

Review

Speciation in fungi

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Abstract

In this review on fungal speciation, we first contrast the issues of species definition and species criteria and show that by distinguishing the two concepts the approaches to studying the speciation can be clarified. We then review recent developments in the understanding of modes of speciation in fungi. Allopatric speciation raises no theoretical problem and numerous fungal examples exist from nature. We explain the theoretical difficulties raised by sympatric speciation, review the most recent models, and provide some natural examples consistent with speciation in sympatry. We describe the nature of prezygotic and postzygotic reproductive isolation in fungi and examine their evolution as functions of temporal and of the geographical distributions. We then review the theory and evidence for roles of cospeciation, host shifts, hybridization, karyotypic rearrangement, and epigenetic mechanisms in fungal speciation. Finally, we review the available data on the genetics of speciation in fungi and address the issue of speciation in asexual species.

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1. Introduction

Speciation, the splitting of one species into two, is one of the most fundamental problems of biology, being the process by which biodiversity is generated. Understanding how the 1.5 millions of fungal species (Hawksworth, 1991) have arisen is of fundamental interest and has tremendous applied consequences in the cases of agricultural pathogens, emerging human diseases, or fungal species used in industry and biotechnology. Although much progress on the origin of species has been made since the book of Darwin (1859), the subject remains heavily debated.

Fungi are excellent models for the study of eukaryotic speciation in general (Burnett, 2003; Kohn, 2005), although they are still rarely included in general reviews on this sub-

ject (e.g. Coyne and Orr, 2004). First, many fungi can be cultured and crossed under laboratory conditions, and mycologists have long reported numerous mating experiments among fungal species (reviewed in Le Gac and Giraud, in press). Second, fungi display a huge variety of life cycles and geographical distributions, allowing the study of which parameters most significantly influence the speciation processes. Third, numerous species complexes are known in fungi, encompassing multiple recently diverged sibling species (e.g. Dettman et al., 2003a; Fournier et al., 2005; Johnson et al., 2005; Le Gac and Giraud, in press; Le Gac et al., 2007a; Pringle et al., 2005), which allows investigations on the early stages of speciation.

Excellent reviews on the modes of speciation in fungi have already been published (Burnett, 2003; Kohn, 2005; Natvig and May, 1996), and we therefore focus mostly on recent developments. We first address the question of species definitions and species criteria and then review the patterns of speciation in fungi, situating them in the general

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theory as applied to eukaryotes. We focus particularly on the aspects that have not been extensively reviewed previously and/or that have seen recent and significant developments, such as sympatric speciation, cospeciation, hosts shifts, reproductive character displacement, and the time course of speciation. For other aspects, readers should refer to the previous reviews to have more exhaustive discussions and references (Burnett, 2003; Kohn, 2005; Natvig and May, 1996; Olson and Stenlid, 2002; Taylor et al. 2000; Schardl and Craven, 2003).

2. Species definition vs species criteria

To study speciation, it seems necessary to first define species. The continual proposal of new species concepts may lead one to think that there is no general agreement about what species are. To the contrary, it has been argued that all modern biologists agree that species correspond to segments of evolutionary lineages that evolve independently from one another (de Queiroz, 1998). The apparently endless dispute about species concepts stems from the confusion between a species definition (describing the kind of entity that is a species) and species criteria (standard for judging or recognizing whether individuals should be considered members of the same species). Many so-called “species concepts” actually correspond to species criteria, i.e., practical means to recognize and delimit species (De Queiroz, 2007; Hey, 2006; Taylor et al., 2000). The Biological Species Concept (BSC) for instance emphasizes reproductive isolation, the Morphological Species Concept (MSC) emphasizes morphological divergence, the Ecological Species Concept (ESC) emphasizes adaptation to a particular ecological niche, and the Phylogenetic Species Concept (PSC) emphasizes nucleotide divergence. These species criteria correspond to the different events that occur during lineage separation and divergence, rather than to fundamental differences in what is considered to represent a species. One may wonder why there are conflicts over which species criterion we adopt. There are three main reasons why such criteria cannot be universal: (i) speciation is a temporally extended process, but one which varies tremendously in its pace among different types of organisms, (ii) several modes of speciation can occur, during which the phenomena used for species recognition do not necessarily appear in the same chronological order, (iii) characteristics of certain organisms render some criteria difficult to apply.

Let us take as example the most popular yet the most challenged species criterion, the BSC. For proponents of the BSC, the capacity to interbreed delimits the infraspecies level, and “Biological Species” are intersterile groups (Mayr, 1942). This criterion is based on reproductive isolation, but this is only one of the many stages of speciation. Depending on the mode of speciation, intersterility can occur at early or late stages of speciation, and can constitute the critical stage (in sympatric speciation), or it may be only a by-product of genetic divergence (in allopatric speciation). Obviously, the BSC will be most useful in the

first case (sympatric speciation), whereas species criteria based on evidence for lack of gene flow using molecular markers will be more discriminating in the latter case. Intersterility is the stage at which the process has become irreversible, but this stage may take very long to reach. Many well-recognized species of plants and animals are still interfertile.

Until quite recently, the most commonly used species criterion for fungi has been the MSC. However, many cryptic species have been discovered within morphological species, using the BSC (e.g. Anderson and Ullrich, 1978), or the GCPSR (Genealogical Concordance Phylogenetic Species Recognition, Taylor et al., 2000), an extension of the PSC. This latter species criterion uses the phylogenetic concordance of multiple unlinked genes to indicate a lack of genetic exchange and thus evolutionary independence of lineages. Species can thus be identified that cannot be recognized using other species criteria due to the lack of morphological characters or incomplete prezygotic isolation. The GCPSR criterion has proved immensely useful in fungi, because it is more finely discriminating than the other criteria in many cases, or more convenient (e.g. for species that we are not able to cross), and is currently the most widely used within the fungal kingdom (e.g. Dettman et al., 2003a; Fournier et al., 2005; Johnson et al., 2005; Koufopanou et al., 2001; Le Gac and Giraud, in press; Le Gac et al., 2007a; Pringle et al., 2005).

3. Allopatric speciation

How new species arise in nature is still a highly active field of research. It has long been believed that species originate mostly through allopatric divergence (Mayr, 1963), because extrinsic geographic barriers seemed obvious impediments to gene flow. Fungi could appear as exceptions because eukaryotic micro-organisms have long been considered to have global geographic ranges (ubiquitous dispersal hypothesis; Finlay, 2002), at least for those not dependent on a host having a restricted range. This was in particular true for airborne fungal pathogens because their spores can be dispersed over very long distance (Brown and Hovmoller, 2002). Among the numerous complexes of sibling species recently uncovered using the GCPSR criterion, many however appear consistent with allopatric divergence, because the cryptic species occupy non-overlapping areas separated by geographic barriers (Taylor et al. 2006).

Among the recent examples, a multiple gene genealogies approach revealed the existence of cryptic species among the morphological species *Neurospora crassa* (Dettman et al., 2003a). They had non-overlapping geographical ranges, suggesting allopatric speciation: one phylogenetic species was located in the Congo, another in the Caribbean and Africa (but not Congo), and a third one was restricted to India. In yeasts, Kuehne et al. (2007) showed, also using a multiple gene genealogies approach, that *Saccharomyces paradoxus*, a close relative of *Saccharomyces cerevisiae*

present in temperate woodlands in the northern hemisphere, was composed of two distinct genetic groups, A and B. The majority of isolates from group A were from Eurasia whereas all isolates from group B had been collected in North America, suggesting a differentiation of these incipient species in separate continents. Another example comes from *Fusarium graminearum*, a fungus responsible for scab on wheat and barley, which had long been considered as a panmictic species with a broad distribution. Recent studies however identified at least nine phylogenetically distinct and geographically separated species (O'Donnell et al., 2004). Four of them were clearly endemic to South America, one was found only in Central America, one in India and one in Australia (O'Donnell et al., 2000; O'Donnell et al., 2004). Yet another example is *Coccidioides immitis*, responsible for coccidioidomycosis (Valley fever) in humans and other mammals in America. An analysis conducted after a burst of infected patients in California revealed that this pathogen was composed of two cryptic species. One was located in California and the other all around in America (Koufopanou et al., 1997,1998). These groups were estimated to have been genetically isolated from one another for 11–12.8 million years and had largely distinct geographical distributions, suggesting that their genetic isolation had a biogeographic origin. Examples can also be found among basidiomycetes (Kohn, 2005; Le Gac and Giraud in press), for instance in *Armillaria mellea*, where North American and European strains have been shown to belong to different species (Anderson et al., 1989, 1980).

4. Theoretical issues of sympatric speciation

In contrast to the wide acceptance of allopatric speciation, the possibility of sympatric speciation in sexual populations had long been dismissed. This is because recombination between different subsets of a population that are adapting to different resources or habitats counteracts natural selection for locally adapted gene combinations (e.g. Felsenstein, 1981; Rice, 1984). Recombination indeed prevents both the building of linkage disequilibrium between adaptive alleles at different loci and divergence at loci not under disruptive selection. Population genetics has shown that a very low level of gene flow, such as one migrant per generation, is sufficient to prevent differentiation (Slatkin, 1987). This is true regardless of population size, because while one migrant has a more diluted effect in a large population, the effect of drift is also smaller.

Theoretical models have shown that the simplest way to eliminate the role of recombination in breaking down the effects of selection, and thereby allow sympatric speciation, is to have the same gene(s) controlling pleiotropically both enhanced fitness in a specialized habitat and assortative mating (mate choice, i.e., prezygotic isolation) or both fitness and habitat choice if mating takes place within habitats (Rice, 1984). Such “magic traits” (Gavrilets, 2004) have however proved difficult to find in nature. Another

way to reduce recombination between two populations specialized on different niches is to build up an association (linkage disequilibrium) between habitat-based fitness genes and either assortative mating genes and/or habitat choice genes if mating is restricted within habitats (e. g. Dickinson and Antonovics, 1973; Johnson et al., 1996). Theoretical models have shown that this is plausible under certain conditions, although the limitations to the process are far from trivial.

Fungi cannot actively choose the habitat in which they will grow, but for many fungal species sex must occur in the habitat after mycelial development (e.g. on or within the host for fungal parasites). A recent model has shown that, due to this important characteristic of the life style (inability of disperse between development on the host or habitat and mating), mutations providing adaptation on a new habitat can affect pleiotropically both the fitness on the habitat and the ability to mate in this habitat. Adaptation to a new habitat can thus be sufficient to restrict gene flow in sympatry in fungi for which mating occurs within their specialized host or habitat, without requiring active assortative mating, i.e., prezygotic intersterility (Giraud, 2006a; Giraud et al., 2006). Specialization would act in these fungi as a “magic trait” (Gavrilets, 2004), pleiotropically allowing both adaptation to the new host or habitat and reproductive isolation, thus facilitating sympatric speciation.

Sympatry is often said to be difficult to define for micro-organisms and parasites. For instance, parasites specialized on different sympatric hosts are sometimes considered allopatric (e.g. Huyse et al., 2005). A simple, widely applicable definition of sympatry is however available: “in sympatry, the probability of mating between two individuals depends only on their genotypes” and not on extrinsic barriers (Kondrashov, 1986). Following this definition, parasite individuals infecting two different host species will only be in allopatry when: (i) the host species are allopatric, i.e., there is an extrinsic, geographic barrier to gene flow that prevents dispersal for both hosts and parasites; or (ii) the host species are sympatric but their parasites cannot disperse to other host because of extrinsic barriers other than geographic ones; examples are rare, but include cases where parasite vectors are strictly specific to the different host species. Because the probability of mating among parasites then depends on host genotypes and not on parasite genotypes (the vector chooses the host species), the reproductive barriers can be considered as extrinsic to the parasites. In all other cases, the problem of speciation in sympatry remains: mechanisms must evolve that will prevent crosses between individuals specialized on different niches (Giraud, 2006b; Le Gac and Giraud, 2004). These can be assortative mating or specialization if mating occurs within hosts or habitats (Giraud et al., 2006). This latter case is still sympatric speciation because the probability of mating only depends on the genotypes of fungal individuals (at specialization loci) and not on extrinsic barriers.

The possibility of sympatric speciation in fungi thus heavily relies on the specialization of individuals to different ecological niches, possibly causing directly reproductive isolation or selecting for assortative mating. An important issue is then what are the selective pressures that determine whether fungi become niche specialists or generalists. At first sight, being able to exploit a variety of ecological niches, or hosts, seems a better strategy (Felsenstein, 1981). The most commonly invoked explanation for specialization is the existence of trade-offs. In parasites, trade-offs imply that an allele allowing greater exploitation of a given host reduces the ability or benefit of infecting other hosts (van Tienderen, 1991). However, despite numerous works looking for such trade-offs, they have been difficult to find in nature. An alternative and interesting hypothesis is that specialist parasites can evolve faster in response to their single host, which provides them a great advantage in the endless coevolution associated with antagonistic systems (Kawecki, 1998; Whitlock, 1996).

5. Possible cases of sympatric speciation

Compelling evidence for the sympatric divergence is extremely difficult to provide, because excluding a past period of allopatry is almost always impossible (Coyne and Orr, 2004, p. 142). Even the most famous candidate cases are still debated, such as the phytophagous insect *Rhagoletis pomonella* (Coyne and Orr, 2004, pp. 159–162) or the cichlid fishes in African lakes (Coyne and Orr, 2004, pp. 145–154). Evidence consistent with sympatric divergence of fungal populations driven by parasitic adaptation to different hosts has however been reported.

An example is provided by *Ascochyta* pathogens, where recent multilocus phylogenetic analyses of a worldwide sample of *Ascochyta* fungi causing blights of chickpea, faba bean, lentil, and pea have revealed that fungi causing disease on each of these hosts form distinct species (Peever, 2007). Experimental inoculations demonstrated that infection was highly host-specific, yet *in vitro* crosses showed that the species were completely interfertile. The host specificity of these fungi may therefore constitute a strong reproductive barrier, and the sole one (Peever, 2007), following a mechanism of sympatric divergence by host usage (Giraud, 2006a; Giraud et al., 2006). The coexistence in sympatry of interfertile populations specialized on different hosts that remain reproductively isolated cannot indeed be explained currently by models other than the reduced viability of immigrants (Giraud, 2006a; Giraud et al., 2006; Nosil et al., 2005). This mechanism seems to be able to maintain the species differentiated in sympatry and could similarly have created the divergence in sympatry. It is however difficult to exclude a period of allopatry in the past that would have facilitated specialization, i.e., the accumulation of different alleles beneficial on alternate hosts, as has been proposed for the well-studied case of the phytophagous insect *Rhagoletis pomonella* (Coyne and Orr, 2004, pp. 159–162).

An elegant way to demonstrate the sympatric occurrence of speciation is to show that gene flow has occurred after initial divergence (Wu and Ting, 2004). This approach is very promising and has been used so far in fungi only on *Mycosphaerella graminicola*, showing that this wheat pathogen arose recently, most probably during wheat domestication in the fertile crescent, by sympatric differentiation from *Mycosphaerella* species pathogens of natural grasses (Stukenbrock et al., 2007).

6. Nature of reproductive isolation

As seen above, a *sine qua non* of speciation in sexually reproducing organisms is the decrease of gene flow between incipient species due to the development of reproductive barriers. Two types of reproductive barriers are usually distinguished, prezygotic and postzygotic, depending on their time of occurrence, before or after fertilization. In fungi having a long dikaryotic stage, nuclear fusion occurs long after individual or gamete fusion, which may render the term postzygotic ambiguous. We will therefore here use the terms pre- and postmating for fungi, which qualifies time before or after cell fusion.

Premating isolation may include different kinds of barriers: (1) for organisms depending on biotic vectors, specialization of these vectors can prevent contact between two populations even if they lie close to one another, yielding ecological isolation. For example, the endophyte *Epichloë typhina* is preferentially chosen by their fly vectors *Botanophila* as opposed to *Epichloë clarkia* (Bultman and Leuchtmann, 2003), which may promote a certain degree of reproductive isolation. Another example is the complex *Microbotryum violaceum*, where the insect vectors are different to some extent between host species, leading to a reduction in mating opportunities among strains from different plants, although the barrier is not complete (van Putten et al., 2007). (2) Specialization may also allow for ecological premating isolation if mating occurs within habitats (hosts for parasites), as discussed above (Giraud, 2006a; Giraud et al., 2006). (3) Allochrony, i.e., differences in the time of reproduction, may also be efficient to promote premating isolation. The sister species *Saccharomyces cerevisiae* and *S. paradoxus* exhibit for instance different cell growth kinetics; this allows most individuals of one species to undergo homospecific crosses before or after reproduction of the individuals of the other species. Proportion of interspecific matings can therefore be significantly reduced without the need of incompatibility factors (Murphy et al., 2006). (4) As has been invoked in plants (Fishman and Wyatt, 1999), a high rate of selfing may be efficient in limiting interspecific matings. Selfing has been suggested to act as a reproductive barrier in the anther smut fungus *M. violaceum* (Giraud et al., in press). (5) Assortative mating due to mate recognition occurs if individuals or gametes are able to discriminate between conspecifics and heterospecifics. Assortative mating seems to be especially important in the reproductive isolation of *Homobasidiomycota*, where clamp connections

between mycelia of opposite types are almost exclusively observed when the tested mycelia belong to the same species (Le Gac and Giraud, in press).

Postmating isolation refers to barriers associated with hybrid inviability and sterility and is expected to arise as a result of the divergence of incipient species. In the case of postmating isolation, heterospecific crosses occur and lead to the production of unfit offspring. Hybrids may be inviable or sterile due to genetic incompatibilities if mutations fixed independently in the diverging lineages display negative epistatic interactions when brought together in the same individual, a phenomenon known as Dobzansky–Müller incompatibilities (Orr and Turelli, 2001). This kind of intrinsic postmating reproductive isolation is responsible for the numerous reported cases in fungi of crosses that initiate and subsequently abort during *in vitro* experiments. For instance, heterospecific crosses among *Microbotryum* species produce *in vitro* fewer viable mycelia than conspecific ones (Le Gac et al., 2007b), and crosses among *Neurospora* species lead to few or abnormal perithecia or to few viable ascospores (Dettman et al., 2003b). Postmating isolation may also be linked to ecological factors. Hybrids are then perfectly viable and fertile in a benign environment, such as *in vitro* conditions, but unfit in a natural environment. This can be the case if hybrids display intermediate traits between parental phenotypes and, as a result, are poor competitors in either parental environment. Despite its potential importance to reduce gene flow, such ecological, postmating barriers have rarely been investigated in fungi. In the species complex *M. violaceum*, hybrids between two close species were inoculated onto both parental host species. In one of the host species, hybrids performed as well as the parental species specialized for this host, indicating that there are no genetic incompatibilities in hybrids. However, when inoculated in the reciprocal host, hybrids did not perform as well as the parental species specialized for this host, showing that in other environmental conditions the same hybrids had a lower viability (Le Gac et al., 2007b).

Mycologists have extensively studied the pre and postmating reproductive barriers that are accessible via *in vitro* crosses, namely intrinsic premating mate recognition and intrinsic postmating barriers. Despite the potential importance of ecological barriers to gene flow, they are still understudied in fungi. Using fungal systems to investigate reproductive isolation both in the lab and in nature would be a great approach to the virtually unexplored question of the relative contributions of the various reproductive barriers to the decrease of gene flow between sibling species (see Ramsey et al., 2003, for one of the rare examples of such an approach, in plants) and to understand which barriers arise first during speciation.

7. Evolution of reproductive isolation

How does reproductive isolation evolve with time? This question of primary importance for the understanding of

the speciation process has been investigated both theoretically and experimentally during the last decades. In the few biological models studied so far (mainly animals), some trends start to emerge (Coyne and Orr, 2004): (1) pre- and postmating isolation evolve gradually; (2) premating isolation evolves faster or at the same rate as postmating isolation; (3) postmating hybrid sterility evolves faster than postmating inviability; (4) reproductive isolation evolves at different rates in different groups. Despite their great potential as models for studying speciation, only few studies have investigated the evolution of reproductive isolation in fungi. Dettman et al. (2003b) investigated reproductive isolation among five phylogenetic species of *Neurospora*. Reproductive isolation was scored according to a scale of seven categories ranging from the ability to produce numerous viable ascospores to the inability to produce any perithecia, and thus encompassed both pre- and postzygotic isolation. Liti et al. (2006) investigated the evolution of postmating isolation among the six species of the genus *S. sensus stricto* by measuring hybrid sterility. In these two studies on *Ascomycota*, reproductive isolation increased with genetic distance among species, in agreement with findings in other organisms. Another study investigating the evolution of reproductive isolation in fungal organisms used the species complex *M. violaceum*. Reproductive isolation was measured among 10 species as the ability to mate, to produce hybrid mycelium, and to infect plants. Postmating isolation increased with genetic divergence, but premating isolation did not (Le Gac et al., 2007b). The absence of correlation between premating isolation and genetic distance in the *M. violaceum* complex strongly contrasts with results obtained in other biological systems, and might be explained by a high selfing rate (Giraud et al., in press). Indeed, if selfing is the rule, the proportion of interspecific matings may be too low for a selection pressure for assortative mating to have an effect.

Evolution of reproductive isolation is expected to differ according to the geographic distribution of incipient species. Those evolving in allopatry have no opportunity to mate with each other, so reproductive isolation is expected to arise gradually and slowly as a result of independent mutation, genetic drift, and indirect effects of natural selection driving local adaptation (Coyne and Orr, 2004, pp. 83–110). In contrast, close species in sympatry may have the opportunity to mate with each other and a strong selection for avoiding interspecific crosses is expected (Coyne and Orr, 2004, chapters 4 and 10). Altogether, stronger reproductive isolation is therefore expected between sympatric than allopatric sibling species. Such reproductive character displacement (i.e., the pattern of enhanced reproductive isolation in sympatry) is expected to mainly affect premating reproductive isolation and has extensively been studied in the theoretical literature dealing with reinforcement (Servedio, 2000). This pattern has been detected in many natural cases, for instance among *Drosophila*, damselflies, frogs, fish, crickets, toads, birds, marine organisms and rodents (Coyne and Orr, 2004, pp. 357–360). In a

comparative study, Le Gac and Giraud (in press) analyzed published works reporting crossing experiments within 16 species complexes of *Ascomycota* and 16 of *Homobasidiomycota*, representing a total of 431 species pairs. In *Homobasidiomycota*, sympatric species were virtually always isolated by very strong pre-mating isolation, as shown by the absence of clamp connections between sympatric species during *in vitro* assays, while allopatric species pairs often display a higher compatibility levels. *Homobasidiomycota* fungi thus show a pattern of reproductive character displacement and are a potential empirical model to study the details of this phenomenon in nature. In *Ascomycota* in contrast, both sympatric and allopatric species pairs display a similarly low level of reproductive isolation, mainly post-mating (i.e., reproductive isolation linked to the inability to produce normal perithecia containing viable and fertile ascospores). The lack of consistent pre-mating isolation due to mate recognition among sympatric pairs of *Ascomycota* may be due to the presence of species in this group that mate within their hosts or habitat and for which specialization could act as a pre-mating barrier (Giraud, 2006a; Giraud et al., 2006).

8. Speciation by hybridization

Many fungal species do not exhibit complete intersterility (Le Gac and Giraud, in press), which gives the opportunity for hybridization. Hybrid speciation is classified according to the ploidy level of the resulting individuals: when hybrids have a chromosomal number that sums that of the parental species, the process is called allopolyploid speciation, whereas hybrids with ploidy identical to that of the parents are referred to as allodiploids or homoploids.

Allopolyploids have a higher ploidy level than the parental lines, but their karyotype is interestingly often not the exact addition of the two parental genomes, due to losses of chromosomes (Leitch and Bennett, 2004). As a consequence, many ancient polyploidy speciation events may have been overlooked. Recent allopolyploid hybrids have however, been identified in diverse genera: *Botrytis allii*, the agent of gray mold neck rot of onion and garlic (Staats et al., 2005), several *Neotyphodium* species, symbiotic endophytes of grasses (Moon et al., 2004), and several *Saccharomyces* species empirically selected for brewing (Masneuf et al., 1998); see Olson and Stenlid (2002) for more examples.

The presence of multiple hybrids in some taxa suggests that hybrids could have selective advantages over parental species, at least in some cases. Indeed, by combining traits, allopolyploids of animals and plants exhibit a wider range of phenotypes making them able to exploit vacant ecological niches (Mallet, 2007; Rieseberg and Willis, 2007a). Allopolyploidy would provide simultaneously instant reproductive isolation, due to triploidy in backcrosses, and a new ecological niche. Examples can be found among grass endophytes, whose perfect forms belong to the *Epichloe*

genus and the imperfect stage to the *Neotyphodium* genus, which is constituted by hybrids between *Epichloe* species. Most of the *Neotyphodium* hybrid species parasitize grass species that are not hosts for *Epichloe* species. Some *Neotyphodium* hybrid species exploit the same host as one of their *Epichloe* parental species, but exhibit different life-history traits. Indeed, *Neotyphodium* are mutualistic and asexual, while *Epichloe* species are sexual and parasitic, sterilizing their host during their reproduction (Schardl et al., 1997). The asexual form of reproduction in *Neotyphodium*, which occurs only as vertical seed-borne transmission, is thought to be a direct result of the hybridization (Kuldau et al., 1999). These asexual hybrids may thus have been selected for mutualism: they avoid sterilizing their host, which is the only way for them to be transmitted (Selosse and Schardl, 2007). *Neotyphodium* hybrids further contribute to host fitness through the combination of several genes involved in alkaloid production, as these compounds protect the host plants from herbivores (Tanaka et al., 2005). *Neotyphodium* grass endophytes may thus have come to occupy a different ecological niche as the result of hybrid-induced asexuality, even when they infect the same host as their ancestors. Another example of fungal allopolyploids can be found in *Cryptococcus neoformans*, a human pathogen causing meningoencephalitis. Diploid hybrids between two different serotypes have been found to be highly prevalent in nature and to show higher fitness than the parental haploid forms, both *in vivo* and *in vitro* (Lin et al., 2007).

Evidence for homoploid speciation comes from a ploidy level identical to that of its parents and a broad heterozygosity. Contrary to allopolyploids that are reproductively isolated from their parents, homoploid hybrids are in competition not only with their parents but also with backcrossed individuals, which renders stable allodiploid species much more unlikely than polyploid ones. A well-described case of homoploid speciation is that of the rust *Melampsora × columbiana* that emerged from hybridization of *M. medusa*, parasite of *Populus deltoides*, and *M. occidentalis*, parasite of *P. trichocarpa* (Newcombe et al., 2000). This hybrid emerged in 1997 when a poplar hybrid resistant to the two parental rust species was widely grown in California, the hybrid rust being able to infect the hybrid poplar. In this case, the homoploid hybrid clearly had a novel ecological niche, a new host. Another question is why many loci actually stay heterozygous despite potential recombination among F1 hybrids? This may be due to a selective advantage of simultaneous heterozygosity at many loci.

Interestingly, recent focus on the gene expression in hybrids provides a potential mechanism for such an advantage. Studies on hybrid strains of *Drosophila* and *Arabidopsis* showed that most of the genes exhibiting different expression levels in the two parental species were not expressed at an intermediate level in the hybrid. In average, half of them were underexpressed as compared to the expression in each of the two species, and one fourth was

overexpressed (Landry et al., 2007). Hence, hybridization may allow exploring fitness landscapes outside that of the parental species. This would facilitate the maintenance of hybrids in a new niche and thereby their persistence as a new species.

9. Chromosomal speciation

Another mechanism allowing instant speciation is chromosomal speciation. The first model of chromosomal speciation (speciation due to chromosomal rearrangements) considered that if two isolated populations had fixed karyotypic differences, and that recombination between rearranged chromosomes were generating unbalanced gametes that lowered fitness, between-population gene flow could be prevented upon secondary contact (White, 1978). This model was then dismissed on the rationale that rearrangements that cause a sufficient reduction in fitness in heterozygotes could not be fixed in a population precisely because of this reduction in fitness. When at low frequency, the rearrangement will indeed always be in a heterozygous state, and should not be able to increase in frequency. Fungi may however be some of the rare organisms where this speciation scenario could occur because of asexual reproduction and selfing that allow mutants with karyotypic rearrangements to reproduce without loss of fitness.

New models of chromosomal speciation consider that the effects of chromosomal rearrangements on recombination rates are more important than those on fitness to explain speciation: a chromosomal rearrangement creates a large region of suppressed recombination where one or more specialization genes can accumulate and lead to the localized restriction of gene flow, which could eventually drive the populations to speciation (Noor et al., 2001; Rieseberg, 2001). Chromosomal rearrangements seem frequently involved in plant speciation, as indicated by the findings that sterile plant hybrids often recover fertility after chromosomal doubling, which furnishes an exact homolog for each chromosome, and by mapping of genetic incompatibilities, which frequently fall into chromosomal rearrangements (Rieseberg and Willis, 2007).

In fungi, the small size of chromosomes has long been a barrier to the study of chromosomal rearrangements and chromosomal speciation. The invention of the pulsed-field-gradient-gel-electrophoresis allowed the separation of intact fungal chromosomes and revealed that an extremely high proportion of fungal species exhibited chromosome-length-polymorphism (CLP). Chromosomal rearrangements leading to CLP reported in fungi include deletions, reciprocal and insertional translocations, chromosome breakage and fusion or complete chromosome loss, which may in large part be due to transposable elements and other dispersed repetitive sequences (Burnett, 2003; Zolan, 1995).

In the ascomycete *Sordaria macrospora* and in the basidiomycete *Coprinus cinereus*, intraspecific sexual crossing of strains harboring different karyotypes resulted in low ferti-

ity in the progeny, concordant with the idea that chromosomal rearrangement can play a role in the speciation process (Poggeler et al., 2000; Zolan et al., 1994). However, these karyotypically differentiated strains may also have differed in their genic content. In order to isolate the effect of karyotypic rearrangement, Delneri et al. (2003) elegantly constructed strains of *Saccharomyces cerevisiae* differing uniquely by the presence of reciprocal translocations but otherwise completely isogenic. They showed that crosses between such strains had lowered spore fertility and proposed that chromosomal rearrangements, for yeasts at least, are able to provide partial isolation. Chromosomal rearrangements can thus theoretically have a role in speciation in fungi, but showing that the rearrangements were a cause of the divergence, and not only its consequence, remains a challenging task.

10. Role of epigenetic mechanisms in speciation

Major factors responsible for postmating reproductive isolation are inherited directly as part of the parental DNA, such as rapidly evolving genes that may interact incompatibly in hybrids (the Dobzhansky–Müller model) or chromosomal rearrangements that produce segmental deletions in meiotic products. However, the recent appreciation that genetic systems function largely under epigenetic control mechanisms should let us consider that these may also be involved in reproductive isolation.

Epigenetic mechanisms have important roles in preserving genomic integrity and in defending the genome against selfish DNA elements, such as transposons. The types of sequence and structural irregularities in hybrid genomes may interact with epigenetic controls in ways that limit survival, and in several cases the disabling of epigenetic mechanisms increases the viability of hybrid crosses. For example, in the *Saccharomyces sensu stricto* yeasts, a mismatch repair system prevents chiasma formation when DNA sequences show marked dissimilarity. Overall divergence can so strongly prevent recombination that chromosomes fail to segregate properly during meiosis. Mutation to *MSH2* in the mismatch repair system increased the relative fertility of crosses, even for intraspecific combinations between distantly allopatric populations, suggesting that the epigenetic mechanism may play a role in the early stages of speciation (Greig et al., 2003).

Another hallmark of hybrid genomes is the presence of repeated and non-syntenic DNA sequences that result from allopolyploidy or smaller-scale chromosomal and segmental duplications. Failure of proper meiotic divisions is the cause of unbalanced gametes, and haploids possessing duplications are often more likely to survive than those suffering major deletions. Epigenetic defenses in fungi against autonomously replicating DNA elements, such as retrotransposons, are able to detect and silence repetitive sequences. In *Neurospora crassa*, sequences in regions of the genome that fail to pair normally during meiosis are silenced by a process involving bi-directional transcription

that presumably feeds into an RNA-interference pathway (Matzke and Birchler, 2005). This “Meiotic Silencing of Unpaired DNA » depends partly upon an RNA-dependent RNA polymerase (*Sad1*), which when disabled restores fertility to sterile hybrids between *Neurospora* species (Shiu et al., 2001). In a similar way, the genome defense against transposable elements known as Repeat-Induced Point Mutation (Galagan and Selher, 2004) may be expected to act upon the segmental duplications and aneuploidy in hybrids. This system is found in diverse fungi and causes hypermutation of sequence repeats that lead to their non-functionalization. Thus a variety of epigenetic mechanisms may contribute to postmating isolation in fungi, but the extent to which they play an important role in the speciation process in nature remains largely unknown.

11. Cospeciation and host shifts

In host–parasite and host–symbiont associations, speciation in one of the interacting organisms, particularly the host, can lead to speciation in the other. As a consequence of such cospeciations, a strong congruence (topological similarity) is usually expected between the host and parasite/symbiont phylogenies. High congruence has in fact been observed in some host–parasite associations involving animals, such as gophers and lice (Hafner et al., 1994), but such patterns are not the rule. Host shifts, sorting events (e.g. extinctions), and duplications of parasite lineages have been invoked to account for reduced similarity between the phylogenetic trees of hosts and their parasites or symbionts (for a review, see Page, 2003). In order to elucidate whether cospeciations or host shifts have been prevalent, one has therefore to test if the level of congruence is significantly higher than that expected by chance (de Vienne et al., 2007a; Legendre et al., 2002), and then to reconstruct the most likely evolutionary history of the association. Methods have been developed to do so, implemented in software such as Treefitter or Treemap (reviewed in Page, 2003, Chapter 1). These methods however rely on *a priori* assumptions about the relative likelihoods of host shift and cospeciation events, which can be challenging to estimate.

Many fungi interact with other species, as symbionts or parasites, but also sometimes as hosts. Most fungi develop in association with a restricted number of host species and are therefore good candidates for cophylogenetic studies. Because fungal spores are most often widely disseminated in the environment, a high number of spores are likely to land on non-suitable hosts where for obligate pathogens and symbionts they must either cause infection or die. Both opportunities and selection for the utilization of a new host should therefore be frequent. As a consequence we expect cophylogenetic analyses dealing with fungi to reveal high levels of incongruence between host and parasite trees. This is indeed the case in many plant–fungal associations (Jackson, 2004), such as in the *Puccinia*-Crucifer (Roy, 2001) or the *Microbotryum*-Caryophyllaceae (Refrégier et al., in

press) systems. Host shifts are therefore frequent in many fungal pathogens, showing that apparently strict host specificity is not sufficient to impede host shifts over the long term. This idea has also been supported by the observation of incipient host shifts (Antonovics et al., 2002; Lopez-Vilavicencio et al., 2005).

In other fungi however, high levels of congruence are observed, as in the *Golovinomyces*-Asteraceae (Jackson, 2004) or the *Epichloe*-grass associations (Schardl et al., 1997, in press). Another interesting case of highly congruent phylogenetic histories is that of fungi belonging to the Agaricaceae and Tricholomataceae families, being simultaneously cultivated by fungus-growing ants and hosts of the parasitic *Escovopsis* species of fungi. Research over the last decade has revealed a high degree of congruence between both ant and cultivated fungi and between the cultivated fungi and *Escovopsis* (Currie et al., 2003 and references therein), suggesting that cocladogenesis occurred in this tripartite interaction. Interestingly, the fungal symbionts do not appear to have free dispersal stages, which could restrain the possibility of host shifts. However, congruence between host and parasite trees is not always evidence for rampant cospeciations because host shifts can give rise to congruent phylogenies if they occur preferentially towards closely related hosts (de Vienne et al., 2007b). Temporal concordance therefore needs to be assessed with appropriate tests in order to elucidate whether cospeciation has occurred (de Vienne et al., 2007b; Hirose et al., 2005; Schardl et al., in press).

In any case, cospeciations and host shifts are not strictly speaking modes of speciation. They are rather related to the evolutionary forces of ecological divergence leading to speciation. To occur, cospeciation and host shifts require allopatry or the evolution of reproductive isolation, following the mechanisms explained above.

12. Asexual fungi

In asexual fungi, the theoretical issues of species formation are completely different from those in sexual organisms. There is no recombination to break down combinations of multiple alleles adapted to a given habitat, and the selective pressure on one gene has an effect on the whole genome. Any new allele allowing adaptation on a new niche can thus give rise to a new “species”. The difficulty in asexual organisms is rather to understand if, and why, discrete entities exist that we can recognize as species, instead of continuous distributions of phenotypes/genotypes. Asexual organisms in fact seem to form discrete species (Fontaneto et al., 2007), and the hypotheses invoked to explain their existence despite lack of homogenizing gene flow are the existence of discrete ecological niches, random processes of extinctions of intermediate genotypes/phenotypes (Coyne and Orr, 2004, pp. 17–22), or the recurrent apparition of asexual species from sexual ones (LoBuglio et al., 1993).

The clonal fungus *Penicillium marneffe*, the causal agent of disease in immuno-compromised humans, exhibits geo-

graphic endemicity despite long-distance migration via aerially dispersed spores. DNA multilocus typing showed that different clones of the fungus are associated with different environments (Fisher et al., 2005), which suggested that adaptation to these environments is constraining the organism's ability to successfully disperse in nature. The population structure in asexual parasites may thus directly reflect host or habitat adaptation, at all loci, because selection at one locus results in hitchhiking of the whole genome.

Another example is the *Magnaporthe grisea* complex, many species of which are strictly asexual and host-specific. One of the species of this complex, *M. oryzae*, an important fungal pathogen of rice, has been shown to have arisen recently, possibly in association with rice domestication (Couch et al., 2005). Isolates from rice, millet, cutgrass, and torpedo grass appeared also strictly asexual, and to constitute recent host-specific lineages. These patterns in the *M. grisea* complex appear consistent with the idea that acquisition of abilities to infect new hosts in asexual parasitic fungi can readily form new species because recombination will not prevent the differentiation from the ancestral populations.

Fungi, with their enormous diversity of modes of reproduction, seem ideal subjects to test the different hypotheses on the nature of species in non-recombining organisms (Coyne and Orr, 2004, pp. 17–22). In some asexual fungi however, recombination can still occur between individuals via somatic recombination (Bos, 1996), which can be considered as equivalent to sex as regards the speciation issue. Hyphal fusions between genetically different individuals is controlled by elaborate vegetative compatibility systems (Bos, 1996), resulting in a condition of heterokaryosis. The exchange of nuclei and organelles can lead to parasexuality via highly transient nuclear fusion and subsequent chromosomal segregation and/or ameiotic recombination (Fincham et al., 1979). In fungi undergoing such somatic recombinations, vegetative compatibility groups (VCG) could be considered as reproductively isolated from each other and therefore as distinct species. This has been suggested in *Aspergillus flavus*, where the different VCGs indeed formed genetically distinct lineages (Ehrlich et al., 2007).

13. Genetics of speciation

Knowing which genes are involved in reproductive isolation may help get a better understanding of speciation processes. The genetics of speciation has just begun to shed some light on the evolution of reproductive isolation, but the focus so far has been mostly on insects (Wu and Ting, 2004). Genes reported to cause premating isolation are often involved in sexual pheromones (Dallerac et al., 2000; Thomas et al., 2003), in host choice for parasites mating on their host (Dambroski et al., 2005), and in gametic compatibility (Galindo et al., 2003; Lee et al., 1995). Genes involved in postmating isolation have been found to be

strongly influenced by positive selection, with functions not directly involved in reproductive isolation (Wu and Ting, 2004). This is consistent with the Dobzhansky–Müller model (Orr and Turelli, 2001), with the reunion of rapidly evolving genes (for reasons other than reproductive isolation) with interacting gene products causing incompatibilities in hybrids and thus postmating isolation.

In fungi, little investigations have been undertaken to understand the genetics of speciation. Fives genes have been shown to be involved in the intersterility among *Heterobasidion* species (Chase and Ullrich, 1990), but they have not been characterized yet. They do not include the mating type genes, although we could have expected that the mating type locus could play a role in sexual isolation (Natvig and May, 1996). Mating types in fact appear not to be involved in sexual isolation in general among fungal species, where mating type alleles indeed exhibit extensive trans-specific polymorphism (James et al., 2006) and can be functional when transferred in other species (Arnaise et al., 1993). Mating types may play a role in sexual isolation in other species. It has for instance been shown that the pheromones and pheromone receptor of *Ustilago hordei* MAT-1 were necessary and sufficient to make *U. maydis* compatible with *U. hordei* MAT-2 (Bakkeren and Kronsstad, 1996). The pheromone signal system may also play a role in reproductive isolation among heterothallic *Neurospora* species (Karlsson et al., 2008).

In the cases of reproductive isolation by mere specialization, the genes allowing specialization should also be the speciation genes (Giraud, 2006a; Giraud et al., 2006). In asexual species too, genes responsible for host shift could also be those present at the origin of new species. More generally, the genes involved in ecological divergence between incipient species may also be fascinating to discover, as we have seen that they play prime roles in speciation. Also, as discussed above, genes of the mismatch repair or silencing machineries could be considered as speciation genes in some cases. Because postmating isolation seems to arise gradually with time (Le Gac and Giraud, in press; Le Gac et al., 2007b), it is likely that many genes are involved, possibly by incompatibility between loci (Orr and Turelli, 2001). Several fungal species complexes should be amenable to genetic dissection of the postmating incompatibility, which should provide highly illuminating results.

14. Conclusion

In conclusion, important advances have been made recently on the speciation in fungi, and they have proved tractable biological models for the general study of speciation. Fungi also exhibit some specific and interesting modes of speciation, and many open questions remain which will be fascinating to explore. Recently developed analytical methods for studying past gene flow and differentiation should be useful to determine in which cases fungal speciation by specialization onto novel hosts has occurred in sympatry (Hey and Nielsen, 2004; Hey et al., 2004). Deci-

phering the genetics of speciation should also prove to be fascinating, for instance by finding markers segregating with inviability or sterility in interspecific progeny. Another promising approach to understand speciation may be experimental evolution, which has already been used in a few excellent works in yeast. These experimental evolution studies showed that assortative mating could evolve under selection against hybrids as expected by reinforcement theory (Leu and Murray, 2006), and that adaptation to divergent environments promotes the evolution of post-mating isolation (Dettman et al., 2007), as expected in the Dobzhansky–Müller model. Fungi should bring many other unique results to the field of speciation in the coming years.

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