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REVIEW

Mate-recognition and species boundaries in the ascomycetes

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Abstract Reproductive gene evolution is commonly invoked as a source of reproductive isolation during speciation. This possibility has not been adequately explored in the Ascomycota, the most species-rich fungal phylum. The mechanisms of mate-recognition in this group are relatively simple: a "mating type" locus determines reproductive mode and sexual compatibility, and two pheromone/receptor pairs control sexual attraction. However, ascomycete reproductive genes can experience unique and interesting evolutionary forces, which could lead to rapid divergence. In this review, we examine the mechanisms of sexual interaction in ascomycetes and explore current evidence as to whether these mechanisms allow for species-specificity in mate-recognition. We discuss the evolutionary forces that can drive reproductive gene divergence, how these may apply in the world of ascomycetes, and their possible consequences for speciation.

Keywords Ascomycota · Speciation · Mating type · Pheromone · Receptor

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Introduction

Genes involved in mate-recognition are known to evolve rapidly in many lineages (Swanson and Vacquier 2002; Clark et al. 2006), and the ascomycete fungi are no exception (Pöggeler and Kück 2001; Turgeon 1998; Wik et al. 2008; Martin et al. 2011a, b; Seibel et al. 2012). This trend is of special interest in evolutionary biology as the divergence of mate-recognition mechanisms could be directly related to the development of sexual isolation and speciation. Speciation as a result of sexual isolation may be particularly relevant in micro-organisms such as fungi, where large ranges and nearly ubiquitous dispersal (Brown and Hovmøller 2002) make geographical isolation (allopatry) less common.

Although the phylum Ascomycota is defined by an aspect of sexual morphology (the production of "ascospores"), most ascomycete species reproduce far more frequently (and probably more effectively) via asexual (clonal) means. Indeed, many have only ever been been observed in the asexual state (anamorph), and never in the sexual state (teleomorph). This fact complicated taxonomy and species recognition until molecular methods were introduced (Taylor and Jacobson 1999), showing that sexual and asexual (or "perfect" and "imperfect") species are polyphyletic and can both occur within the same genus (e.g. Berbee and Taylor 1992; Lobuglio et al. 1993). Another complication is that the concept of species arguably becomes blurred in completely asexual organisms, as the exchange of genetic material between members of a species is considered to be important to maintain species cohesiveness (Coyne and Orr 2004). Surprisingly, numerous studies have revealed functional reproductive genes and patterns of recombination consistent with sexuality in species previously designated asexual (Dyer and O'Gorman 2011). This suggests that many ascomycetes may have cryptic sexual or parasexual cycles that are yet to be observed, and that truly asexual species may be rare.

Biological species recognition, which defines species based on sexual compatibility (Taylor et al. 2000), has been limited to genera for which sexual reproduction can be stimulated in a laboratory setting, such as *Neurospora* (Turner et al. 2001) and *Fusarium* (Kuhlman 1982). Phylogenetic analyses have revealed impressive concordance between biological and phylogenetic species (Menkis et al. 2010; O'Donnell et al. 1998; Kvas et al. 2009). While reproductive incompatibility can easily evolve as a consequence of speciation, these patterns, taken together with the rapid rate of reproductive gene evolution, highlight the possibility that divergence in mate-recognition mechanisms may be an important contributor to speciation in ascomycetes, particularly when speciation has occurred in sympatry.

The importance of behavioural (interaction between mates) and gametic (interaction between gametes) isolating mechanisms in animal speciation is well recognised, as are the evolutionary forces that can give rise to such barriers (Coyne and Orr 2004). In the Ascomyceta, the primary phase of the life cycle is haploid, so "mate" recognition is indistinct from gamete recognition, and the molecular mechanisms that govern this interaction are comparatively simple. However, only a few studies have begun to explore the potential for species-specificity in ascomycete mate-recognition (Turgeon et al. 1995; Karlsson et al. 2008; Martin et al. 2011a; Seike et al. 2012). This review serves to interrogate current knowledge of the molecular mechanisms of ascomycete mate-recognition, with a view as to whether their evolutionary modification could be associated with the formation of reproductive boundaries between species. We focus predominantly on the potential development of pre-zygotic barriers and less on post zygotic isolation via genetic and genomic incompatibilities, which has been addressed elsewhere (Kohn 2005; Giraud et al. 2008; Louis 2011; Giraud and Gourbière 2012). Lastly, the types of evolutionary forces that could drive the divergence of mate-recognition mechanisms in this phylum are discussed.

Mating in ascomycetes

Mating begins with a pheromone-based interaction between compatible haploid cells (Stage 1 in Fig 1). In hemiascomycete yeasts, projections called "schmoos" grow from each cell to initiate fertilization. In filamentous species, mating is more complex, and usually involves distinct male and female elements. The male (donor) element, or antheridium, can be either a conidium or a hypha. The female (acceptor) element is a coiled lateral hypha known as an ascogonium. In some species, such as *Neurospora crassa*, the ascogonium produces a receptive hypha called the trichogyne, which actively grows toward the male element and initiates fertilization (Bistis 1981, 1983).

Cellular fusion (plasmogamy), is followed immediately by nuclear fusion (karyogamy) in yeasts, but karyogamy is delayed in filamentous species (stage 2 in Fig. 1). Instead the two nuclei proliferate through synchronized mitotic divisions, giving rise to multinucleate cells within a developing fruiting body (ascocarp) (Coppin et al. 1997; Pöggeler et al. 2006). Nuclei from each parent then pair up and a single cognate pair is packaged into an ascus mother cell where karyogamy finally occurs.

Yeast and filamentous species also differ in the stability of the zygote (stage 3 in Fig. 1). In yeasts, the diploid cell type is stable and can reproduce by mitosis, although it must undergo meiosis to produce haploid ascospores (stage 4 in Fig. 1) before mating can again commence. In filamentous species, the zygote is the only diploid stage in the life cycle, and it immediately undergoes meiosis to produce ascospores (Pöggeler et al. 2006). As a result of the proliferation of nuclei prior to karyogamy in filamentous species, thousands of ascospores are produced by a single mating event, all housed within an ascocarp.

The MAT locus

The MAT locus is the distal controller of sexual compatibility

Ascomycetes are either self-fertile (homothallic) or self-sterile (heterothallic). In heterothallic species, only individuals of distinct "mating type" can mate. Most heterothallic ascomycetes have two mating types (MAT-1 & MAT-2 or MATa & MAT α ect.) which are determined by the identity of a single genomic locus. Mating occurs only between haploid individuals dissimilar at the MAT locus. The two alternative sequences at the MAT locus share no obvious homology and sometimes contain more than one gene. They are therefore referred to as "idiomorphs" rather than alleles (Metzenberg and Glass 1990). The borders of the MAT idiomorphs are defined by the points at which homology is restored between the genomes. The dissimilarity between the two idiomorphs is maintained by the suppression of recombination in this region (Coppin et al. 1997; Kronstad and Staben 1997).

The genes in the MAT locus are believed to encode transcription factors. They function in general sexual development, but are most fundamentally responsible for determining "nuclear identity" (i.e. whether a cell/nucleus is MAT-1 or MAT-2) by directing expression of mating-type-specific genes such as pheromones and receptors (reviewed by Lee et al. 2010). There is great variability in the composition of the MAT locus among species. Furthermore, MAT proteins

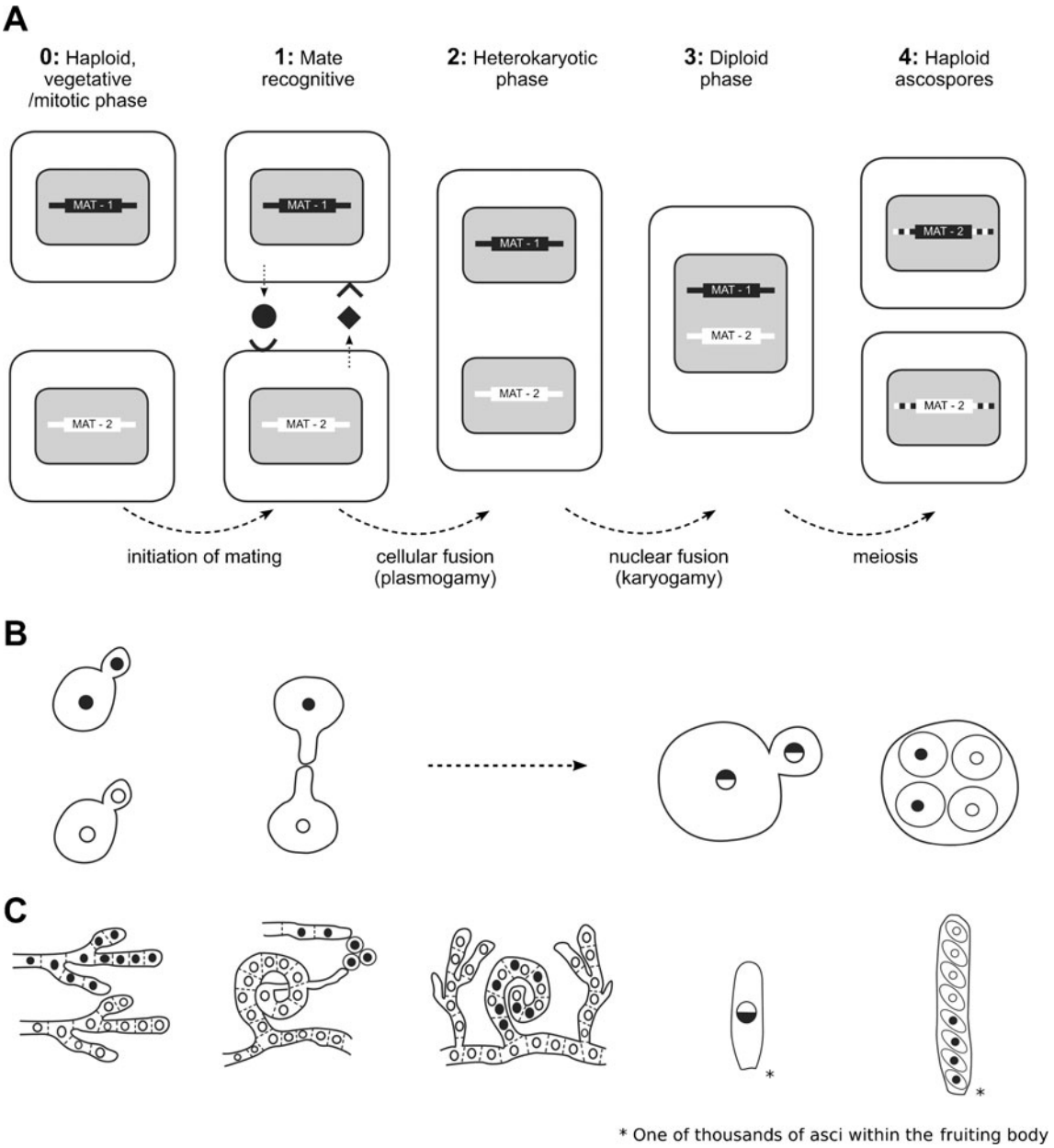

Fig. 1 Stages of Mating in Heterothallic Ascomycetes. **a** shows a diagramatic representation of the major stages of mating, indicating the MAT idiomorphs. **b** and **c** show the general differences between yeasts (**b**) and filamentous taxa (**c**) (diagram modelled on figure from Coppin et al. 1997). The two different mating types are represented by open and filled nuclei. The predominant phase of the life cycle is haploid (*stage 0*). Mating starts with pheromone-based recognition between cells of opposite mating type (*stage 1*). Cellular fusion occurs to produce a heterokaryotic hypha in filamentous species (*stage 2*), but this stage is skipped in yeasts, which proceed directly to nuclear fusion, yielding a diploid zygote (*stage 3*). The diploid stage is stable and can reproduce asexually in yeasts. In filamentous taxa meiosis occurs immediately to yield haploid ascospores (*stage 4*). The ascospores have recombinant genomes, but recombination is supressed in the MAT locus

are highly variable and evolve rapidly (Turgeon 1998; Wik et al. 2008; Martin et al. 2011b), which originally made them notoriously difficult to identify (Cisar et al. 1994).

In homothallic species, any two individuals/cells are theoretically sexually compatible, including hyphae/spores derived from the same clone—making homothallic species self-fertile. Homothallic filamentous species usually carry genes for both mating types in a single genome, often, but not always, juxtaposed (Cisar et al. 1994; Beatty et al. 1994; Pöggeler et al. 1997; Yun et al. 1999; Yun et al. 2000; Galagan et al. 2005; Paoletti et al. 2007; Amselem et al. 2011). In some cases, it is apparent that homothallism has arisen via the merging of the two MAT loci in a heterothallic ancestor, most likely via an unequal crossover event (Yun et

al. 1999; O'Donnell et al. 2004). It was proposed by Coppin et al. (1997) that sexual reproduction in homothallic species may rely on alternate expression of genes for one or the other mating type in different cells. Another route to self-fertility in filamentous species is via "pseudohomothallism", whereby ascospores are heterokaryotic, carrying two haploid nuclei of different mating type. Such ascospores can, therefore, give rise to hyphae and conidia of opposite mating types, thus displaying apparent self-fertility (Kronstad and Staben 1997). In some yeast species, clonal cells can become sexually compatible by switching their mating type (Herskowitz 1989). These species harbour both MAT idiomorphs at silent loci in the genome, and switch mating type by swapping out the DNA at the active idiomorph with one of the silent loci via non-homologous recombination. These independent routes to self-fertility all have in common some alteration of the mating type of the cell.

In a few cases, recombination is apparently achieved without the presence of both sets of MAT genes. In some homothallic *Neurospora* species, genes for one of the mating types are either absent or have accumulated deleterious mutations, yet self-fertility persists (Glass et al. 1990; Glass and Smith 1994; Wik et al. 2008). The pathogenic yeast *Cryptocuccus Neoformans* has two compatible mating types (a and α), but α cells are also capable of "same-sex" mating (reviewed by Heitman 2010). Finally, parasexual cycles (recombination in the absence of meiosis) has been observed in the yeast *Candida albicans* (Bennett and Johnson 2003) as well as the filamentous *Aspergillus nidulans* (Schoustra et al. 2007). The molecular control of these unusual processes is not well understood, and whether mating type genes are at all relevant is unclear.

Overall, the multiple forms of self-fertility in the Ascomycota suggests that it may have strong selective advantages, at least in certain conditions. Phylogenetic evidence indicates that transitions from one reproductive mode to another have occurred in both directions, and can be frequent on the evolutionary time-scale (Lee et al. 2010; Nygren et al. 2011). This raises the question as to the potential consequences of such transitions for species evolution.

MAT loci and reproductive barriers

Despite their rapid rate of evolution, it is unlikely that the MAT genes, which encode transcription factors, could directly affect mate-recognition between species. A more likely means by which MAT genes could be involved in speciation is indirectly, by orchestrating differential reproductive behaviour. Many ascomycetes require unique and specific conditions before the sexual cycle is initiated (Pöggeler et al. 2006). Temporal differences in reproduction can also reduce the potential for hybridization. For example, differential rates of reproduction may be an important factor preventing hybridization between *Saccharomyces cerevisiae* and *Saccharomyces paradoxis* (Murphy et al. 2006) and in *N. crassa*, circadian rhythms affect the expression of both pheromone precursor genes (Bobrowicz et al. 2002). Quantitative differences in pheromone regulation have also been observed between successful and unsuccessful hybridizations in *Neurospora*, and were proposed as a possible cause of inter-sterility between certain species (Karlsson et al. 2008), although it is unclear precisely how quantitative differences in pheromone production could cause inter-sterility.

Indirect evidence for the importance of MAT loci in speciation can be found in the fact that mating type gene trees sometimes reflect the species phylogeny (Turgeon 1998; O'Donnell et al. 2004). This is not always the case however. There is phylogenetic evidence of introgression of MAT genes between non-sister species in *Neurospora* (Strandberg et al. 2010), and *Fusarium* (Martin et al. 2011b). In *Ophiostoma*, adaptive introgression of the MAT locus between species has occurred at least once, restoring sexuality to a single-mating-type population (Paoletti et al. 2006). Even more convincing is the fact that the equivalent processes has been performed artificially, by translocating MAT genes between species or even between genera (Arnaise et al. 1993; Turgeon et al. 1995; Pöggeler et al. 1997; Yun et al. 1999; Pöggeler et al. 2008; Lu et al. 2011). These observations show that MAT genes can sometimes function in highly divergent genetic backgrounds, suggesting that the functional domains of these proteins are highly conserved, and that the inter-specific differences in MAT protein sequences may be of little functional consequence.

Reproductive modes and speciation

It has been suggested that a transition in reproductive mode, say, from heterothallism to homothallism, is in itself a form of speciation, as the resulting populations differ with regard to their reproductive system (Idnurm 2011). A potentially more interesting question is whether such a transition could result in genetic divergence between populations, by directly or indirectly causing reproductive isolation. Giraud et al. (2008) proposed that high levels of selfing, and the consequent reduction in out-crossing, can reduce gene flow between two species. Coyne and Orr (2004) made the point that a conversion to self-fertility or asexuality reduces gene flow between individuals of the same population just as much as between two different populations. In the extreme circumstance, a completely selfing or asexual species, each individual will give rise to a genetically isolated lineage or "microspecies". Thus, a conversion to self-fertility or asexuality in itself should perhaps not be considered a true isolating mechanism. However, Gibson et al. (2011) proposed that partial selfing, where a single mating event can give rise to both "selfed" and out-crossed (or hybrid)

progeny, might reduce inter-specific gene flow, provided that hybrids are less fit. This is because partial selfing can promote sibling-competition, potentially leading to the systematic eradication of the weaker hybrids, thereby reducing gene-flow between species more than within species (Gibson et al. 2011). It is yet to be seen whether this process may occur in any ascomycetes. Nevertheless, it is probably only important in strengthening existing reproductive barriers, rather than generating new ones, because it hinges on reduced hybrid fitness. Finally, there may be secondary consequences of a transition to a selfing lifestyle that might contribute to species divergence. For example, the reduction in effective population size brought on by selfing can result in increased genetic drift (Coyne and Orr 2004; Whittle et al. 2011), particularly at sex-related genes, some of which may become redundant in homothallic species (as discussed below).

Mating pheromones and receptors

Pheromones and receptors mediate proximal sexual attraction

If there is any deliberate mate "preference" for conspecifics in ascomycetes, it is most likely determined by the pheromone/receptor system. Studies in *S. cerevisiae* have formed the basis of our understanding of ascomycete pheromones (Kurjan 1993; Duntze et al. 1991; Caldwell et al. 1995; Chen et al. 1997). The two mating types (MAT a and MAT α in this case) each produce a specific pheromone (a-factor and α-factor), as well as a membrane-bound receptor that is receptive to the pheromone produced by the opposite mating type. Both pheromones consist of short peptides, cleaved from larger precursor proteins (see Jones and Bennett 2011 for a comprehensive review). The a-factor precursor is a typical fungal pheromone precursor with a C-terminal "CaaX" motif, the signal for the addition of farnesyl and carboxymethyl groups to the mature peptide (Brake et al. 1985). The α factor precursor is unique to the Ascomycota, and contains multiple repeats of the mature peptide, each bordered by cleavage sites (Singh et al. 1983). It is thought that all of the peptide repeats are cleaved and released by the cell (Caplan et al. 1991). The cognate receptors for the two pheromone classes are both G-protein coupled receptors with seven trans-membrane domains (Nakayama et al. 1985). The pheromone and receptor genes are unlinked from the MAT locus (unlike in the Basidiomycota). Hence, individuals of both mating types carry both sets of pheromone and receptor genes, yet their expression is mating-type-dependent. A number of elegant studies involving genetic manipulation of *S. cerevisiae* have shown that expression of compatible pheromones and receptors is necesary and sufficient to induce mating, regardless of the underlying mating types (Bender and Sprague 1989; Gonçalves-Sá and Murray 2011). This proves that pheromones and receptors are the principal determinants of mating specificity.

In filamentous species of the class Sordariomycetes, both classes of pheromone precursor gene (referred to here as "a-class" and "α-class" for simplicity) have been identified (see Martin et al. 2011a for references). In other filamentous ascomycetes, only the α-class gene has been identified, yet the a-class gene may evade detection due to small size and a rapid rate of divergence. In heterothallic species, production of mating pheromones is essential for male fertility (Kim et al. 2002; Turina et al. 2003; Coppin et al. 2005). Kim and Borkovich (2006) showed that male spores need to release pheromones in order to attract growing (female) trichogynes for fertilization. Furthermore, mating pheromone expression is potentially unnecessary for female fertility (Coppin et al. 2005; Kim and Borkovich 2006), as the female hyphae need only be receptive to the pheromones released by the male spores. This is consistent with the observation that pheromone expression in *Podospora anserina* is almost entirely limited to microconidia (Coppin et al. 2005). Pheromone receptor mutants are female sterile, probably due to a failure to sense the presence of pheromone-secreting spores, but possibly also because receptors are required for post-mating sexual development (Kim and Borkovich 2004; Seibel et al. 2012; Kim et al. 2012).

The strict laws of the pheromone receptor system appear to break down in homothallic species. *Sordaria macrospora* expresses both pheromones and receptors (Pöggeler 2000; Pöggeler and Kück 2001; Mayrhofer et al. 2006), which is perhaps not surprising as each nucleus carries the genes for both mating types. Nevertheless, a limited level of self-fertilization is possible even when both pheromone genes are knocked out, although at least one of the two receptors must be expressed (Mayrhofer et al. 2006). This suggests that the mating signal transduction cascade is initiated in the absence of pheromone-receptor interaction. In *Fusariam graminearum*, cells appear to utilize only the α-factor-like pheromone, while the a-factor-like pheromone encoded in the genome of this fungus appears to be obsolete (Lee et al. 2008; Kim et al. 2008). Interestingly, *F. graminearum* mutants lacking both pheromone and receptor genes are self-fertile (Lee et al. 2008; Kim et al. 2008). Kim et al. (2008) proposed that contact between haploid structures (for example, mycelia and conidia) may be sufficient to initiate the mating signal transduction cascade in *F. graminearum*.

Pheromone evolution and species boundaries

Analysis of pheromone peptide variation among 69 ascomycete species revealed that pheromones are sometimes highly divergent between species, yet they are strongly

conserved in certain lineages (Martin et al. 2011a). As the primary (and possibly only) determinants of sexual attraction, the relevance of pheromone peptide evolution for speciation is an important consideration. While examples of reproductive isolation due to shifts in pheromone signals are well known in insects (reviewed by Nosil et al. 2007; Smadja and Butlin 2009), these are usually chemical compounds quite different from the peptide pheromones of fungi.

Research into the functional relevance of inter-specific differences in ascomycete pheromones has been limited to only a few species. Early work on yeasts demonstrated a correlation between hybridization ability and pheromone peptide similarity (Burke et al. 1980). However, this pattern would also be expected if pheromone divergence was merely a by-product of reproductive isolation rather than the cause. Other studies have shown that the a-factor pheromones of three S*accharomyces* species have some interspecific activity, despite their distinct amino acid sequences (McCullough and Herskowitz 1979; Hisatomi et al. 1988). In-depth studies of the *S. cerevisiae* α-factor peptide showed that the N-terminal residues control binding to the receptor, while certain C-terminal residues, although unnecessary for receptor binding, are required to stimulate the receptor, initiating the mating response (see Naider and Becker 2004 for a detailed synthesis). Similarly, comprehensive mutagenesis of the *Schizosacharomyces pombe* M-factor (homologous to α-factor), revealed that only a few C-terminal residues are necessary for mate-recognition (Seike et al. 2012). It is therefore likely that only certain inter-specific differences would disrupt mate recognition, while others may have no effect. Further study is needed to determine how widely these findings are applicable.

The fact that multiple tandem copies of the α-factor-like pheromone in certain ascomycetes are completely (or nearly) identical offers insight into the significance of peptide sequence variation for functionality. Tandemly-repeated sequences are sometimes homogenised by concerted evolution (via gene-conversion or non-homologous recombination) (Nei and Rooney 2005). This is not the case in the α-class precursor, where different copies of the mature pheromone can be highly divergent at synonymous sites and only conserved at non-synonymous sites. This pattern implies that functional constraint—rather than concerted evolution—acts to maintain a specific pheromone peptide (Martin et al. 2011a). Selection acting to conserve the peptide sequence could indicate that the interaction between pheromone and receptor may be quite sequence-specific. However, there is accumulating evidence that pheromones might have additional functions outside of mating that could explain the additional constraint. These include "conglutination", the cementing of hyphae to form resilient sclerotial structures in filamentous species (Kim et al. 2002) and biofilm formation in yeasts (Daniels et al. 2006; Alby et al. 2010; Sahni et al. 2010). The specificity of the pheromone-receptor interaction during mating therefore requires more direct testing.

One major hurdle to the theory of reproductive isolation via divergence in mating cues is the fact that selection should act strongly against mating cue divergence, unless it is accompanied by a corresponding co-evolutionary alteration of the mate preference. This may be less relevant in organisms capable of asexual reproduction, where the cost associated with reduced sexual compatibility may be minimal. However, ascomycetes may possess a more ingenious solution to this problem. The modular organisation of the α-class precursor makes it possible to harbour multiple variants of the mature pheromone. These repeats appear to evolve through a process of gradual succession via duplications and losses (birth-and-death evolution, Nei and Rooney 2005) (Martin et al. 2011a). This arrangement could therefore facilitate gradual pheromone-receptor co-evolution. This would be restricted to the α-class pheromone, although in at least one ascomycete lineage the a-class precursor gene appears to have evolved a similar modular repeat structure (Schmoll et al. 2010; Martin et al. 2011a). The presence of multiple copies of the precursor genes, which is common in ascomycetes, would also allow such flexibilty (Seike et al. 2012).

A number of ascomycetes harbour two or more variants of the mature pheromone peptide, differing at one or two residues, within the α-class precursor. Such species include *S. cerevisiae* (Singh et al. 1983), *Aspergillus nidulans* (Dyer et al. 2003) and various *Fusarium* spp. (Kim et al. 2008; Lee et al. 2008; Martin et al. 2011a). These cases may represent transitions from one pheromone peptide to another that are mid-way: if one of these variants confers higher fitness, it is likely that the fitter variant will eventually replace the other entirely. Even in the absence of differential selection, birth-and-death evolution lead to a loss of one variant by chance. This is one possible explanation for the rapid rate of pheromone divergence observed in some lineages. However, multiple distinct species within the same genus often harbour the same sets of variants (Martin et al. 2011a), suggesting that they may persist for many millions of years. This highlights the possibility that multiple variants may all function equally well, or that the presence of the distinct variants is necessary for successful mate recognition, perhaps in a specific ratio. The relevance of distinct pheromone variants and the numbers of copies of each will become clearer as larger population samples are studied.

Reproductive genes experience unique evolutionary forces

Numerous explanations have been proposed for the trend of reproductive genes to evolve rapidly (reviewed by Swanson

and Vacquier 2002; Clark et al. 2006). While only one evolutionary force, reinforcement, involves direct selection for enhanced reproductive isolation (Howard 1993), others could have the same effect as a by-product of divergence (Templeton 1989; Palumbi 2008). They are therefore all potentially important for species boundaries. A discussion of some relevant theories, and how they might apply to mate-recognition in ascomycetes follows.

Reinforcement

If two species diverge in allopatry and return to a sympatric distribution where hybrids experience reduced fitness; natural selection might favour alleles that enhance prezygotic isolation between the two species. Hence, reinforcement is thought to cause a pattern of "reproductive character displacement", whereby sympatric populations display stronger sexual isolation than allopatric populations (Howard 1993). Pre-mating barriers might be favoured, to avoid the physical costs associated with mating (Coyne and Orr 2004). Reinforcement has been proposed as a driving force behind the diversification of reproductive proteins (Howard 1993) and signalling molecules such as pheromones (Symonds and Elgar 2008).

Meta-analyses of data from numerous crossing experiments suggest that reproductive character displacement and pre-mating isolation is rare in ascomycetes and more common in Basidiomycetes (Le Gac and Giraud 2008; Giraud and Gourbière 2012). These authors proposed that this distinction could reflect the distinct life styles of the two groups. Generally, basidiomycetes mate in the soil where encounters with hetero-specifics could be frequent, while ascomycetes mate on or within their host, potentially reducing the likelihood of such encounters. Hence, ascomycetes may not experience the same selective pressures to develop species-specificity in mate-recognition. This explanation applies only to species with strong specificity for a unique host. Nevertheless, there is only a single well-documented case of reproductive character displacement in this Phylum. Dettman et al. (2003) reported lower reproductive success in sympatric than allopatric crosses between *N. crassa* and *Neurospora intermedia*, although this incompatibility occurs post-mating.

The fact that pre-mating barriers are not more common in ascomycetes might indicate that the costs associated with occasional attempted hybridizations do not outweigh the cost associated with alteration of the mate-recognition machinery. Turner et al. (2010, 2011) have argued that reinforcement may favour post-mating barriers if selective constraint acting upon pheromone sequences is too great to allow divergence. Indeed three *Neurospora* species as well as *S. macrospora* (from a sister genus), all share identical pheromones, implying strong purifying selection in this clade. Similar limited variability in pheromone peptides was observed among eleven species of the *Gibberella fujikuroi* species complex (Martin et al. 2011a), even though all of these species are reproductively incompatible (presumably due to post-mating incompatibility). As discussed above, purifying selection acting to conserve pheromone peptide sequences may be bolstered if they perform additional cellular functions.

Selection dependent on sex

Fitness costs associated with playing the female role may be an important factor in the evolution of reproductive genes. Females generally have a greater energy investment in the offspring, but it is unclear how many ascomycetes have true male and female "sexes" in terms of reproductive investment. This is probably not the case in yeasts, where mating involves the simple fusion of haploid cells. By contrast, in some filamentous species such as *N. crassa*, the male parent simply donates a haploid nucleus while the female parent must often locate and grow towards this nucleus for fertilization, and then produces a complex ascocarp structure which protects the developing ascospores (Pöggeler et al. 2006). Ascocarp development could significantly limit investment into asexual reproduction as well as subsequent sexual reproduction for the female parent (Turner et al. 2011). Proliferation of the male nucleus within the ascocarp allows an amplification of the male parent's genotype at the direct cost of the female parent. Finally, since spores that are not fertilized function as asexual propagules, there is apparently no cost at all associated with the male role. Hence, sexual reproduction itself may be a trait under sexually antogonistic selection (i.e. sexual conflict) as it increases male fitness and potentially decreases female fitness.

There is some circumstantial evidence of a disproportionate cost to the female parent during mating. For example, abortion of hybrid ascospores between *N. crassa* and *N. intermedia* is initiated by the female parent (Turner et al. 2010), which is consistent with Coyne and Orr's (2004) prediction that reinforcement should be greater in the parent likely to incur the greater cost of reproduction. Even during same-species mating, the costs associated with playing the female role might lead to selection favouring reduced female fertility. Interestingly, female sterility (either partial or complete) has been reported in many populations of ascomycetes (Leslie and Klein 1996). If this hypothetical selective pressure continued indefinitely, a complete loss of female fertility could result, giving rise to an "asexual" species. However, the advantages associated with sexual reproduction may be expected to cause antagonistic selection to retain some level of female fertility.

There is a direct connection between sexual conflict and mate-recognition in filamentous ascomycetes, because

functional pheromone receptors are required for female function but may be dispensable for male function (Turina et al. 2003; Coppin et al. 2005; Kim and Borkovich 2006). It is tempting to speculate that sexual conflict could result in a co-evolutionary chase, with receptors continually evolving to avoid reception by pheromones (to avoid performing the female role). Models have shown that such a process could cause reproductive signals to diversify in arbitrary directions, potentially even causing reproductive isolation between two populations of the same species (Gavrilets 2000; Gavrilets et al. 2001). This phenomenon has been proposed as a possible force driving the rapid evolution of receptors responsible for sperm-binding on the eggs of Abalone (*Haliotis rufescens*), which are thought to be associated with speciation in this genus (Galindo et al. 2003). Elevated divergence in receptor genes has been observed in *Neurospora* (Karlsson et al. 2008). Similar increased polymorphism in one receptor was observed in *Hypocrea jecorina* (Seibel et al. 2012). However, in both cases, the sites implicated are in the intracellular C-terminal domain of the receptor protein, and are more likely to be involved in G-protein signalling and than ligand binding.

While selection may drive reduced female fertility, the benefits of sex for the male parent could also drive selection for increased male "attractiveness". Wik et al. (2008) found that positive selection acting upon mating type genes in *Neurospora* was restricted to heterothallic species. They postulated that this selection may be driven by strong competition among spores to attract trychogynes—a selective force that may be far greater in heterothallic and obligately out-crossing fungi than selfing species. This would be analogous to sperm competition, which occurs commonly in polyandrous animals, and is thought to be an important source of selection on reproductive genes, particularly if coupled with sexual conflict (Clark et al. 2006).

Ecological adaptation

Paterson (1985) proposed that reproductive systems would inevitably diverge between species as they are optimized by natural selection to suit specific habitats or life cycles. An extreme example in fungi might be a transition from heterothallism to homothallism to suit specific environmental conditions. Templeton (1989) elaborated on the idea by suggesting that selection on reproductive systems could act not only to optimize reproduction per se, but also due to other environmental factors, for example to avoid predation. Ascomycetes that reproduce within host tissue may experience strong selective forces imposed upon their reproductive biology by this enviropnment.

All signals that are intended for conspecific individuals are vulnerable to exploitation by unintended recipients (Haynes and Yeargan 1999). Reproductive signals could experience selective pressure to avoid detection by predators and parasites (Symonds and Elgar 2008) or microbial pathogens (Vacquier et al. 1997). In the same way, pheromones of fungi might experience selective pressure to avoid stimulating the specific defence systems of their plant or animal hosts. Ascomycete pathogens are unique in that spores that inoculate host tissue also act as male gametes and, therefore, produce pheromones abundantly. In fact, pheromone precursors are among the most highly expressed genes (Nelson et al. 1997; Kim et al. 2002) and it is therefore, plausible that pheromones could function as effectors of host defence. This, in turn, could result in positive selection for changes in the nature of the pheromone signal, enabling pathogens to avoid detection.

Genetic Drift

Relaxed purifying selection can make genes vulnerable to divergence due to drift. Both selfing and asexuality reduce the effective recombination rate. This is expected to result in lower effective population size (N_e), and thus weaker selective constraint throughout the genome, as well as mutation accumulation via Muller's Ratchet in both homothallic and asexual species (Whittle et al. 2011). Indeed, rates of protein divergence are generally higher among homothallic than heterothallic *Neurospora* spp. (Nygren et al. 2011). Ironically, the "benefit" of outcrossing in heterothallics in this regard is not felt by the MAT locus, because of its lack of recombination and lower effective population size than the rest of the genome (Idnurm 2011). In homothallic genomes, there is presumably no region of suppressed recombination, so selection upon MAT genes could arguably be more efficient, and Muller's Ratchet avoided. Thus, while the genomes of heterothallics may be generally less vulnerable to divergence due to drift and mutational accumulation, the heterothallic MAT locus specifically may be more vulnerable.

The fate of reproductive genes is further complicated by the ability of most fungi to reproduce effectively by asexual means, which can weaken purifying selection on all reproductive genes. The sexual cycle may become dispensable when there is limited advantage to be gained from sexual reproduction. This may be the case in relatively clonal populations or in uniform environments such as crops and plantations. In *Magnaporthe oryzae*, female fertility can be lost rapidly during periods of asexual reproduction (Saleh et al. 2012). Sexuality can also be lost inadvertently during invasion if founder populations of only a single mating type become established (e.g. Paoletti et al. 2006). A possible advantage of homothallism is the ability to maintain sexuality during invasion, even if only a single individual becomes established. Homothallics might thus avoid the decay of reproductive genes through lack of use. However,

there is also contrasting evidence of relaxed selective constraint on reproductive genes in homothallic relative to heterothallic species (e.g. Martin et al. 2011b; Wik et al. 2008). This could be a result of functional redundancy (e.g. pheromones in *F. graminearum* [Lee et al. 2008; Kim et al. 2008]). The effects of reproductive mode on reproductive gene divergence are therefore difficult to predict.

Future directions

The relatively simple mechanism of mate-recognition in ascomycetes makes this an ideal group in which to study the consequences of reproductive system evolution. The hypothesis that divergence of mate recognition genes can result in reproductive isolation leads to several testable predictions. In general, high divergence at mating type or pheromone loci should be correlated with inter-sterility. If changes in reproductive mode can result in speciation, the frequency of these changes should be correlated with species richness. The ability to perform gene knock-outs and complementation in an increasing number of species now makes it possible to take these predictions to the next level, by specifically scrutinizing the effects of different genes on reproductive compatibility. Transformation studies have already shown that alteration of MAT and pheromone genes can alter sexual compatibility (Lee et al. 2003; Lu et al. 2011, Gonçalves-Sá and Murray 2011). A decisive test would be to determine whether two sexual, but inter-sterile species could be made inter-fertile by complementation of reproductive genes (e.g. Turgeon et al. 1995). Future research on the reproduction of ascomycetes will thus provide numerous insights into the mechanisms of speciation, the source of biodiversity.

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