleeanus.*

Evolution of *MAT* loci in fungi

The *P. blakesleeanus MAT* locus is unique so far in having alternative alleles encoding proteins belonging to the same transcription factor family, both are HMG domain proteins. Could the ascomycete and zygomycete loci have a common origin? It seems very likely. In ascomycete fungi, the simplest *MAT* locus (characteristic of the Dothidiomycetes) also consists of just one gene in each allele, one encodes an HMG domain protein and the other an alpha-box protein.[16] Because the Zygomycota represent an early branch in the fungal evolutionary tree, Idnurm et al.[1] naturally draw the conclusion that genes encoding HMG domain proteins represent an early form of the fungal *MAT* loci. They note, however, that the sequences of the sexM and sexP proteins are sufficiently similar to indicate that they may have diverged from a common ancestor. It would require only small changes in the DNA-binding domain to confer different promoter specificity, and sequence divergence within and around the genes would suppress recombination. Particularly relevant to this evolutionary argument are comparisons of the two MAT proteins encoded by the alternative *MAT* alleles of the ascomycete *Cochliobolus* and other members of the Dothidiomycetes. Studies of Gillian Turgeon and her colleagues[17] have shown that, although the gene at one of the *MAT* loci (*MAT1*) encodes an alpha-box protein, downstream of this DNA-binding site is a novel HMG domain. This HMG domain has, moreover, some shared homology with the HMG domain found in the other MAT protein encoded by the alternative allele *(MAT2)*, and there are other shared sequence motifs that suggest that like the *P. blakesleeanus* MAT proteins, these too could have been derived from a common ancestor. Whether or not the HMG domain in the MAT1 protein has a functional role is not known. Transcription factors are modular in structure and the acquisition by an HMG protein of a new DNA-binding domain, the alpha-domain, is a possible first step in the evolution of the ascomycete *MAT* locus from the situation that we now see in the Zygomycota (see Fig. 1).

In yeasts we have a good understanding of how the *MAT* genes determine haploid and diploid cell identity.[5,10,18] The *MAT* loci have more genes than those of the Dothidiomycetes. In *Candida albicans,* for example, the alternative forms of *MAT* encode an alpha-box protein and a homeodomain protein belonging to the HD1 class (*MAT*α), or an HMG domain protein and a homeodomain protein belonging to the HD2 class (*MATa*).[18] In cells expressing the MATα genes, the alpha-box protein activates transcription of genes required to synthesise α-sex pheromone processing and secretion and a-sex pheromone receptors. In *MATa* cells, the HMG domain protein activates genes required for processing and secreting the structurally different a-sex pheromone and α-sex pheromone receptors. Pheromone binding to a compatible cell surface receptor triggers an intracellular signal transduction pathway (a MAP kinase cascade) that leads to expression or upregulation of all genes involved in mating.[19] Once the mating cells have fused, the HD1 and HD2 homeodomain proteins that were originally separated in different haploid cells heterodimerise to form a mated cell-specific transcription factor that shuts off all mating functions and, in sexually reproducing forms, prepares cells for meiosis.

It is puzzling to many fungal biologists why the basidiomycete *MAT* loci are so different from those of ascomycete fungi

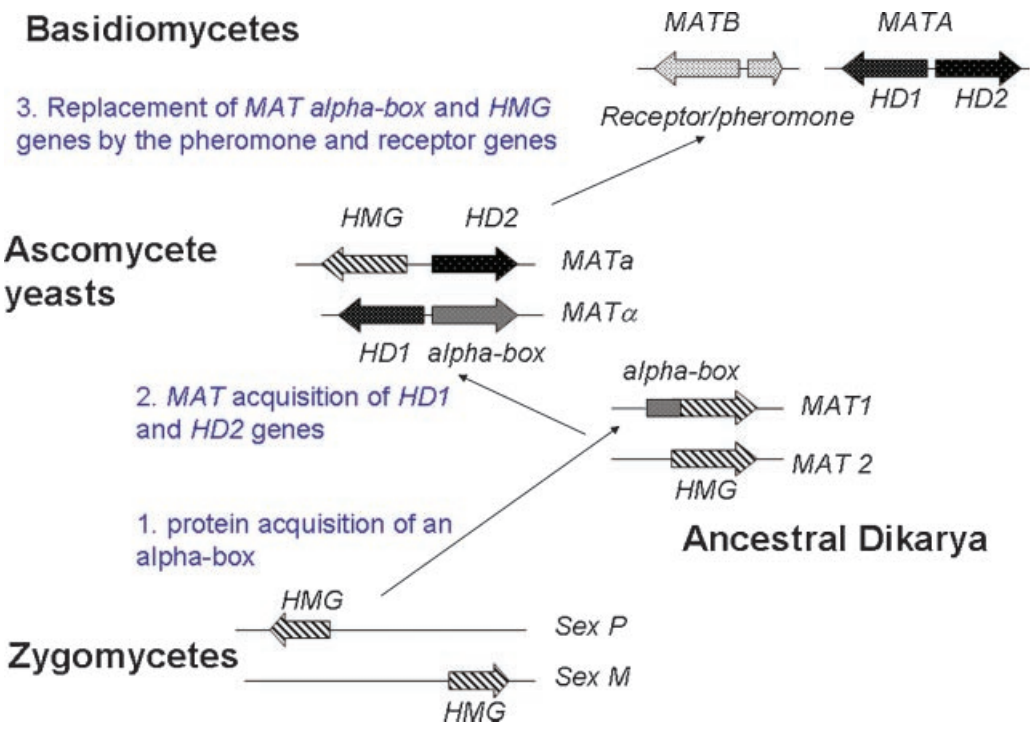

Figure 1. Suggested steps in the evolution of different fungal mating type loci. **1:** A first step from the situation seen in Zygomycota assumes the acquisition in an early Dikarya ancestor of an alpha-box DNA-binding domain by one of the HMG domain transcription factors. This could have given rise to the *MAT* loci seen in modern day Dothidiomycetes. **2:** A second step involves the acquisition of the *HD1* and *HD2* homeobox genes seen in yeast and basidiomycete *MAT* loci. **3:** A third step is the replacement of genes encoding the HMG domain and alpha-box transcription factors by genes encoding pheromones and receptors. This latter step enabled the evolution of multiple mating types in mushrooms where genes at both *MATA* and *MATB* became multiallelic.

even though their mating pathways are very similar.[20,21] There are commonly two *MAT* loci in basidiomycetes (although the genes may all be present at a fused locus).[13,14] One is quite different from all ascomycete *MAT* loci because it encodes the pheromone receptors and pheromone precursor genes. The other contains pairs of genes encoding HD1 and HD2 homeodomain proteins similar to those found in yeasts. In mushrooms, both sets of genes are multiallelic and their various combinations permit the generation of thousands of different mating types. How can we fit the structure of basidiomycete *MAT* loci into an evolutionary scheme that illustrates conservation of *MAT* function? Compatible mates have a complement of pheromones and receptors that trigger a MAP kinase signalling pathway to activate genes necessary for mating,[22] and combinations of HD1 and HD2 proteins that can heterodimerise to generate a mated cell-specific transcription factor.[23,24] The pathway is conserved, it is just the way that it is regulated by the *MAT* genes that is different. In ascomycetes, the MAT transcription factors regulate the genes required for sex-specific pheromone signalling, whereas in basidiomycetes the pheromone and receptor genes have been sequestered into the *MAT* locus and determine mating type directly. A comparison of these different *MAT* loci and steps by which they may be related are illustrated in Fig. 1.

The likely role of sexM and sexP

Central to mating and *MAT* gene regulation in the Dikarya is sex pheromone signalling. The receptors belong to the ubiquitous G protein-coupled receptor families and the secreted pheromones are small peptides. In *P. blakesleeanus*, the sex pheromones are derivatives of β-carotene. They are MAT specific, (methyl trisporates in *plus* strains and trisporates in *minus* strains) and are converted to trisporic acids by the opposite mating type during mating.[25,26] A new challenge will be to determine how the matP and matM proteins regulate synthesis of different pheromones from the same precursors.

Conclusion

With many fungal genome sequences now available, there has been a resurgence of interest in the role that *MAT* genes play in determining the different life styles that have evolved. Heterothallic species have until recently been the main focus of attention, but it is now known that homothallic species may

contain a full complement of *MAT* genes and that pheromone signalling still plays an essential role in fertility.[27] Even more remarkable, species long assumed to be asexual, such as the human pathogen *Aspergillus fumigatus* and industrially valuable *A. oryzae*, have typically heterothallic *MAT* loci.[28] There is considerable excitement at the prospect that genetic manipulation of these species may now be possible by activating a previously unknown sexual cycle. It is appropriate, therefore, to follow the lead of Idnurm et al.[1] in discovering more about the *MAT* genes in other fungal groups, particularly those of industrial or medical interest, so that their genomes also are open to exploitation through sexual manipulation.

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