Rapidly Evolving Genes and Genetic Systems

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CHAPTER 19

Ancient yet fast: rapid evolution of mating genes and mating systems in fungi

Timothy Y. James

19.1 Introduction

Though seldom observed directly, the life cycle of most filamentous fungi includes countless cryptic encounters between conspecific fungal cells as they grow within their substrate. Complex substrates such as soil contain hundreds of species of fungi per gram (Buée et al. 2009) and hyphal interactions are unavoidable. Most fungi are sexual, and completing the life cycle necessitates that hyphal encounters with potential mates are distinguished from enemies. Recognition of self from potential mate or enemy is governed by a set of genes known as incompatibility genes. Unlike mating in the majority of eukaryotes in which sex involves the fusion of two cells, interactions between filamentous fungal individuals are fundamentally different and may be played out in a theater of numerous redundant and synchronous interactions between nuclei and the cells that house them. Following an encounter with a compatible mate, a genetically merged and dynamic colony is formed (Rayner 1991). Hyphal fusion is seldom followed by nuclear fusion, and instead filamentous fungi postpone the formation of a diploid zygote by forming a heterokaryon in which nuclei of compatible genotype coexist in the same cell and continue to divide synchronously by mitosis as the network grows. Only immediately before meiosis, does the zygote nucleus form by karyogamy of the mated partner nuclei (Fig. 19.1).

Incompatibility genes in fungi control both sexual and competitive conspecific interactions through two distinct molecular pathways. The mating-type incompatibility genes (*MAT*) control sexual attraction and heterokaryon (see Box 19.1 for a glossary of terms) maintenance through the production of communication and signal transduction molecules (Hiscock and Kües 1999). The genes of the vegetative incompatibility pathway (*HET* genes), function to prevent illicit vegetative fusions and nuclear exchange between incompatible conspecific mycelia while allowing the network of one genotype to proliferate by branching and re-fusion to self. Vegetative incompatibility genes define 'individuality' in mycelial fungi, and vegetative incompatibility can often be observed in nature as lines in the substrate (physical boundaries) that demarcate genetically distinct individuals (Rayner 1991; Worrall 1997). In Ascomycota, a monophyletic group including most lichenized fungi, cup fungi, many molds and yeasts such as *Saccharomyces cerevisiae*, individualistic behavior occurs between haploids (homokaryons), whereas in Basidiomycota, including the rusts, smuts, and a diversity of mushroom-like groups, it occurs between heterokaryons (Fig. 19.1). In contrast to heterokaryons, filamentous homokaryons in basidiomycetes are highly promiscuous cells that are able to mate with most other conspecific homokaryons they encounter due to increased compatibility imparted by a mating system with a large number of mating types. Thus the life cycle of the basidiomycete is primarily mated or heterokaryotic, while in that of the ascomycetes, with much more limited compatibility by possession of only two mating types, sex occurs only at precisely the right condition, place, and time, and most of the life cycle exists in a primarily haploid or homokaryotic stage.

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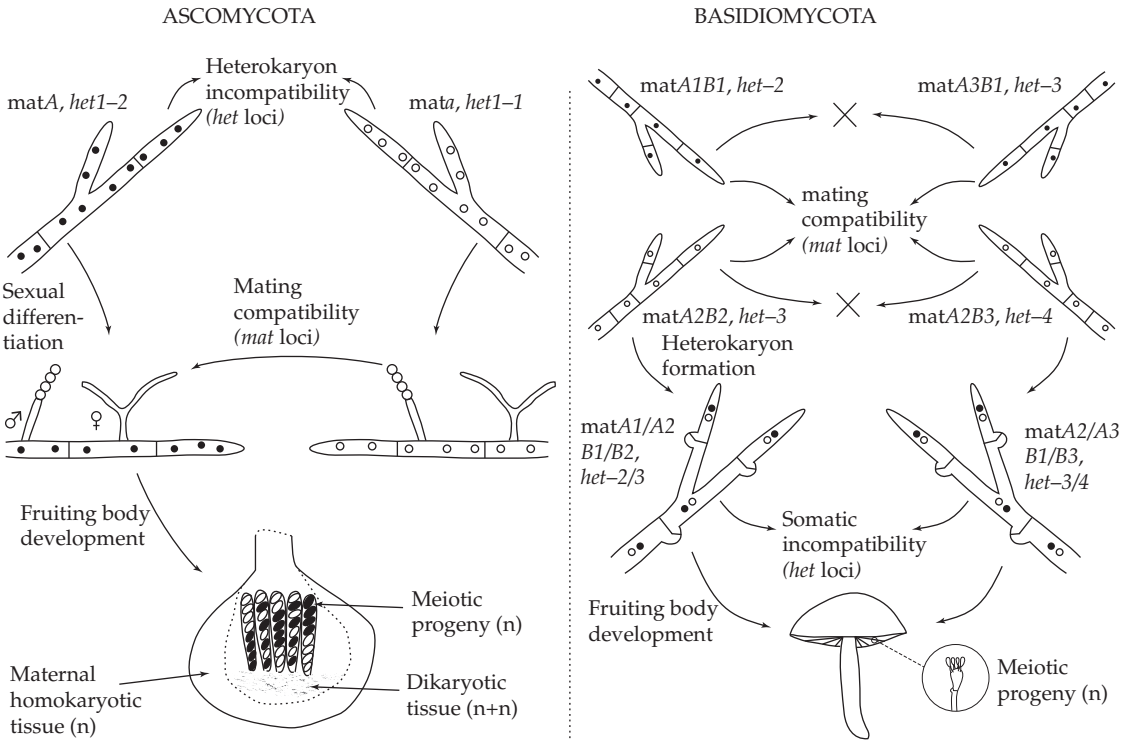

Figure 19.1 Contrasting life cycles of the filamentous Ascomycota and Basidiomycota. Major differences include the nuclear condition in which *HET* loci determine the ability to fuse (homokaryotic stage in Ascomycota, heterokaryotic stage in Basidiomycota) and genetic condition of the fruiting body (homokaryotic in Ascomycota, heterokaryotic in Basidiomycota). Shown is a tetrapolar basidiomycete with two mating-type loci (mat-*A* and mat-*B*). Both life cycles are drawn with a single *HET* locus, but most species typically have several.

Box 19.1 Glossary

Anisogamy: in fungi, occurs when a spermatium (small aerially dispersed gamete) fuses with a trichogyne (receptive female hypha).

Bipolar: a homoallelic mating incompatibility system with only a single locus. At meiosis two mating types are produced per meiocyte.

Heterokaryon: a cell type comprised of multiple nuclear genotypes maintained in an unfused state through multiple rounds of mitosis.

Homeodomain (HD) locus: a mating type locus encoding one or more transcription factors that possess a distinctive homeodomain DNA binding motif.

Homokaryon: a cell type comprised of only a single nuclear genotype.

Homothallic: a non outcrossing mating system in which a single spore is capable of giving rise to a fruiting body.

Mating type: equivalent to 'sexes' in isogamous or hermaphroditic organisms; individuals of the same mating type are sexually incompatible.

Pheromone receptor (P/R) locus: a mating type locus encoding at least one G-protein coupled transmembrane receptor and one pheromone peptide.

Tetrapolar: a homoallelic mating incompatibility system with two loci. At meiosis four mating types are produced per meiocyte.

As the result of decades of intensive research, much is known about the genes regulating hyphal fusion and mating (Hiscock and Kües 1999; Lee et al. 2010), and some of these pathways are among the best known in eukaryotes, such as the pheromone response pathway in yeast (Herskowitz 1989). Incompatibility genes in fungi have many similarities to genes controlling incompatibility and sex determination in animals and plants. The reproductive and incompatibility proteins in the plant and animal kingdoms have provided some of the clearest examples of rapid evolution. In fungi, however, little is known about the evolutionary dynamics of reproductive proteins, particularly whether incompatibility genes evolve rapidly as they do in plants and animals.

In this review I will discuss the expectations for fungal incompatibility systems generated by the plant/animal systems and provide examples of key studies that can shed light on whether these expectations hold for fungi. I will discuss evidence for accelerated evolution as well as balancing selection on incompatibility genes, loci, and mating systems in fungi. Emphasis will be placed on mushroom fungi as these species have evolved a multiallelic system that has many of the characteristics of other fast-evolving incompatibility systems such as those observed in flowering plants.

19.2 Incompatibility systems in fungi

The two types of incompatibility systems in fungi function through two distinct mechanisms leading to two distinct signal cascades. Vegetative incompatibility is a heteroallelic allorecognition system, whereas mating incompatibility is a homoallelic autorecognition system (Hiscock and Kües 1999).

In the allorecognition system, individuals that possess different alleles at one or more incompatibility loci are unable to form a heterokaryon. Depending on the species, the number of loci involved ranges from a single *HET* locus to typically several, each with two or more alleles. In *Neurospora*, which has 11 such loci, the number of potential vegetative compatibility (VC) types based on combinations of the alleles is in the thousands, and thus, random individuals pulled from the population are unlikely to be compatible. However, in species with fewer VC types, such as *Aspergillus nidulans* with six *HET* loci (Jinks et al. 1966), genetically distinct individuals may by chance or through shared ancestry possess common alleles and be able to fuse and form heterokaryons. In basidiomycetes, heterokaryons can also be formed outside of the mating cycle through somatic compatibility between genetically distinct heterokaryons, leading to the formation of higher-order heterokaryons with three or more nuclear types (Johannesson and Stenlid 2004). Currently, nothing is known of the molecular basis for vegetative incompatibility in basidiomycetes, and in ascomycetes, the genes are only known from two classes of this group, the Sordariomycetes and Eurotiomycetes. The gene products of the known *HET* loci vary widely but often include a 150 amino acid HET domain. How the HET proteins function is unknown, but the cellular result is compartmentalization and activation of the programmed cell death pathway in fused incompatible cells, which leads to boundary formation among individuals (Glass and Dementhon 2006).

The allorecognition system of fungi is analogous in function to the systems controlling tissue rejection in vertebrates (animals), for example, the major histocompatibility complex (MHC) that controls tissue and fetal rejection and the cell surface recognition molecules alr1 and alr2 controlling fusion in colonial cnidarians (Nicotra et al. 2009). Maintenance of a high diversity of alleles is necessary for these systems to function properly and failure to reject results in chimerism.

In the autorecognition system controlling mating, individuals that share alleles at *MAT* loci are the same mating type and are incompatible. Thus *MAT* loci segregate for alleles that determine a cell's mating type. Most fungi have a single *MAT* locus (bipolarity), but in many basidiomycetes, there are two *MAT* loci (tetrapolarity) and heteroallelism at both loci is required for full compatibility. MAT proteins regulate steps in mate attraction and fusion (e.g. pheromones) and once mated maintain the heterokaryotic state by nuclear signaling. The gene products of ascomycete *MAT* are primarily transcription factors, including proteins with homeodomain and high mobility group (HMG) DNA binding domains (Lee et al. 2010).

Basidiomycete *MAT* loci also encode homeodomain transcription factors, but may also encode peptide pheromones and the G protein-coupled pheromone receptors they stimulate. This is another departure from ascomycetes, wherein pheromones and receptor alleles are differentially regulated but not differentially present between the genomes of mating types. In tetrapolar basidiomycetes, one locus encodes at least one incompatible receptor and pheromone combination (P/R) and the other locus typically encodes two types of homeodomain proteins (HD1 and HD2) that are self-incompatible but can form a HD1–HD2 heterodimer in heteroallelic encounters. In bipolar basidiomycetes the *MAT* locus encodes homeodomain proteins solely or both homeodomain proteins and pheromone/receptors, but never a pheromone/receptor solely (James 2007). As with the allorecognition system, the typical model postulates that the outcome of mating interactions in filamentous fungi is largely determined post-cell fusion by coordination of nuclear migration and nuclear acceptance of the mating mycelia.

The autorecognition system is analogous in function to the genes that control sex determination in animals and plants but has more important similarities to the well-known self-incompatibility (SI) system in flowering plants where interactions occur on the cellular level independently of the sex of the parent. Here, a dichotomy can again be made between ascomycetes and basidiomycetes. Filamentous ascomycetes have anisogamous sex (Fig. 19.1) with only two mating types. Agaricomycetes (mushrooms), on the other hand, often have multiple mating types (as many as 100s) and always isogamous sex. Maintenance of an equal frequency of each of the mating types or incompatibility alleles is assured by frequency dependent selection that favors rare alleles. This parallel between a high diversity of plant SI alleles and Agaricomycetes *MAT* alleles has been long recognized and suggests similar evolutionary dynamics (Uyenoyama 2005; Newbigin and Uyenoyama 2005). Why the number of mating types in ascomycetes has been limited to two is unknown but may relate to a lower cost of mating or ancestral anisogamy (Billiard et al. 2011). This stable equilibrium of the two mating type system is similar to the sex determining systems in animals and plants with two sexes in equal frequencies (Hurst 1996). In the multiallelic mating system of basidiomycetes, however, *MAT* alleles can be lost by drift or by replacement with a slightly higher fitness allele. In the following section, I address how fitness differences between MAT alleles in the multiallelic basidiomycete system could lead to accelerated evolution.

19.3 Fungal reproductive proteins show evidence for positive and balancing selection

Non-neutral evolution takes several forms, but this review focuses on selective mechanisms that cause rapid evolution of genes within and between species, i.e., positive selection, and the selective forces that maintain polymorphism within a species, i.e., balancing selection. In this review, positive selection is used to refer to selection that increases the fitness, and therefore the frequency, of an allele relative to its ancestral allele, whereas balancing selection is used to refer to forces that prevent allele loss or fixation. Because all incompatibility systems require the maintenance of multiple alleles in order to function, balancing selection prevents allele fixation by positive selection. However, balancing selection can also act to accelerate amino acid replacement at sites under selection under certain selective regimes (e.g., negative frequency dependent selection and heterosis).

Reproductive proteins in animals and plants have often been demonstrated to evolve rapidly relative to other regions of the genome. Evidence for rapid evolution can be obtained along multiple lines. One line of evidence is based on traditional tests for positive selection by detecting an increased rate of nonsynonymous substitution over neutral expectations. These data have often shown that reproductive proteins involved in sperm–egg (Panhuis et al. 2006) or pollen–ovule interactions (Takebayashi et al. 2003) have rates of nonsynonymous substitution greater than synonymous substitution. A second line of evidence comes from studies demonstrating increased divergence in reproductive protein sequences between species relative to other proteins in animals and

plants (Jagadeeshan and Singh 2005). A final line of evidence comes from comparisons of gene duplicates that have undergone neofunctionalization. For example, a testes-specific homeobox variant in mammals showed an elevated rate of substitution relative to the ubiquitously expressed copy from which it diverged (Wang and Zhang 2004).

Balancing selection extends the genealogical depth of a collection of alleles, and depending the selection coefficient may impart a very high level sequence diversity within and among species (Takahata 1990). In scenarios in which homozygous genotypes cannot be formed, such as in homoallelic incompatibility systems, the selection is very strong, and alleles are expected to be maintained for very large numbers of generations, thereby increasing the divergence among functionally distinct allelic lineages at both functionally relevant and linked neutral sites. In extreme cases, this may result in a pattern of trans-specific polymorphism wherein lineages diverged before the species in which they are found. Trans-species polymorphism has been observed for lineages of plant SI genes (Richman et al. 1996) and the complementary sex determination genes in hymenoptera, one of the largest orders of insects (Cho et al. 2006). What is unknown is whether the alleles are really functionally equivalent (in some plant SI systems they clearly are not) and whether demographic forces, mutational limitation, or both can lead to the observed differences in numbers of alleles across species.

Despite the widespread demonstration of rapid evolution of reproductive proteins, the nature of selection on the proteins is actually not often clear (Swanson and Vacquier 2002). Several general models have been put forward to explain the rapid evolution of reproductive proteins, but they are likely applicable to only a subset of proteins (Swanson and Vacquier 2002). Following this logic, I propose that at least five forces may be particularly relevant for rapid evolution and/or extreme sequence divergence of incompatibility proteins in fungi:

1. *Rare advantage:* The multiallelic MAT incompatibility systems of Agaricomycetes may maintain hundreds of alleles at a single locus (James et al. 2004). Even if a population is at equilibrium for the number of alleles they may maintain (a function of population size and mutation rate), new mutations that generate novel specificities have an initial compatibility (fitness) advantage, and this should cause increased allele turnover. This constant favoring of novel alleles could be observed as an increase in nonsynonymous substitution rates over synonymous rates because alleles containing new amino acid replacements are on average less likely to be lost than those without them during the replacement process. See Newbigin and Uyenoyama (2005) for one model of how the replacement and acceleration could work in the plant SI system. Gaps in knowledge: vanishingly little is known about the origin of new alleles, and most alleles appear to be deeply divergent in sequence and therefore, time. One explanation for the deep sequence divergence of mating type and self-incompatibility alleles hypothesizes that the absence of recombination in and near mating type loci causes the accumulation of genetic load due to a reduced efficacy of purifying selection (Uyenoyama 2005). The accumulation of genetic load then favors pairs of alleles of deeper divergence as they are less likely to display homozygosity of deleterious alleles linked to the mating specificity. Thus, new alleles are most likely to replace the closest related allele, extending the overall coalescence time of the genealogy and reducing the rare advantage effect. However, it is unclear whether this genetic load is expected at mushroom mating type loci in which recombination immediately outside of *MAT* appears to be high (James et al. 2006) and cells have the potential to purge deleterious alleles due to selection in the free-living haploid stage.
2. *Competition/sexual selection model:* because of the high density of potential mating partners, access to mates through selection on rate of nuclear migration may be a fierce arena for competition. Opportunities for 'female' strains to choose among potential nuclear donors has been observed and linked to differences among mating types (Nieuwenhuis et al. 2011). Analogies to sperm competition are clear, with the end result that proteins in control of

access to mycelia (e.g. the pheromone proteins), attractiveness to potential 'female partners' (nuclear acceptors), or proteins involved in nuclear migration such as dynein and the cytoskeletal proteins (Gladfelter and Berman 2009) may be subject to strong selection. Gaps in knowledge: how extranuclear reproductive proteins can remain specific to the nucleus in which the allele is encoded. Do the male/female roles during fungal provide an avenue for sexually antagonistic mutations to develop by divergence in male/female nuclear behavior?

3. *Constant tinkering/Red queen model:* with constant sequence diversification caused by selection and drift, the molecular interactions between proteins in the multiallelic MAT systems must be complex and dynamic. Pheromone stimulation of the receptors with seven transmembrane helices appears to involve tertiary structure, and dimerization of HD1 and HD2 proteins involves broadly defined dimerization motifs in the specificity determining regions. Are some alleles more fit than others, and are fitness improvements difficult or easy to achieve through mutation? Variance in compatibility of alleles is likely to be pronounced in fungal mating systems because MAT proteins continue to act after fertilization. This differs from some of the plant SI systems because in that model of incompatibility, proteins in the style must only identify and inhibit fertilization by pollen with the same SI type (Wheeler et al. 2009). Once fertilization is secured, there is no role for the SI system. In contrast, after fertilization in filamentous fungi, the MAT genes function to regulate and maintain a heterokaryotic state, typically through trans-acting dimerization or pheromone-receptor stimulation. Because the proteins must recognize and cooperatively interact with each and every other MAT allele, it could be speculated that not all proteins are equivalent in dimerization or activation ability, and given the variation in numbers of genes per MAT allele, alleles should be expected differ quantitatively in their degree of compatibility/recognition of other alleles (Fig. 19.2). For example at the P/R locus of *Coprinopsis cinerea*, some haplotypes encode

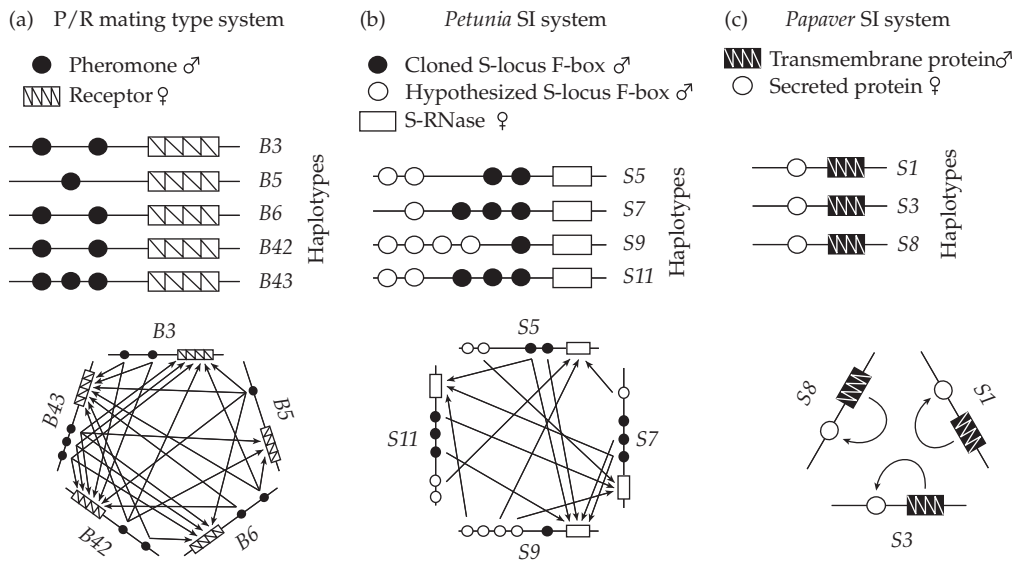

Figure 19.2 The pheromone/ pheromone receptors (P/R) in mushroom fungi use redundancy and versatile binding interactions to ensure all alleles are able to recognize and activate all other alleles (the pheromone response pathway). Positive interactions are shown using single arrows. The P/R system of mushrooms is most similar to that of *Petunia* SI in which each allele has a number of pollen-specific F-box proteins that collaboratively work to deactivate the stylar RNases of all other SI alleles (Kubo et al. 2010). The *Papaver* SI system (Wheeler et al. 2009) shows a very different interaction network, where the pollen-specific transmembrane protein need only recognize the homoallelic stylar protein to terminate pollen tube growth.

multiple pheromones (B43) that can stimulate the same receptor, while others, e.g., B5, encode only a single pheromone (Fig. 19.2). The ability of a single pheromone to stimulate multiple receptors is reminiscent of the *Petunia* SI system in which multiple S-locus F-box pollen proteins are encoded by each allele, and many are capable of deactivating multiple stylar S-RNases (Fig. 19.2). In the constant tinkering model, each allele must continuously evolve at the sites in the protein sequence that interact with partner MAT proteins in order to compensate for mutations in these partners. If the rare advantage model is also incorporated, then the global optimum is always changing because of the constant introduction of new alleles and thus there will be constant need for adaptation.

Gaps in knowledge: Because each allele interacts with a broad spectrum of other alleles (promiscuity) yet must prevent self-compatibility (Figure 19.2), it stands to reason that the fitness landscape is very rugged and exploration limited by mutation. However, mutagenesis screens have essentially never identified new or higher fitness alleles (Raper et al. 1965). Also, the biochemistry of the interactions among mating proteins is only weakly predicted by primary and secondary sequence structure.

4. *Mycoparasites:* the early-diverging fungal lineages comprising zygomycetes have a conserved use of trisporic acid as a pheromone for hyphal homing that has been exploited by related parasites for illicit invasion (Schultze et al. 2005). Diversification of pheromones and receptors and especially *HET* genes in fungi could work to prevent mycoparasites from tricking host cells into invasion as well as diminish interspecies fusions. This argues for an arms race between hosts and parasites that would facilitate rapid evolution of reproductive proteins. Gaps in knowledge: Hormones in zygomycetes are conserved and thus relatively easy for parasites to track. In the Dikarya (Ascomycota + Basidiomycota), pheromones appear to evolve rapidly, but *Candida albicans* was recently demonstrated to respond to a broad range of interspecific mating pheromones (Alby and Bennett 2011). Examining species-specific mycoparasites and their host reproductive proteins for coevolutionary patterns could test this hypothesis.
5. *Population size fluctuation model*: Given that the equilibrium number of MAT alleles is solely a function of census population size and mutation rate (Wright 1939), dramatic changes in effective population size are likely to cause departures from equilibrium that will increase the effectiveness of selection by rare advantage. For example, if a population undergoes a severe bottleneck and concomitant loss of mating type alleles followed by a recovery of population size, the number of alleles will be far below the equilibrium number and selection favoring novel alleles arising from mutation will increase. Recurrent episodes of population size change will thus speed the evolution of amino acid substitutions in specificity-determining regions of MAT alleles.

   Gaps in knowledge: Fungal demography is a large unknown, and estimates of effective population size over time have never been obtained for any species. Evidence for recent range expansions exist (Kauserud et al. 2007), but whether recurrent changes in effective population size in fungi should be expected are unknown because the spatial definitions of populations are also largely unknown (James et al. 1999). In species like *Serpula lacrymans* that have undergone a recent range expansion, it would be interesting to determine whether increased rates of nonsynonymous substitutions could be detected in MAT and HET proteins.

19.4 Evidence for rapid evolution of fungal incompatibility genes and systems

It will come as no surprise that fungal incompatibility genes appear to share many of the characteristics of rapidly evolving animal and plant reproductive loci. Three aspects of rapid evolution are considered here and shown to apply to fungal incompatibility genes: rapid evolution of mating gene/protein sequences, rapid transitions among mating systems, and rapid changes in *MAT* loci.

19.4.1 Sequence evolution

Multiallelic MAT loci have been shown to evolve faster than biallelic loci based on DNA cross-hybridization of MAT genes within and among species (Specht et al. 1994; Metzenberg and Randall 1995). Sequence variation at the multiallelic loci among species was found to be much greater than biallelic loci. In the biallelic Ascomycota system, allele diversification within a species is a moot point because the two mating types alleles are 'idiomorphs,' meaning the two mating-type alleles encode entirely different gene products. Nonetheless, *MAT* genes in Ascomycota appear to evolve more quickly relative to other 'neutral' genes used to study species-level phylogeny (Barve et al. 2003).

Most of the evidence for rapid evolution and selection comes from the multiallelic MAT loci of basidiomycetes which supports the rare advantage model. Sequencing of *MAT* alleles from three model species (the split gill mushroom *Schizophyllum commune*, the inky cap mushroom *Coprinopsis cinerea*, and the corn smut *Ustilago maydis*) have revealed that alleles are invariably characterized by hyperdiverse amino acid sequences, with pairwise identities of 37–78% among alleles (Schulz et al. 1990; Stankis et al. 1992; Halsall et al. 2000). Heightened polymorphism in these systems appears to results from both positive selection and balancing selection. However, the evidence for positive selection on mating type genes has been difficult to obtain because alleles appear to be so ancient that silent mutations are saturated and insertion/deletions have made protein alignments challenging (Badrane and May 1999). Moreover, identifying and isolating these proteins from non-model organisms has remained a challenge due to their large sequence diversity that complicates isolation by PCR (polymerase chain reaction). Standard approaches for detecting positive selection include identifying proteins for which the ratio of nonsynonymous substitution rate to synonymous substitution rate (ω) is significantly greater than 1. However, this type of data is typically unrealistic in molecular evolution, because most proteins, especially incompatibility proteins, will have both conserved domains and domains that actually impart specificity, for example the dimerization (specificity) and transactivation (conserved) domains in homeodomain proteins. More sensitive tests that allow codons to behave independently within a coding sequence allow discrimination between various evolutionary models incorporating neutrality or selection (Yang et al. 2000).

As a first pass to test for non-neutral evolution in basidiomycete MAT proteins, I applied the codon models in PAML to test selection versus neutral models for homeodomain protein alignments of *Coprinellus disseminatus*, *Coprinopsis cinerea*, and *Ustilago maydis* (Table 19.1). While the average ω is considerably less than 1 for all of the proteins, likelihood ratio tests supported codon-based models that incorporated positive selection over neutral models. An additional finding from these explorations is that the number positively selected codons differed between the HD1 and HD2 genes in both *C. disseminatus* and *U. maydis*, consistent with divergent roles between the two protein types (Spit et al. 1998). Nonetheless, the data show a strong indication that for all of the species investigated, positive selection on selected codons is more likely than neutral or purifying selection only models.

Investigations of multiallelic pheromone receptors are likely to be fruitful, as these proteins are typically alignable over the seven transmembrane regions at the N-terminus even across all of Basidiomycota. Moreover, evidence that these genes may undergo positive selection has been obtained for heterothallic members of the genus *Neurospora* (Karlsson et al. 2008). Here, the genes for pheromone receptors are not mating type genes, but instead genes involved in mating and have a much lower polymorphism than in the multiallelic system. The multiallelic systems that govern some *HET* loci in fungi also show hyper-polymorphism and evidence for positive selection (Table 19.1). Both pheromones and *HET* loci are intimately involved in controlling interactions preventing illicit fusions and would be predicted to be under greater selection in more intensely competitive, highly species-rich niches such as soil.

19.4.2 Mating systems and loci

One of the most frequent transitions in the evolution of fungal mating systems has been the origin

Table 19.1 Elevated polymorphism in basidiomycete homeodomain *MAT* genes and *HET* genes from Sordariaceae and tests for positive selection using PAML (Yang et al. 2000). Model M1a is a nearly neutral model, and M2a is a variant of M1a that allows for sites with $\omega > 1$. M7 models variation in using a beta distribution with ω in the range of 0–1. M8 allows for an additional class of codons with $\omega > 1$. # selected codons with posterior probabilities > 0.5 (>0.95 in parentheses) using the empirical Bayes method. HD2 motif proteins are shown in bold.

Species	N	n	π	ω (d_n/d_s)	*P* M1a vs. M2a	*P* M7 vs. M8	# selected codons	Reference
Coprinellus disseminatus CDA1	9	1860	0.366	0.349	< 0.0001	< 0.0001	36 (4)	James et al. (2006)
Coprinellus disseminatus **CDA2**	9	1524	0.213	0.400	< 0.0001	< 0.0001	86 (23)	James et al. (2006)
Coprinopsis cinerea b1	17	1941	0.271	0.301	> 0.5	< 0.0001	18 (0)	Badrane and May (1999)
Ustilago maydis **bW**	23	966	0.133	0.547	< 0.0001	< 0.0001	106 (52)	Gillissen et al. (1992) + GenBank
Ustilago maydis bE	18	648	0.154	0.544	< 0.001	< 0.0001	6 (4)	Gillissen et al. (1992) + GenBank
Sordariaceae *het-c*	39	375	0.112	0.325	< 0.001	< 0.001	11 (3)	Wu et al. (1998)

of homothallic (non-outcorssing) mating systems from heterothallic (outcrossing) ones (Lin and Heitman 2007). There is no clear-cut evidence that the reverse transition has occurred. Our understanding of mating type gene function provides a reasonable explanation for this pattern. Early efforts to produce novel mating alleles by mutagenesis failed to create new alleles, but instead recovered a large number of self-compatible alleles that activated the mating response pathway (Raper et al. 1965). Thus, the MAT genes can become self-activating through simple changes to their molecular structure whereas the evolution of novel specificities requires multiple substitutions. Furthermore, self-compatible combinations can be readily created by recombination within the MAT locus (which normally is suppressed). Merely bringing the two-component system that is normally provided separately by two mating types together into a single genome has led to the evolution of homothallic species repeatedly in the Ascomycota (Yun et al. 1999; Nygren et al. 2011).

Within Basidiomycota, transitions from tetrapolar to bipolar have occurred numerous times (Hibbett and Donoghue 2001). These transitions appear to arise from either physical linkage of the two mating type loci or the inactivation of one of the two loci, possibly by formation of a self-compatible allele. In each of many tetrapolar to bipolar transitions investigated in Agaricomycetes, it has been the P/R mating type locus which has become self-compatible (James et al. 2011). Similar to the absence of evidence for reversal from heterothallic to homothallic, no reversals from bipolar to tetrapolar have been documented. Altogether the data on mating system transitions in fungi provide a genetic explanation for how rapid evolution of mating systems could proceed, because only a single genomic change is required, as well as an explanation for why the process is irreversible, the incompatibility mechanism is fractured in a manner that is difficult to repair.

Variation in the number of genes present within MAT haplotypes of the multiallelic mushroom species is common, and two exemplary studies are provided. These examples involve changes in gene number and organization but not type of genes involved in mating determination, which are much more static. Dynamic numbers of homeodomain genes are known from various *A* mating types of *Coprinopsis cinerea* (Kües et al. 2011). The number of complete gene copies per haplotype varies from four to seven, and among these genes are included both non-functional and non-expressed HD genes (Kües et al. 1994). Whether these result from degenerative processes or could eventually

provide fodder for the future evolution of allelic diversity at the *A* mating type locus of *C. cinerea* is speculation only. Along similar lines, Fowler et al. (2004) demonstrated that the model of organization at the *Schizophyllum commune* P/R mating type locus was not as cleanly divided into two functionally redundant subloci (α and β) as previously hypothesized. Using transformation assays, individual pheromones were shown to stimulate receptors from both α and β subloci. These data, in combination with genome sequencing and phylogenetic analysis of *C. cinerea* receptors (Riquelme et al. 2005), now suggest that reorganization and recombination through time make division of mating genes into clear paralogous classes impossible.

19.5 Evidence for ancient alleles and mating systems

Also contributing to maintenance of diversity at fungal incompatibility genes are stabilizing forces that prevent allele or gene turnover. Based on what we know about the *MAT* genes in Dikarya, the loci controlling mating incompatibility have not been reinvented numerous times, unlike, for example, the repeated evolution of sex chromosomes in animal lineages such as fishes. Evidence that all Dikarya utilize the same mating response pathway through activation of a G protein-coupled pheromone receptor comes from studies that recapitulate the pheromone signaling system in yeast using P/R genes isolated from mushroom fungi (Brown and Casselton 2001). The *MAT* genes themselves are essentially the same in all filamentous ascomycetes which utilize HMG-motif and α-domain proteins as the two alleles of *MAT*. Likewise, all basidiomycetes have HD transcription factors and often P/R genes as *MAT* genes. These systems are thus ancient, having remained the same for hundreds of millions of years of evolution, each heterothallic species having inherited a set of alleles during speciation.

Genomic evidence provides further evidence of the longevity of *MAT* genes rather than the constant recruitment of new HD and HMG genes to the *MAT* locus from the many non-mating type specific copies of these genes throughout the genome. Specifically, conserved gene order near *MAT* has been detected for ascomycetes (Butler 2007) and the *MAT-A* HD genes of basidiomycetes. For example, the genes *SLA2* and *MIP* are known to be adjacent to MAT in most Pezizomycotina and Agaricomycetes, respectively. The Agaricomycete HD *MAT* genes are typically found on the largest chromosome, whereas the P/R genes are typically found on a smaller chromosome and show no evidence of conserved gene order (Kües et al. 2011). It is possible that the conserved location of *MAT* is related to a reduced recombination rate as larger chromosomes appear to have a lower rate of recombination. Alternatively, the larger chromosomes appear to be enriched for essential genes, and this location may reflect the fact that the HD MAT genes play a critical role in the basidiomycete lifecycle.

How long can independent MAT allele lineages be maintained? For biallelic species, the two lineages will be maintained as long as the system does not collapse to asexuality (a single mating type) and as long as a third mating type allele does not emerge. As mentioned above, the biallelic ascomycetes demonstrate this trans-specific inheritance of the two mating type allele lineages throughout all of the evolution of the Pezizomycotina, but instead of alleles, the two MAT variants are considered 'idiomorphs' because they encode different genes. In the basidiomycetes, MAT loci always encode for alleles instead of idiomorphs, and it has been demonstrated that for the biallelic Pucciniomycotina and Ustilaginomycotina, the same two pheromone receptor lineages have been maintained for over 370 million years of evolution (Devier et al. 2009).

Multiallelic systems found in some Ustilaginomycotina and most Agaricomycotina have high allelic and sequence diversity owing in large part to balancing selection that extends the age of the genealogy of alleles. This extension of the coalescence time relative to neutral expectations has been measured for the HD gene *b*1 in *Coprinopsis cinerea* (May et al. 1999). May et al. derived an estimate of the scaling factor by which the genealogy of the *b*1 gene was extended in time relative to neutral expectations as 27.8, which was larger than estimates for vertebrate MHC. This scaling factor is difficult to relate to geological time, and

evidence for trans-species polymorphism in multiallelic systems would provide more data on the timing of sequence divergence at *MAT* loci relative to speciation events. One example of trans-specific polymorphism was suggested for the pheromone receptor genes of the mushroom genus *Pleurotus* (James et al. 2004). However, the absence of positional or functional information in this study makes inferring homology of the studied proteins difficult. Thus, convincing evidence of trans-specific polymorphism in multiallelic species is lacking and should be tested by using recently diverged species. A comparison among the relatively closely related species with clear homology can be drawn using the smuts *Ustilago maydis*, *U. hordei*, and *Sporisorium reilianum*. Approximate divergence times between the species are 20 million years ago (mya) for *U. maydis*–*S. reilianum* and 60 mya for the divergence of *U. hordei* from the other two species based on back of the envelope calculations using 18S rRNA sequence data (Berbee and Taylor 1993). Reciprocal monophyly is observed for each region of the HD2 gene *bW* analyzed separately though diversity is much greater in the specificity-determining region based on branch lengths (Fig. 19.3). One exception is the specificity determining region of the bipolar smut *U. hordei*, which is additionally unusual in that its mating system has been reduced to a biallelic system. Clearly, the alleles in multiallelic species are turning over faster than 20 mya, but the timescale investigated is very limited. Sister species and species complexes need to be investigated.

How frequently new genes are recruited to become *HET* loci is largely unknown as most molecular information concerns a single order of filamentous ascomycetes, Sordariales. However, investigation of the draft genomes of aspergilli (Eurotiomycetes) identified the presence of most of the characterized *HET* genes from *Neurospora crassa* and *Podospora anserina* (Pál et al. 2007). Moreover, within the family Sordariaceae, the *het-c* locus shows clear evidence of ancient trans-specific polymorphism, with three *het-c* lineages found in most of the species studied (Wu et al. 1998). These data show that the heterokaryon incompatibility systems, like *MAT* genes are not merely allelic incompatibilities that arise commonly through genetic drift or isolation but are complex systems that have been long maintained.

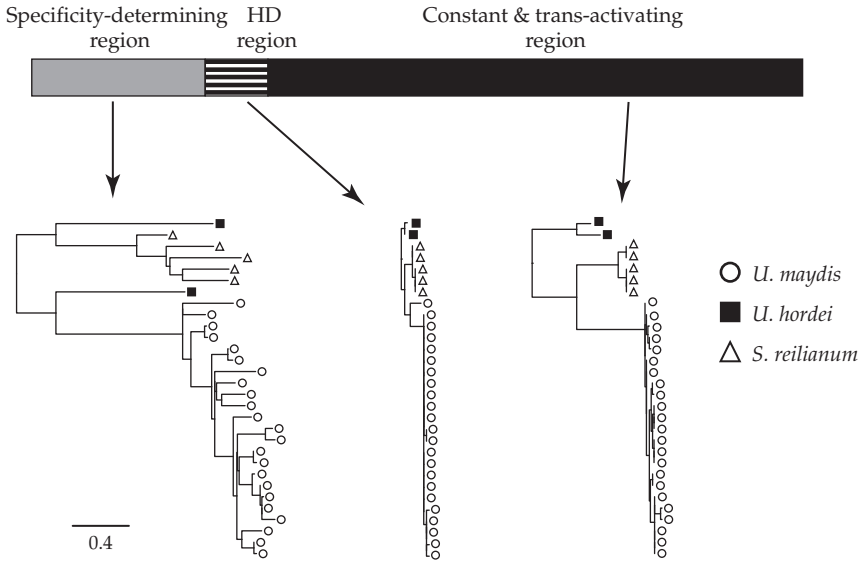

Figure 19.3 The HD2 protein bW of Ustilaginaceae shows variable and constant domains. Phylogenies were constructed for three separate regions of the protein and shown are the phylograms with branch lengths at a constant scale. The specificity-determining region displays the highest level of polymorphism based on branch lengths, but with the exception of the two *U . hordei* alleles, all other alleles are reciprocally monophyletic between species.

19.6 Conclusions

Because fungi lack motility, their interactions with other fungal conspecific and interspecific cells are defining moments during the lifetime of a mycelium. However, individuality is not imparted upon the mycelium, rather, genotypes are more dynamic than other multicellular organisms because nuclear interactions and movements can vary across the mycelium. Critical for these interactions is the pheromone signaling pathway that has been conserved throughout Dikarya. Evidence is presented here that the genes involved in incompatibility in fungi experience rapid evolution and heightened polymorphism due to the effects of selection. Mating-type genes are subject to simple balancing and (mostly) symmetrical selection making them subject to many of the phenomena witnessed in similar systems involved in mate and tissue recognition such as MHC and plant SI. Both *HET* and *MAT* genes are also shown to undergo positive selection at specific codons. Like the animal and plant systems, the forces responsible for positive selection are not entirely clear. Another major unanswered question in the evolution of the multiallelic mating type system is how novel mating type alleles are generated. How many changes are required and are recombination events a source of new alleles? In summary, the multiallelic incompatibility loci are marvels of evolution that are complicated, yet tractable, systems whose study could shed great insight into principals of receptor–ligand signaling, sexual selection, and perhaps even reproductive isolation and speciation.

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