

# MECHANISMS OF FUNGAL SPECIATION

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■ **Abstract** The objective of this review is to provide a synthesis of speciation theory, of what is known about mechanisms of speciation in fungi and from this, what is expected, and of ideas on how speciation can be elucidated in more fungal systems. The emphasis is on process rather than pattern. Phylogeographic studies in some groups, such as the agarics, demonstrate predominantly allopatric speciation, often through vicariance, as seen in many plants and animals. The variety of life history factors in fungi suggests, however, a diversity in speciation mechanisms that is borne out in comparison of some key examples. Life history features in fungi with a bearing on speciation include genetic mechanisms for intra- and interspecies interactions, haploidy as monokaryons, dikaryons, or coenocytes, distinctive types of propagules with distinctive modes of dispersal, as well as characteristic relationships to the substrate or host as specialized or generalist saprotrophs, parasites or mutualists with associated opportunities and selective pressures for hybridization. Approaches are proposed for both retrospective, phylogeographic determination of speciation mechanisms, and experimental studies with the potential for genomic applications, particularly in examining the relationship between adaptation and reproductive isolation.

## INTRODUCTION

This review is about how speciation occurs in fungi—the relatively little that we know based on some signature studies, with insights into how we might find out more. Do fungi conform to general models of speciation? If so, how can fungi provide model systems for targeting speciation genes and their products? Speciation mechanisms in fungi are studied retrospectively, through biogeographical interpretation of phylogenies (phylogeography) based on single or multilocus sequence data, or experimentally, through interfertility studies with the associated genetics, or through in vitro studies of hybridization or evolutionary divergence in experimental populations. The focus in this review is on mechanisms. Previous reviews of the dynamics of speciation (11) and fungal speciation (17, 85) are sources for categorized lists of fungal species with references, bearing in mind that many of us working in this area have attributed mechanisms of speciation without hard data. There is also a recent comprehensive review of hybridization in

plant-associated fungi and Oomycota (102) and as well as a short, provocative treatment on essentially the same topic (9). On the topic of fungal species concepts, readers are referred to several recent treatments (17, 53, 95, 107), and for a comprehensive comparative analysis, to Coyne & Orr's treatise, *Speciation* (32), specifically to the appendix, "A Catalogue and Critique of Species Concepts."

Speciation is usually a splitting of existing species into new ones, although one species may be replaced by the next (cladogenesis versus anagenesis). New species emerge from ancestral species by divergence through adaptation or genomic change, or by hybridization of ancestral species. New species maintain cohesion with respect to other species largely through reproductive isolation. The biological species concept is implicit in most models of speciation, as it will be in this review. There are clear examples in fungi and fungus-like protists of speciation without full reproductive isolation, such as serial hybridization, cryptic speciation in asexual lineages, and partial interfertility between allopatric species—all to be discussed further. Even so, reproductive isolation, if not the sole mechanism of speciation, is certainly the result of complete speciation and is reflected in phylogenies inferred solely from DNA sequence data (e.g., 36). Reproductive isolation arises through changes in chromosome number (autopolyploidy or aneuploidy), hybridization (allopolyploidy or aneuploidy, or some degree of heteroploidy), genetic drift, or selection. The genetic mechanisms conveying adaptation may be only indirectly associated with those conveying reproductive isolation through hitchhiking or pleiotropy (32).

Speciation models build from a general framework of prezygotic or postzygotic barriers imposing reproductive isolation, i.e., impeding gene flow between two or more populations (see next section). These barriers may be extrinsic, i.e., hybrids develop normally but with decreased viability because they cannot locate a suitable niche or obtain mates, or intrinsic, i.e., hybrids are inviable or sterile. In all organisms, interfertility occurs only in the absence of both prezygotic and postzygotic isolation. In fungi, the absence of prezygotic isolation as shown by normal formation of complete clamp connections, sexual fruiting bodies, or meiosporangia in matings is therefore not sufficient to conclude that two individuals are fully interfertile. For full interfertility, postzygotic isolation must also be absent. This means that meiospore formation and viability must also occur at normal rates. The point here is that while the distinction can be demonstrated between prezygotic and postzygotic isolating mechanisms in fungi, interfertility is often evaluated solely on the prezygotic evidence. For ascomycetous fungi and oomycetous protists, interfertility includes the ability to function as either a female or male parent. For all fungi, interfertility includes  $F_1$  (meiospore) viability and fertility, which is the ability to function as a parent in a cross to yield a viable  $F_2$ . Our understanding of reproductive isolation in fungi would be enhanced by quantitative information on all of these components of interfertility for a wider array of species.

The populations isolated by lack of gene flow may be sympatric—spatially conjunct with overlapping ranges even before speciation, or allopatric—disjunct with discrete ranges before and after speciation, or parapatric—ultimately conjunct

after speciation. Clearly, the best evidence for spatial effects on speciation is under conditions of allopatry, which intuitively is the most effective source of prezygotic isolation, or postzygotic failure if hybrids cannot disperse to a suitable host or environment. Allopatric speciation appears to be as important in fungi as in other groups of organisms. In the absence of immigration, populations diverge through mutation accumulation, gene duplication, or polyploidization, with allele frequencies further modulating in “gene sorting” by genetic drift, natural or artificial selection (Figure 1). If adaptation under selection drives divergence, the fitness of the new form is expected to surpass that of the progenitor (or hybrids of the new form and the progenitor) in the environment of the new form. Divergence through adaptation may arise through a host of ecological adaptations. These ecological adaptations include host shifts or changes in pathogenicity in fungi.

Is divergence through adaptation likely to be caused by one “speciation gene”? Let’s start with one gene locus, with allele *A* fixed through selection in one population, and allele *a* fixed in another population, the hybrid *Aa* (which we could imagine as a dikaryon or a premeiotic diploid or a postmeiotic haploid segregant in a fungus) would likely be a suboptimal performer in the habitat of either parent. How could we evolve a new species, and what would be its evolutionary intermediary? Multiple genes make more sense, as shown in Figure 2.

That isolation is caused by multilocus interaction, rather than the effect of one locus, is the prediction of the Dobzhansky-Muller model, which shows how hybrid sterility can evolve without selection purging intermediate steps (32). Coyne & Orr (32) have determined that the model originates with Bateson (8). The simple version of the model postulates two gene loci, but it is possible that three or more are required for hybrid inviability or sterility to occur (84). Also, the model does not require equal rates of evolution in population lineages, but both loci must have both experienced mutations (93). Derived alleles cause incompatibilities more often than ancestral alleles, with a “snowballing” effect as the latest mutations are more likely to cause hybrid problems than earlier mutations. Coyne & Orr (32) provide examples of the substantial support for this model, both indirect, as observed “peak shifts,” and directly as alleles that are fit in one genetic background but are unfit in another (e.g., complimentary lethals in *Drosophila melanogaster*). An excellent fungal example of the incompatibility of mutations evolved in different populations under different selection can be found in *Saccharomyces cerevisiae*. Two types of mutations conveying drug resistance were recovered under each of two different selection regimes: step-wise increase or one-step exposure to stasis-inducing drug concentration. The first type of mutations, from the step-wise regime, mapped to *PDR1*, impacting ABC transporters that efflux drug from yeast cells, whereas the second type, from the one-step regime, mapped to *ERG11*, the drug target gene in the ergosterol biosynthesis pathway. Each type of mutation conveyed increased fitness among replicate populations evolved in each of the two selective regimes. Combining these two types of mutations in a diploid hybrid resulted in a loss of fitness in the presence of drug (3).

It is easy to imagine how, in pathogenic fungi, a genotype with a set of loci conveying avirulence or virulence for one population adapted to one set of plant hosts, when hybridized with a genotype with an alternative set of avirulence loci associated with other plant hosts, would result in a hybrid with either no real or no available host plant. If there are no available host plants and the hybrid is a dead end, we have a good example of an extrinsic isolating barrier in which the fitness of the hybrid is reduced because either a suitable ecological niche or a mate is not available. The extent to which either type of extrinsic barrier obtains, or whether hybrid failure is due to hard-wired, epistatic gene interactions under the Dobzhansky-Muller model, is just the sort of problem that can be addressed in speciation studies of experimental fungal populations, which are discussed further.

Increase of prezygotic reproductive isolation by natural selection in sympatry is termed reinforcement. The scenario presented by Dobzhansky (38) began with two species that arose in allopatry, then met, undergoing some hybridization, producing unfit hybrids. Under this scenario, intraspecific matings would enjoy a fitness advantage, with selection favoring evolution of increased prezygotic isolation. Comparative studies in organisms other than fungi suggest that a pattern of increased prezygotic isolation in sympatry is relatively common in nature, with some evidence that postzygotic isolation may be increased in sympatry (32). There are examples consistent with reinforcement in fungi. In pairings between intersterility groups of *Armillaria* from North America and from Finland, partial intercompatibility was observed between intersterility groups from North America and Finland, but complete intersterility between North American groups (2). It was not determined whether hybrids from pairings between North American intersterility groups were as fit as their parents. A comparable situation pertains in *Heterobasidion annosum* (2, 19, 64, 104). The poor fitness of hybrids of *Ophiostoma ulmi* and *O. novo-ulmi* when the two presumably allopatrically derived species meet in sympatry, discussed below, may also be an example of reinforcement. In *Neurospora*, Dettman et al. (36) reported several lines of evidence consistent with reinforcement in the strict sense (invoking natural selection). The evidence was based on categorical scoring of reproductive success in reciprocal crosses, with full success being ejection of >50% black meiospores, black spores having an expected high germination rate. The mean reproductive success was lower in sympatric than in allopatric interspecific crosses at all spatial scales: local (within same collection site), subregional (within the same country in a region), and regional (India, Caribbean Basin, Africa, East Asia), with increased geographical distance predictive of reproductive success and decreased sympatry associated with lower success. Sympatric and allopatric crosses did not show significant phylogenetic divergence with neutral markers, indicating that genome-wide divergence was not the cause of sympatry-associated sexual failure. Hybrid progeny showed reduced fitness in interspecific matings in general; true interspecies hybrids have not been reported from nature.

Intrinsic postzygotic isolation is when hybrids fail, even under optimal conditions. Failure may occur when the hybrid combines parental genomes with (a) different numbers of chromosome sets, i.e., different ploidies; or (b) different gene orders, i.e., translocations; or (c) different alleles that do not function together in a hybrid; or (d) infection by different endosymbionts (32). Strong cases have been made for chromosomal speciation, in which structural changes in chromosomes, types *a* and *b* above, cause reproductive isolation (99). Certainly the whole-genome duplication in an ancient ancestor of *S. cerevisiae* had a stunning impact in driving not only reproductive isolation but also in facilitating ecological specialization towards independence from oxygen (reviewed below). As an explanation of how postzygotic hybrid incompatibilities arise, duplication may be considered as a special case of the Dobzhansky-Muller model (32). [For general discussions of duplication as a source of intrinsic postzygotic isolation see (105).] Structural changes in chromosomes may implicate groups of genes that cause reproductive isolation [for general discussions see (73, 87, 90, 97)]. Because of their compact genomes and ease of manipulation among eukaryotic model systems, fungi have been key in experimental studies of such mechanisms, as discussed further in this review.

Mechanisms of speciation, like species concepts, are subject to spirited partisan argument. There is discussion on whether ecological adaptations precede or follow reproductive isolation, and whether selection or genetic drift promote parallel speciation, when traits that determine reproductive isolation evolve more than once in closely related populations. There have been demonstrations of assortative mating (departure from random mating) by selective environment, for example in populations of walking stick insects where host plant adaptation, rather than genetic drift, promoted parallel evolution of reproductive isolation, that is, in a parallel fashion in several divergent populations (91). Interpretation of the causes for assortative mating in stickleback fish has been contentious (32), but to this reader the supporting studies provide strong evidence for ecological determinants in speciation. McKinnon et al. (80) found that assortative mating based on one trait, body size, accounted for the accretion of reproductive isolation in stickleback fish. The stickleback results were supported both in experimental studies and in samples of populations across the Northern Hemisphere, separated in some cases for hundreds of thousands of years, within the time frame of speciation, although with no implications of sympatric speciation, a controversial issue for fish in postglacial lakes. The chronology of adaptation (or drift) and reproductive isolation is probably best addressed experimentally, and will be discussed in that context later in this review.

The concept of asexual species—and whether any fungi are truly asexual—has been much discussed recently (reviewed in 80, 106, 107). Multilocus phylogenies of a diversity of fungi have repeatedly provided striking resolution of two or more monophyletic lineages within one entity previously accepted as an asexual morphospecies, or as a sexual morpho- or biological species. The cryptic species

concept has emerged for such lineages with the implication that they are new species that are not yet morphologically or reproductively distinct (e.g., 67). Multilocus DNA sequence phylogenies are the method of choice for detecting cryptic species. This is based on the model that phylogenies inferred from more than one genomic region can capture speciation before the whole genome has diverged to the point of splitting (or working backwards, coalescing) for all alleles (7). Divergence in one locus may give only a history of the locus, not the species. In the general speciation literature, apomyxis (asexuality) and autogamy (selfing, which in a haploid can be indistinguishable from apomyxis) are often considered forms of "mating system isolation." Coyne & Orr (32) argue that apomyxis and selfing are not isolating barriers as they do not impede intertaxon gene flow any more than they prevent intrataxon gene flow. The grass endophyte case study discussed later in this review actually illustrates this point quite well. Coyne & Orr suggest that a set of apomictic or automictic individuals is not a biological species but rather a collection of "microspecies," with each individual propagating its own genetic lineage. They present four conditions under which selfing, and, I propose, asexual reproduction in fungi, can lead to speciation by (a) evolving selfing or asexual reproduction through reinforcement to reduce gene flow from populations not adapted to the local habitat, with adaptation producing reproductive isolation as a byproduct; (b) allowing one individual to colonize a new habitat, again with adaptation and reproductive isolation as byproducts; (c) producing new selective pressures that produce reproductive isolation, e.g., smaller flowers with shorter pollen tubes with selfing pollen are outcompeted by pollen from outcrossers when both types of pollen are on longer pollen tubes (34); (d) reducing the effective population size of a taxon, increasing genetic drift and fixing deleterious chromosome translocations, promoting hybrid sterility and polyploid speciation, and hence reproductive isolation. If we are to find the mechanisms of speciation in cryptic species or fully fledged species, we will need to distinguish process from pattern.

Elucidating mechanisms of speciation is not easy. It is difficult to capture a speciation event unfolding in real time. Looking back retrospectively, speciation events may be too slow or too ancient to be captured with the range of powerful statistical approaches that interpret the history of population divergence from mutations in contemporary samples (21, 23). Genetic analysis of many fungi is a challenge because we may not know how to take them through their sexual cycle or because the cycle takes months not days. The genetics of hybrid failure associated with intrinsic postzygotic isolation may be intractable if the hybrids die.

Although there are unseen events en route to speciation and samples of contemporary taxa may not provide the needed representatives of predivergence events, there are several possible approaches to retrospective study of speciation. In order to associate speciation, particularly allopatric speciation, with geological or other events, time estimates based on molecular evolution or mutation rates are necessary. Fossils and other types of evidence have been used to calibrate molecular

clock estimates of fungal evolution (76). Methods for time estimation from phylogenies are improving and have been recently reviewed (5, 101), with several programs available as freeware. Powerful approaches to phylogeography (6, 51) are also available and have been used in studies of fungal speciation to differentiate allopatry from parapatry or sympatry, as described in some examples below.

The use of biogeography to discern mode of speciation, particularly as a kind of test for sympatric speciation among pairs of species, even pairs of different ages, is well reviewed by Coyne & Orr (32). Range overlap is plotted against divergence time; if speciation is allopatric, species begin with a range overlap of zero, with overlap increasing among older taxa as they invade each other's territory. When speciation is sympatric, species begin with a range overlap of one, with subsequent range movements decreasing range overlap. The downside of this approach is that assumptions about ancient distributions may be based on snapshots of highly labile distributional ranges, and purely biogeographical interpretations may miss adaptational phenomena in speciation (74). Another approach would be comparative phylogenetic studies of many species to (a) determine relative rates at which reproductive isolation arises, or (b) find statistically significant associations of phenotypic traits, life history characteristics, or extrinsic events with species divergence, particularly among speciose taxa (i.e., genera or higher-level taxa with relatively high numbers of species), or (c) determine significant associations with specific events in addition to geological phenomena, such as human population migration, international movement of infected materials, or agricultural practice and plant breeding, with population divergence and speciation. Last, speciation studies can be designed to compare closely related species that differ in one trait, such as being a partner in mutualism/symbiosis versus being a free-living saprobe or parasite (e.g., 75). Another example would be species that are separated by one known reproductive barrier or type of reproduction, in order to find the associated reproductive isolation or life history or phenotypic traits (e.g., 67). From such studies of closely related species it should be possible to determine through phylogenetic analyses the chronology of events in speciation. Of course, if the speciation event was ancient, population factors, such as genetic drift, may be lost in the sands of time. In my opinion, experimental approaches are the only way to assign causality and to solve the "chicken and egg" problem of which came first, population divergence through adaptation or drift, or reproductive isolation, as well as the important question of whether genetic control of adaptation is independent of reproductive isolation. These experimental approaches are discussed in the final section of the review.

The balance of this review considers the "speciation menu" of fungi, from which each unique speciation history is derived, then specific examples of fungal speciation in which at least some aspects of speciation are evident, and finally, studies of speciation in experimental populations of fungi, published and proposed, in which conditions are controlled and hypotheses based on specific mechanisms should be testable.

## THE SPECIATION MENU IN FUNGI

### Genetic Processes Include Some that Operate Only Within Species, and Others that Function Between Species, Both Types Maintaining Species Cohesiveness

**WITHIN SPECIES** Sexual compatibility systems regulated by mating-type genes require two gametes with different mating-type specificities for a compatible mating. Although mating types predate speciation events, mating-type genes evolve under selection and are subject to recombination (79). Homothallism (ability to self-fertilize) can derive from heterothallic ancestors (113) by mechanisms other than mating-type switching. A survey of mating-type switching in fungi can be found in Reference 94. Vegetative incompatibility systems control the outcome of vegetative confrontation within species, with compatibility requiring genetic identity, not difference, at certain loci; a difference at a single genetic locus is sufficient for incompatibility (46, 72). The mechanisms functioning between species, intersterility in basidiomycetes, and general decrease in hybrid fitness (well-demonstrated in the model systems, *Neurospora* and *Saccharomyces*) are of direct interest as mechanisms of speciation.

**BETWEEN SPECIES** Intersterility is described only in basidiomycetes. Specific loci are only identified in *Heterobasidion* in which five loci are involved (25). Like vegetative incompatibility, interfertility occurs only with genetic identity, not difference. Parasexuality, another avenue to genetic exchange and recombination, but without meiosis, is usually considered a within-species mechanism, but has been invoked as a mechanism for interspecies hybridization in grass endophytes and arbuscular mycorrhizal fungi, as reviewed in (102).

### Hyphal Fusions and Interconnectedness

This is a unique biological feature of fungi, with systems for self-signaling (47) in mycelial networks that explore and colonize, move nutrients, and make direct contact with plants, animals, and other fungi. The opportunities for horizontal gene transfer are there, but knowledge is rudimentary (reviewed in 100).

### Extensive Asexual Reproduction

Sexual and asexual reproduction combine in the life cycles of a variety of plants, animals, and protists, but the extent of asexual reproduction in fungi is exceptional (1, 106). Extensive asexual reproduction under selection could facilitate clonal speciation (11). While evidence shows that asexual species derive from sexual ancestors and that many fungi have extensive but not exclusive asexual reproduction (106), there are fungi with extensively clonal populations and relatively long-lived clonal lineages (1, 20, 21).



## Ploidy

While most fungi are haploid, the vegetative dikaryon in basidiomycetes has evolutionary potential different from either haploid monokaryons or diploids (27). As in other organisms, polyploidy and aneuploidy are pathways to reproductive isolation and speciation. Emerson (41) made interspecies laboratory crosses of the chytridiomycete *Allomyces*, which has alternation of haploid and diploid thalli. The crosses produced a polyploid series in hybrids comparable to the range in karyotypes observed among these species in the wild. There was a drop in viability in the  $F_1$  (<5%), with a range of inviable results from simple failure to germinate in the majority to production of aborted thalli. In further crosses, however, meiospores of the  $F_2$  and  $F_3$  showed a striking reversion to their parents' level of high viability. An association between viability and chromosome behavior was demonstrated.

## Metapopulations

These are dynamic, complex populations comprising subpopulations that arise and go extinct, drawing from at least one, relatively stable source subpopulation. Metapopulations may be important units in wild fungi, such as ascomycetes with specific substrates or disturbance-mediated reproduction (e.g., fire) and limited long-range dispersal. In the most thorough investigation of the impact of metapopulation structure (in the host) on coevolution, Burdon & Thrall have studied a range of spatial and temporal scales in the *Melampsora lini*-*Linum marginale* system with consideration of the implications for speciation in the rust (16). A key point in considering metapopulation structure as a route to speciation is the potential for subpopulations to diverge through selection or drift (e.g., 22), potentially becoming reproductively isolated.

## Dissemination of Propagules

Dispersal may be long range or short range via meiospores or mitospores, or through soilborne "spore banks" of melanized propagules, including sclerotia, or through long-lived rhizomorphs or other mycelial aggregates that migrate through substrate and soil from a colonized source. Mechanisms of dispersal may be wind, water, insects, and larger animals, including humans. The outcome may be anything in the range from small discontinuous populations, to large, spatially continuous individuals representing one mitotic lineage. The type of dissemination has an impact on patterns of allopatric speciation and on opportunities for sympatric speciation, most likely by hybridization, discussed in case studies below.

## Relation to Host or Substrate

Saprotrophy, parasitic biotrophy, and mutualistic biotrophy will offer opportunities for interaction among sympatric genotypes, or alternatively isolate fungal species

in very restricted niches, or provide opportunities for parallel speciation. The opportunity for hybridization afforded co-occurring grass endophytes is an example (102). Reinforcement of reproductive isolation among species of wood decay fungi that may coexist as saprotrophs in the same log or patch of soil (e.g., 108) is another type of example. A favorite example of what may be parallel speciation in close quarters of a very distinct nature is the cohort of two distinct, unrelated groups of ophiostomatoid species in serotinous infructescences in *Protea* (77). These fungi inhabit, and sometimes cohabit, the closed floral branches that close after pollination, opening to release seeds only with fire. They are highly host-specific species with the morphological features characteristic of insect association in other ophiostomatoid taxa.

## Host Range and Pathogenicity

Clearly, host range and pathogenicity are factors in speciation if the number of fungal species delimited mainly on the basis of host is any guide. In fungi such as the *Magnaporthe grisea* species complex, it is evident that lineage divergence is strongly associated with host preference, both on the level of host species and host variety/cultivar, and that there are important patterns in gains and losses or losses in function of avirulence genes (29, 30). There is also evidence of a radiation in *M. oryzae*, evidenced by its host range on grasses in all major groups, representing 10,000 species and some antiquity, compared with the relatively recent origin of *M. oryzae*. With the elucidation of avirulence genes and their evolutionary patterns, their role as mechanisms in speciation should become clearer.

## Domestication

Fungal substrates or fungal species may be domesticated. Forestry practice or construction with wood has had impacts, such as creating an opportunity for hybridization or genetic drift through a bottleneck that may be speciation mechanisms and are discussed below. The basidiomycete *Serpula lacrymans* has split into two lineages, one associated with wood in forests and one with structural wood. There is evidence of a bottleneck in the transition from the forest to human habitation (52, 58). There is strong evidence that *Aspergillus oryzae* was domesticated from an *A. flavus* ancestor (44, 45), although the lineage divergence time has not yet, to my knowledge, been tested against time estimates based on use of the fungus for producing food and beverages. Domestication of plant hosts has probably had a strong impact on lineage divergence in species of fungal pathogens, as is apparent in *Magnaporthe* from estimates of divergence time based on a multilocus phylogeny that tested the hypothesis (29), but perhaps too recently for speciation. Domestication of fungal species is an issue mainly for conservation of closely related species, as discussed below.

## CASE STUDIES

### Shiitake Mushrooms: Allopatric Speciation, Short-Range Dispersal, and Old World Interfertility Compared with New World Intersterility

Species of *Lentinula* Earle have bifactorial (tetrapolar) mating systems with multiple alleles, and although they produce aurally dispersed meiospores, they lack both mitospores and perennating structures such as rhizomorphs or sclerotia. *Lentinula* species are saprophytes on hardwood logs. Long-distance dispersal would have to be by meiospores or by rafting of a dikaryon in a hardwood log (54). Despite wide distribution, *Lentinula* is not recorded from Europe or Africa. Old World species are interfertile, whereas Old World and New World species are completely intersterile, and the three New World species are intersterile (78a). Shiitake mushrooms have been cultivated for centuries in China and Japan. Hibbett used DNA sequences for the nuclear large subunit rDNA and mitochondrial small subunit rDNA from a set of 48 isolates from both the Old World (Australia, Borneo, China, India, Japan, Nepal, New Guinea, New Zealand, North Korea, Russian Far East, Tasmania, Thailand) and the New World (Brazil, Costa Rica, Mexico, Puerto Rico, Louisiana) to test for rate constancy (molecular clock). He used the internal transcribed spacer (ITS) sequences, also from the nuclear rDNA repeat, to infer a phylogeny of all isolates. Rate constancy was tested with maximum likelihood. Clocks were calibrated using evidence from fossils, results of other molecular clock studies, or estimates of ages that would be expected under a set of alternative historical scenarios. Hibbett dated *Lentinula* to be approximately 34 million years old, and determined that there were at least seven species of *Lentinula* divided in two major clades, one Old World, the other New World. From the phylogenies and molecular clock estimates, the Old World/New World disjunction is the result of vicariance, probably in the fragmentation of an ancient Laurasian range. An alternative Gondwanan hypothesis was not supported. One long-distance dispersal event was suggested between Australia and New Zealand, which is not surprising since fungal dispersal is known to occur across the Tasman Sea (83). Hibbett inferred that long-distance dispersal maybe rare among heterothallic species that lack mitospores or perennating somatic structures or propagules.

### The Species Complex, *Heterobasidion annosum*, Comprises Three Biological Species that Tracked Their Host Tree Genera, with Prezygotic Isolation Reinforced in Sympatry

The basidiomycete *H. annosum* has a unifactorial (bipolar) heterothallic mating system. It is a leading cause of root- and butt-rot of conifers in managed forest stands, from central Finland to Northern Africa and Central America. *H. annosum* was long considered to be one cosmopolitan species with a wide ecological range.

Three intersterility groups have been identified (19, 24, 25, 63, 104). The S intersterility group attacks a variety of tree genera in North America, but occurs almost exclusively on *Picea abies* in most parts of Europe, in northeastern Europe attacking *Abies sibirica* (65). The P group is mainly associated with pine, but can attack other tree species (63). The F group is facultatively pathogenic or saprotrophic on *Abies* spp. and has a distribution limited to central and southern Europe (66). The S, P, and F groups have been recognized as *H. parviporum*, *H. annosum*, and *H. abietum*, respectively.

Chase & Ullrich (25) demonstrated the segregation of five gene loci, four with alleles, for intersterility and interfertility, distinct from determinants of mating-type compatibility. Homozygosity at the + allele of any of the five loci is required for formation of the dikaryon, an interfertile reaction. Among North American isolates (24) there was a surprisingly high level of interfertility between S and P groups, but intersterility between North American and Finnish isolates. In Europe, although the interfertility of P group isolates with S or F group isolates is low (104), interfertility of S and F group isolates is 25%–75% depending on where the S group isolates originate (64, 65). Sympatric S and F group pairings of isolates were 24% interfertile (based on dikaryon formation), whereas allopatric pairings were about 72% interfertile (64). Although the evidence suggests reinforcement of intersterility among sympatric populations, any amount of interfertility would allow hybridization of intersterility groups in one or two steps, such as through an intermediary genotype with partial interfertility.

Johannesson & Stenlid (57) have reviewed the literature on phylogenetic divergence in *H. annosum*, and have conducted the most definitive study to date based on four genomic regions (loci) in 29 isolates from Europe, Asia, and North America to trace the phylogeography of the S and F intersterility groups. For most loci, and for the combined dataset, Euroasian S, European F, and North American S isolates comprise three phylogenetic species. There is evidence of continental differentiation, with the European and North American S groups forming sister clades and subsequent differentiation of Japanese S from the rest of Euroasian S, and Mexican S from North American S. The authors consider alternatives, but interpret their data to indicate that although the differentiation between S and F groups is ancient, it relates to the migration of the main hosts, *Abies* and *Picea*, into Europe from eastern Asia. One population of *H. annosum* following *A. sibirica* or *P. abies* through Siberia adapted to attack both these species and became the S type. The F type may have originated on the southern migration route of *Abies* (from which *Picea* may have been negligible). Hybrids of S and F might have had reduced fitness, as yet untested, favoring the subsequent reproductive isolation of sympatric S and F populations in Europe. Interestingly, this pattern was consistent with patterns of divergence in the manganese peroxidase and laccase genes, implicated in wood decay, although it is not indicated whether polymorphisms in these genes are in synonymous or nonsynonymous positions. Time estimates based on molecular clocks or associated statistical approaches have not yet been employed; S, P, and SP hybrids have been found on a single stump of ponderosa pine in

California, with stumps hypothesized as a conducive environment for hybridization (43). Further investigation of this natural hybrid demonstrated its genetic stability; it is either a heterokaryon undergoing a primary homothallic phase or a diploid with limited ability to exchange nuclei when mated with homokaryons (42a). SP hybrids have also been reported from Europe (103). More data are needed on the fitness of hybrids. One experimental approach would be to set out sporulating hybrids in the field, allow cycles of dispersal, colonization, and sporulation, and follow hybrid frequency over time.

## New Hybrid Poplars Are Vulnerable to a Hybrid Rust

The basidiomycete *Melampsora medusae* is primarily found east of the Rocky Mountains on *Populus deltoides* and its hybrids, but not on *P. trichocarpa*. *M. occidentalis* was, prior to 1991, the only *Melampsora* species occurring on *P. trichocarpa* in the Pacific Northwest; it also occurs east of the Cascade Mountains on *P. trichocarpa*. In the 1980s, commercial poplar clones (*P. deltoides* × *P. trichocarpa*) that were bred for resistance to *M. occidentalis* were planted extensively in the Pacific Northwest. *M. medusae* first appeared on the hybrid poplar in 1991. Until then, the hybrid clones were free of leaf rust. *M. medusae* populations showed little pathogenic variation until 1994.

Newcombe et al. (89) studied the rusts by means of extensive statistical comparison of morphological characters of urediniospores and teliospores in recent and archival collections (as herbarium specimens), host range inoculation studies, and comparison of sequence divergence in the ITS. They also executed painstaking reconstruction of host ranges in the light of current taxonomic concepts in *Populus*. They found that a hybrid of *M. medusae* and *M. occidentalis*, *M. x columbiana*, had spread through the hybrid poplars to the extent that by 1997, the rust hybrid was the only species recovered. The hybrid has morphological character states intermediate between *M. medusae* and *M. occidentalis* and shows mixed avirulence/virulence on *P. deltoides* and *P. trichocarpa*—in other words, a range of responses on poplar differentials. Hybrids were frequently heterozygous for ITS sequences, but there were also cases with hybrid urediniospore morphology but with the ITS sequence of only *M. medusae* or *M. occidentalis* that were consistent with introgression with one of the parent species. Examination of the herbarium specimens revealed that the rust hybrid had existed back to 1910, from specimens collected from Wisconsin, west to California. The authors suggest that “an old population” of the rust hybrid was distributed where natural poplar hybrids are abundant in the intermountain West and east of the Rocky Mountains. The “new population” would have arisen in the Pacific Northwest with the arrival of *M. medusae*, where only *M. occidentalis* had been established previously. The new population has a wider host range than either parent, as determined by experimental inoculations. The epidemiologically emergent hybrid is a phenomenon noted elsewhere, such as in *Phytophthora*, including the new series of heteroploid hybrids associated with *Alnus* (12, 15), and in *Ophiostoma*, discussed below.

More recent work by Newcombe and colleagues (88) has demonstrated abundant pathogenic variation in the new rust hybrids, matching resistance determinants in both *P. deltoides* and *P. trichocarpa*. The commercial hybrids are extremely vulnerable. A defeated major resistance gene among some of these hybrid poplar clones was determined to have no residual effect, i.e.,  $F_2$ s with the gene had the same degree of partial resistance in 1997 and 2000 as their full siblings lacking the gene (112).

## Extensive Hybridization Among Grass Endophytes Facilitated by Close Cohabitation in the Host, Parasexual Hybridization, Perpetuation of Asexual Heteroploid Hybrids Through Efficient Vertical Transmission, and Hybrid Fitness Advantage

Species of *Epichloë* occupy their grass hosts as haploid mycelia and have sexual phases in stromata on the host, with fertilization by fly-transmitted spermatia. Species of *Neotyphodium* are asexual descendants of *Epichloë* and frequently occur as heteroploid hybrids, with significantly more DNA per nucleus than in the haploid somatic nuclei of sexual species (68). Transmission is either horizontal, by meiospores, or vertical, via infected seed. If heteroploid hybrids are compatible with the host, they are transmitted vertically with high efficiency (102). *Epichloë* partially or completely inhibits host seed production, whereas *Neotyphodium* exploits seed in transmission.

Species of *Epichloë* are reproductively isolated (as evidenced by mating tests). Most species are phylogenetically distinct. This is consistent with reproductive isolation and suggests that sexual hybridization is not frequent, as extensive genetic exchange among clades would be very evident in poorly supported branching topologies. *Neotyphodium* species, in contrast, bear the phylogenetic signature of hybridization. Figure 2 in Reference 102 shows that for the *tub2* ( $\beta$ -tubulin) locus, 8 of 9 *Neotyphodium* species are hybrids, whereas 11 *Epichloë* species are not hybrids. Hybrids have two or three *tub2* alleles, consistent with origins in one or two hybridization events, respectively. The same situation pertains for two other loci: *tef1*, translation elongation factor 1- $\alpha$ , and *act1*,  $\gamma$ -actin (33, 81, 82). The alternative explanations are unlikely as (a) gene duplication, aneuploidy, or polyploidy arising through clonal lineages would not result in combinations of alleles from two or three clades in the phylogeny, or (b) an ancient gene duplication in an ancestor, with loss of genes in descendant lineages, is a less parsimonious explanation than hybridization for the evolutionary pattern observed, with hybrids retaining alleles of their ancestor species. Presence of divergent paralogs alone would not be evidence of hybridization. There is no evidence of vegetative incompatibility in *Epichloë*, and formation of interspecific heterokaryons has been demonstrated (26). With formation of diploids in such a heterokaryon, then karyogamy and mitotic recombination, we would expect to see alleles of the parent *Epichloë* species segregating as in meiotic recombination, although not necessarily in the same frequencies. Mitospores of *Neotyphodium* are uninucleate, with each nucleus of a

hybrid including alleles of both or all of its ancestral *Epichloë* species. This and the phylogenetic evidence against sexual hybridization are consistent with parasexual hybridization. Since the endophyte species do not seem to compete but instead tend to replace each other, the best opportunity for hybridization would be shortly after a secondary infection by meiospores of *Epichloë*.

Hybrid endophytes predominate in some grasses, suggesting that they have a fitness advantage, replacing nonhybrids (102), although this does not seem to have been tested experimentally. The hybrids may reward host grasses; hybrids are seedborne, and many seedborne endophytes produce copious alkaloids with antiherbivore effects (18).

### Speciation is a Dynamic Process when Allopatric Taxa are Brought Together in *Ophiostoma ulmi* and *O. novo-ulmi*

Although previously unreported in Europe and North America, since 1900 two pandemics of Dutch elm disease have swept the entire Northern Hemisphere (reviewed in 13). The first pandemic was caused by the ascomycete *Ophiostoma ulmi*, which spread from northwest Europe eastward via sequential epidemic fronts into southwest Asia and westward via movement of infested timber to the United Kingdom, North America, and Central Asia. This epidemic declined in the 1940s in Europe, but not in North America. The decline has been attributed to an infectious deleterious virus in the European population. The second pandemic, caused by the previously unknown *O. novo-ulmi*, has been inferred from geographic sampling to have two origins starting in the 1940s. The Eurasian form (EAN or *O. novo-ulmi* subspecies *novo-ulmi*) appeared in eastern Europe and then migrated westward across Europe and eastward to Asia (with some steps likely through importation). The North American form (NAN or *O. novo-ulmi* subsp. *americana*) appeared in the southern Great Lakes and reached both coasts of the continent by the 1970s and 1980s. It moved in an importation jump from North America to Britain in the 1960s, from which it has spread across western Europe. The patterns of descent are as yet unknown for *O. ulmi*, *O. novo-ulmi*, the two subspecies of *O. novo-ulmi*, and the relatively recently discovered Himalayan endemic, *O. himal-ulmi*.

Of interest here are the consequences of sequential introductions of *O. novo-ulmi* in replacing *O. ulmi* populations, and the interaction between the EAN and NAN subspecies. Intraspecies crosses are highly fertile. Interspecies crosses encounter both pre- and postzygotic barriers, although reproductive isolation is not complete. Interspecies crosses are incompatible when *O. ulmi* is the male but can be compatible when *O. ulmi* is the female. Among the wide range of nonparental phenotypes displayed among progeny are female sterility, reduced fitness and lowered aggressiveness, even in comparison to the *O. ulmi* parent. The strong but partial incompatibility has been interpreted as evidence of their allopatric origins (61). Although *O. novo-ulmi* has a fitness advantage over *O. ulmi* and replaces it, when the two species initially co-occur they may be in close contact in scolytid bark beetle galleries affording the opportunity for hybridization. Of 11,000 isolates from

bark sampled from Europe and North America (10), 9 isolates were recovered with composites of phenotypic and molecular characters indicative of hybridization between *O. ulmi* and *O. novo-ulmi* and consistent with the range of phenotypic variants and patterns of sexuality, such as female sterility, observed in interspecies laboratory matings. Molecular characters included diagnostic polymorphisms in a cerato-ulmin toxin gene. Based on experimental and circumstantial evidence, these hybrids were unfit, rare, and transient—likely a step in the replacement of *O. ulmi* by *O. novo-ulmi*, but possibly a genetic bridge between the two species. Among these and other lines of evidence for introgression between the two species summarized by Brasier (13) have been increases in vegetative incompatibility groups (VCGs) in populations of *O. novo-ulmi*. *O. novo-ulmi* invades a new location as one clone of one VCG type and one mating type. Deleterious, infective viruses can spread rapidly through such a clone, but may be impeded from transmission by vegetative incompatibility. Within a few years after introduction, a population of *O. novo-ulmi* diversifies into multiple VCGs (with a decrease in frequency of the viruses) and shows increased phenotypic variability. Based on observations that this process occurs where *O. novo-ulmi*, *O. ulmi*, and the viruses co-occur, Brasier and coworkers have proposed that the VC genes (and possibly viruses) in *O. novo-ulmi* are acquired from *O. ulmi*, with the selection pressure imposed by the viruses favoring new VCGs over those associated with the invasive clone or clones.

Despite partial prezygotic reproductive isolation, the EAN and NAN forms can hybridize. EAN, as a female parent, partially rejects NAN as a male parent, whereas NAN as female accepts EAN as male (14). Konrad et al. (62) provided evidence of a fixed mutation in a cerato-ulmin toxin gene associated with NAN and one associated with a colony-type gene associated with EAN coexisting in isolates of putative hybrids (62). There is now evidence that the EAN and NAN forms are freely hybridizing in Europe with no loss of aggressiveness on elm (14, 62).

## Whole-Genome Duplication Was a Source of Novel Genes Facilitating Divergence in *Saccharomyces*: Retrospective Studies of Ancient Events with the Aid of Large-Scale Comparative Genomics

While phylogenetic studies of single or multilocus DNA sequence are a form of comparative genomics, comparison of complete, annotated genomes is beginning to yield an understanding of the mechanisms of evolutionary divergence. This retrospective analysis should suggest mechanisms of speciation that can be tested in comparative and experimental studies. The fungi are already a model group in this endeavor. Because of their compact genomes: an increasing number of species are being completely sequenced, including the model species *Neurospora crassa*, *Schizosaccharomyces pombe*, and *Saccharomyces cerevisiae*, as well as many more species of medical, industrial, or agricultural importance. Currently, the best-characterized group of fungi for comparative genomics on a whole-genome scale are the Saccharomycetes (classification underlying GenBank and BLAST,



from the National Center for Biotechnology Information, NCBI) also referred to as the Hemiascomycetes (see 40).

Dujon et al. (40), Kellis et al. (59), and Dietrich et al. (37) have identified key molecular events in the evolution of the Saccharomycetes, bearing in mind that all of these taxa are now highly evolved, their ancestors long gone. Dujon and coworkers, in the most taxonomically comprehensive comparison (40), proposed four key steps in transitions represented by five species: (a) *S. cerevisiae*, baker's yeast, with a predominantly diploid lifecycle that is heterothallic with a type of homothallism based on mating-type switching; (b) *Candida glabrata*, with no known sexual cycle but two mating types, a human pathogen more closely related to *Saccharomyces* than to *Candida albicans*; (c) *Kluyveromyces lactis*, a heterothallic with a mainly haploid life cycle, with an interesting placement in hemiascomycete phylogenies; (d) *Debaryomyces hansenii*, a homothallic, mainly haploid, halotolerant yeast related to *C. albicans* and other pathogenic yeasts; and (e) *Yarrowia lipolytica*, an alkane-utilizing yeast and a haplo-diplontic genetic system, i.e., alternation of generations, of more distant relatedness to the other yeasts. *D. hansenii* and *Y. lipolytica* have only one mating-type (MAT) locus, whereas *C. glabrata* and *K. lactis* have two silent mating-type cassette homologues, similar to *S. cerevisiae*. For each species the haploid "type strain" was sequenced. The key events were, sequentially, (a) genome size control in some lineages via loss of DNA transposons and introns, with the result that new paralogs and new genes would mainly be created through coordinated duplication of genes, (b) next, tandem gene repeats or in other lineages, (c) the appearance of new centromeres facilitating chromosome segregation and the appearance of three new mating-type (MAT) cassettes, altering sexual potential, and finally, (d) whole-genome duplication discussed further below, with more extensive loss of paralogs in some lineages, such as *C. glabrata*, with consequent loss of function and a kind of reductive evolution, compared with evolution by accretion in others, such as *S. cerevisiae*, which retained many paralogs with consequent increase in function.

Independent groups of organisms have found a source of novel genes by means of genome duplication. Eukaryotic genomes generally show relatively high redundancy. While duplicated genes are dispersed in some organisms, such as *Drosophila melanogaster* and the yeast *Candida albicans*, large segments of duplication are the rule in humans, *Arabidopsis thaliana*, and *S. cerevisiae* (111). Whole-genome duplication occurred in an ancestor of *Candida glabrata* and a group of species including the *sensu stricto* clade of *Saccharomyces* (Clade 1 in 69). *Kluyveromyces waitii* and *Ashbya gossipii* diverged from this lineage before the gene duplication event. Genome comparisons demonstrate that the duplication was of the whole genome and therefore was a polyploidization event, not aneuploidization or another form of large-scale duplication (110).

Once the 1:2 relationship of the genomes is taken into consideration, the colinearity of gene order, also termed synteny, among these species allows comparison of *S. cerevisiae* with *A. gossipii* and *K. waitii* and facilitates identification of those surviving gene pairs formed by gene duplication (37, 59). In *S. cerevisiae*,

approximately 500 paralogs indicate that an ancestral 5000-gene genome was duplicated, with subsequent loss of 90% of one gene copy, leaving the original 5000 genes plus 500 extras. One major group of surviving genes, including almost all of the cytosolic ribosomal protein genes and translation genes such as elongation factors, demonstrates almost no sequence divergence between paralogs, possibly as a result of gene conversion (59, 71). The other, larger group of surviving genes includes pairs that are highly divergent. Evolutionary rates between these pairs are asymmetric, and Kellis et al. (59) proposed that the function of the slower-evolving copy is more similar to the ancestral, preduplication function of the gene, with the faster-evolving copy moving toward the derived function. Some of these genes are so highly divergent that they fail to match their partner paralog in BLAST searches.

New studies suggest that genome duplication has been important in evolutionary divergence and ecological specialization of *S. cerevisiae* in such areas as fermentation, response to glucose, budding pattern, and colony morphology (reviewed in 110). For example, unlike *K. waltii* and *A. gossypii*, *S. cerevisiae* can grow vigorously under almost anaerobic conditions in the presence of glucose. Among many of the paralog pairs, transcription in one member is induced under aerobic conditions while the other is induced under hypoxic conditions (70). Gene duplication has facilitated oxygen independence and efficient use of glucose. This may have coincided with the radiation of fruit-bearing plants, 100–200 mya (96). Such yeast lineages would have been able to grow vigorously with or without oxygen and they would have produced ethanol, toxic to bacterial competitors.

## Experimental Removal of Reproductive Barriers to Hybridization

“ENGINEERED EVOLUTION” OF COLLINEAR *SACCHAROMYCES* SPECIES REDUCES POSTZYGOTIC REPRODUCTIVE ISOLATION AND PRODUCES ANEUPLOID HYBRIDS Chromosomal speciation theory proposes that structural changes in chromosomes impose reproductive isolation. As a kind of alternative, more consistent with genic speciation theory (32), structural changes in chromosomes may implicate suites of genes imposing reproductive isolation (90, 97).

*Saccharomyces sensu stricto* (Clade 1 in 69), includes six species: *S. cerevisiae*, *S. paradoxus*, *S. bayanus*, *S. cariocanus*, *S. mikatae*, and *S. kudriavzevii*. These species show little prezygotic isolation, hybridizing readily both in nature and in the laboratory, but postzygotic isolation is strong, with greater than 95% ascospore inviability (86). Three of these species have known reciprocal translocations, with four in *S. bayanus* and *S. cariocanus*, and one and two, respectively, for two laboratory strains of *S. mikatae*. Recently such reciprocal translocations have been demonstrated to convey a fitness benefit in strains of *S. cerevisiae* engineered to include them, when competed against the reference *S. cerevisiae* strain that lack them (28). Although it has been proposed that by reducing hybrid viability, translocations are a mechanism of speciation, no association was found between

the pattern of translocations and the phylogeny of these species (42). Delneri and coworkers (35) reconfigured three chromosomes of *S. cerevisiae* to produce the two reciprocal translocations found in the two strains of *S. mikatae*. The engineered strain of *S. cerevisiae* was collinear with *S. mikatae*; in other words, it had the same gene order with respect to the translocations as *S. mikatae*. Interspecies crosses were made among several species in the *sensu stricto* group. Interspecies and intraspecies crosses between collinear strains showed significantly greater ascospore viability than those between noncollinear strains. Viability ranged from zero in some collinear hybrids to 15%–30% in others. So while this study provides evidence that chromosomal speciation can contribute to reproductive isolation, the extensive sterility among meiospores of hybrids does not predict that this mechanism of speciation is common (32). Of course, two strains that are apparently collinear may carry additional, smaller differences, such as a gene deletion in an otherwise conserved chromosome segment and comparable studies of collinearity on a finer scale (“microsynteny”) could, in theory, produce hybrids with higher fertility (109).

Delneri and coworkers also found that viable spores from the engineered strain of *S. cerevisiae* and one of the strains of *S. mikatae* demonstrated extensive aneuploidy. Spores from many interspecies hybrids contained a complete set of chromosomes of one parent (unexpected after meiosis) and half from the other parent (as expected after meiosis). The authors suggest that the chromosome numbers were imbalanced in the zygote, well before meiosis. The pattern of spore karyotypes suggested duplications of one parent species in the hybrid. This is consistent with results from hybrid zygotes in wider interspecies crosses between more distantly related *Saccharomyces* species, in which chromosomes of one parent are eliminated (78).

Delneri and coworkers (35) suggested that genome duplication may have occurred by allopolyploidization or prevalent aneuploidy in this group of yeasts, proposing aneuploidy as a “major route” to speciation, and the source of the composite nature of the genome in *Saccharomyces cerevisiae*. However, translocations are sufficient but not necessary as an isolating mechanism in these yeasts: Many extant species in this group have collinear genomes, some producing sterile hybrids.

CHROMOSOME DOUBLING THROUGH “AUTOFERTILIZATION” IN MATING-TYPE SWITCHING REMOVES POSTZYGOTIC REPRODUCTIVE BARRIERS IN *SACCHAROMYCES* Greig et al. (49) hybridized *S. cerevisiae* and *S. paradoxus*, which though closely related (69) are postzygotically isolated, with a high level of aneuploidy in hybrids and about 1% viability among meiospores formed from the  $F_1$  (86). Despite this low viability, yeast populations are so large that viable meiospores can be found. By allowing the  $F_1$  meiospores to germinate, form clonal colonies by mitosis and undergo mating-type switching, then mate intraclonally, a homozygous, diploid  $F_2$  generation was formed by “autofertilization,” also termed autodiploidization. In the  $F_2$ , both sporulation and meiospore viability were high,

around 80%, although lower than the parents, which approached 100%. F<sub>2</sub> hybrids had high fertility when crossed with themselves, but low fertility when crossed with their parents. This would suggest that in the backcrosses to parents, many gene combinations between genes of *S. cerevisiae* and *S. paradoxus* have negative effects on fertility. Aneuploidy in F<sub>2</sub>s is also a possibility. Full tetraploid F<sub>1</sub> hybrids between these two species have high fertility (48). Given that incompatibilities are recessive, the authors estimate that the genetic variability they represent accounts for 50% of the variation in self-fertility in F<sub>2</sub> hybrids (49). In comparisons of fitness at temperatures favoring each of the parents (30°C for *S. cerevisiae* and 10°C for *S. paradoxus*), F<sub>2</sub>s showed a high genetic variation for fitness, were less fit than one of the parents at each temperature, and demonstrated a correlation between fitness at one temperature with fitness at the other. This suggests that selection of hybrids might occur under intermediate or fluctuating conditions, although this hypothesis was not tested. The authors propose that “autofertilization” facilitates “homoploid” hybrid speciation by producing identical chromosomal homologues at every gene pair, except the mating-type locus, escaping incompatibilities in mating with other gametes “even from the same parent.” They suggest that this mechanism of speciation may be common in other organisms with gametophytic selfing, including other fungi.

EXPERIMENTAL DEACTIVATION OF THE MISMATCH REPAIR SYSTEM IN MEIOSIS REMOVES POSTZYGOTIC REPRODUCTIVE ISOLATION IN HYBRIDS OF *SACCHAROMYCES*

The previous examples have demonstrated that hybrid sterility could be mitigated by elimination of chromosomal imbalances, such as translocations or mismatches in ploidy. Alternatively, elimination of genetic incompatibilities conveying an adaptive or housekeeping disadvantage to hybrids could be effective. Disabling of mismatch repair (MMR) in interspecies crosses in *Saccharomyces* (56) increased fertility compared with crosses of strains with functional MMR genes. MMR corrects mismatched bases after DNA replication by detecting mispaired heteroduplex DNA and blocking recombination. The result is chromosome loss and aneuploidy in the meiotic products. MMR disrupts postzygotic fertility in diploid, but not tetraploid hybrids, where all chromosomes have pairing partners. Greig et al. (50) approached MMR from the point of incipient speciation in populations of *Saccharomyces*. They disrupted two MMR genes in *S. cerevisiae* and used the knockout strains to make intraspecies hybrids, differentiating the resulting effect of increased mutation from the effects decreased mismatch repair. They crossed two laboratory strains of *S. cerevisiae*, and also did wider crosses of wild isolates of *S. paradoxus* representing allozyme types indicative of incipient speciation. In crosses of *S. cerevisiae*, fertility was significantly increased with disabling of either mismatch repair gene. Six interstrain crosses of six Far Eastern with six European/North American strains of *S. paradoxus* demonstrated reduced fertility compared with intrastrain crosses. Deletion of a MMR gene increased fertility. In crosses of both *S. cerevisiae* and *S. paradoxus*, approximately 50% of the fertility reduction in crosses could be attributed to the MMR genes.

The authors proposed that mismatch repair, associated as it is with the ubiquitous process of meiosis, could drive gradual divergence in speciation in these and perhaps other eukaryotes. In disputing the universality of this mechanism, Coyne & Orr (32) commented that MMR may be more important in species pairs with higher sequence divergence than between most crossable plant species, indicating as well that no evidence exists for mismatch repair causing postzygotic isolation in metazoans.

## SUMMARY: KNOWN MECHANISMS OF SPECIATION IN FUNGI

Most of our current information is based on retrospective studies, with few workers yet exploiting the full power of statistical methods for phylogenetic inference, including estimates of divergence time. Experimental studies have been mainly with yeast and are still limited compared with the body of experimental speciation research in *Drosophila* (reviewed in 32). There are also major groups of fungi that receive little attention. For example, not much has been done on the Zygomycota, although parasexual hybridization has been proposed among arbuscular mycorrhizal fungi (102), or since the work of Emerson on *Allomyces*, in the Chytridiomycota. Wild ascomycetes, including many genera that are rich with species, are poorly understood. For that matter, although patterns of speciation and lineage divergence in pathogenic ascomycetes point to movement of host material (including human hosts), domestication, loss of sex, and deployment of host genetic material and fungicides as important mechanisms influencing gene flow and reproductive cohesiveness of populations, we cannot speak with confidence on the mechanisms of speciation.

The dominance of asexual reproduction, with loss of sex in some lineages, especially among the ascomycetes, can result in lineage splitting through accretion of mutations (with evolutionary divergence perhaps accelerated by genetic exchange and recombination in sexual reproduction or parasexuality). Clearly, this evolutionary divergence is a process that can lead to speciation, noting that (a) the effects of selection versus genetic drift should, and can, be addressed in retrospective studies (112a), and (b) the status of such lineages as asexual species requires strong support in concordant multilocus phylogenies. The dynamics of epidemics of fungi among populations of plants or animals and their impact on clonal speciation, and the more rapid and responsive process of hybridization, have been elegantly conceptualized (11, 12). But the evidence, such as the emergence of highly fit and aggressive clones with loss of a mating type, is in a contemporary time scale, indicative of lineage divergence. This is not the protracted process of speciation. The divergence of lineages compared with speciation in *Sclerotinia* (21) is a good example of the challenge of working back to relatively ancient speciation events from reconstruction of contemporary mitotic lineages, even with multilocus molecular data and strong statistical methods. There is a distinction

between finding patterns of lineage divergence and speciation, and demonstrating mechanisms of divergence and speciation.

The ancient genome duplication in an ancestor of *S. cerevisiae* was a major mechanism of divergence likely leading to speciation. The whole-genome duplication was the grist for the evolution of new genes, allowing the original gene to maintain continuity in the gene product and in cell function, while providing a paralog that could “experiment,” diverging in product and function (92). More such examples are probably yet to be discovered as more fungal genomes are elucidated and compared.

I have put some emphasis on speciation in the Basidiomycota. Here, examples of classic allopatric speciation associated with key geological events are consistent with considerable evidence for evolution of prezygotic reproductive isolation in allopatry in plants and animals. Partial reproductive isolation observed in some cases in allopatry is consistent with reinforcement of isolation in sympatry. Partial compatibility in sympatry is likely to be countered by a lack of fitness in hybrids, such as in *H. annosum*, although this has not been confirmed experimentally in all cases where it has been suggested.

Across all fungal phyla, hybridization is likely the most direct route to sympatric speciation, although there are as yet only a few examples of hybridization adequately supported by evidence. For examples of sympatric speciation, we could look toward pairs of sister species with different ecological specificities, including host, although it is very difficult to prove retrospectively that such pairs were not originally allopatric (e.g., some species of *Armillaria*).

Host shifts, another potential route to sympatric or allopatric speciation, are difficult to observe in the time frame of speciation and will be addressed further in the next section. Domestication of a host or substrate, or of a fungal species, is particularly interesting. For host or substrate, the likely outcome is a bottleneck in the fungus, as in *Serpula lacrymans*, assuming that only some individuals have the subset of pathogenicity determinants, degradative capabilities, or other adaptive traits to move to the new situation. When the fungus is domesticated, a possible outcome is replacement of noncultivated, closely related species, a conservation problem (55, 60).

## PROSPECTS: EXPERIMENTAL APPROACHES TO ELUCIDATING SPECIATION MECHANISMS IN FUNGI

There are three main approaches to experimental studies of speciation. First, testing a mechanism through genetic engineering, as described for the engineering of collinear genomes or disabling of mismatch repair to overcome intrinsic postzygotic reproductive isolation in *Saccharomyces*, as described previously. This approach can establish causality and demonstrate that the association with speciation is sufficient, but not that the mechanism is widespread or necessary for speciation. Because speciation studies thrive in a dialectical culture, an elegant experimental

study establishing causality is a contribution to the debate. That the mechanism is the one, most important mechanism of speciation would have to be established through extensive comparative study.

The second approach is more ecological (and phytopathological). The example is actually a study of a host shift of anther smut *Microbotryum* from *Silene alba* to *Silene vulgaris* (4). *Microbotryum violaceum* infects over 100 species in the Caryophyllaceae and, based on patterns of host specificity and morphological variation, is considered by some to be a species complex. Anther smuts cause systemic infections during which all flowers of a plant evidence disease with smut spore-filled anthers. Following vector transmission, spores germinate on a healthy host, undergo meiosis, and mate mainly by plasmogamy and karyogamy within the tetrad, i.e. in each tetrad, sisters mate. Karyotype comparison of *Microbotryum* from *S. alba* and *S. vulgaris* indicated recent transmission between hosts, likely from *S. alba* to *S. vulgaris*. Spatial analysis of field populations indicated that the host shift was dependant on the close co-occurrence of the hosts. Reciprocal inoculations showed intraspecies variation in susceptibility, consistent with potential for rapid evolution of resistance. Disease expression on the new host was abnormal, consistent with maladaptation to the new host. In experimental populations, transmission within the populations of the old host, *S. alba*, surpassed that in populations of the new host, *S. vulgaris*, with substantial transmission back to the old host. This study indicated that while *Microbotryum* might have diverged a new host race on *S. vulgaris*, continued gene flow between *S. alba* and *S. vulgaris* might limit divergence. I cite this example because the host shift may represent an incipient speciation event. The authors continued population studies by replacing dead plants with plants grown from seed recovered from the same population in the previous season. They also proceeded to experiments in the laboratory and greenhouse, passaging the pathogen through sequential inoculation, to determine the rate of adaptation to the new host with respect to a potential loss of pathogenicity on the old host.

The third approach is experimental evolution of speciation, following experiments (in organisms other than fungi) that have demonstrated that replicate populations reared under different conditions coincidentally evolve reproductive isolation. Coyne & Orr (32) wrote, "Given enough time, and barring extinction, any pair of geographically isolated taxa is likely to evolve reproductive barriers. Laboratory selection experiments (unfortunately limited to Diptera) support the basic premise of allopatric speciation—that divergent selection indirectly produces isolating barriers. Reproductive isolation in such studies arises surprisingly fast. This isolation is probably a byproduct of the same genes that underlie the response to selection, but exactly how this happens remains unknown." In a well-cited selection experiment, eight populations of *Drosophila pseudoobscura* were derived from one wild population (39). Four lines were serially reared on a stressful starch medium and four on a stressful maltose medium. After a year of adaptation, populations reared on different media were reproductively isolated, but showed significant assortative mating in starch  $\times$  starch and maltose  $\times$  maltose matings. Rice & Hostert (98)

found a significant change from interfertility to prezygotic isolation between populations reared under divergent conditions in 11 of 14 such experiments in the literature, with the remaining 3 not showing significant change. The beauty of such experiments is that one can start with a uniform population with as much genetic variation as one wants, and from this, create replicate populations. All of the advantages of experimental evolution are there, including control of population size, with the potential to test founder effects under genetic drift by putting populations through bottlenecks. Whether or not reproductive isolation is genetically independent of adaptation can be determined. Determinants of adaptation and of isolation (prezygotic or postzygotic) may be genetically interrelated, either through pleiotropy (one gene influences more than one phenotype) or hitchhiking (genes with close physical linkage to genes conveying increased fitness come along for the ride). With a genomic approach, adaptation genes, isolation genes, and stress response genes could be distinguished (or not, in the case of pleiotropy or hitchhiking). Many fungi are haploid, making interpretation of mutational effects and use of microarrays somewhat more direct. We see promise for this experimental approach, especially given the ample evidence of rapid adaptation in experimentally evolved populations of both yeasts and filamentous fungi (3, 27, 31).

## CONCLUSIONS

In preparing this review, one of the most striking things I have discovered is that fungi are missing in the speciation debate. Coyne & Orr (32) discussed some specifics: ancient gene duplication and engineering of colinearity or mismatch repair disfunction in yeast, and also evidence for reinforcement in *Neurospora*, “and the references therein” cited in (36). But it is obvious that the canon is based on plants and animals. My point is not just that the roughly 100,000 to 1,500,000 species of fungi deserve more respect. Rather, that basing speciation theory on plants and animals—and to a very small extent on bacteria—is missing something important in the synthesis. The book on fungal speciation is yet to be written, and we need to do more hypothesis-driven research in order to write it. From the more local perspective of phytopathology, speciation is the window on the future of pathogens. Issues such as disease control, quarantine and free trade, and conservation of ecosystems and fungal species depend on our understanding not just of species diagnosis, but also of how species come to be.

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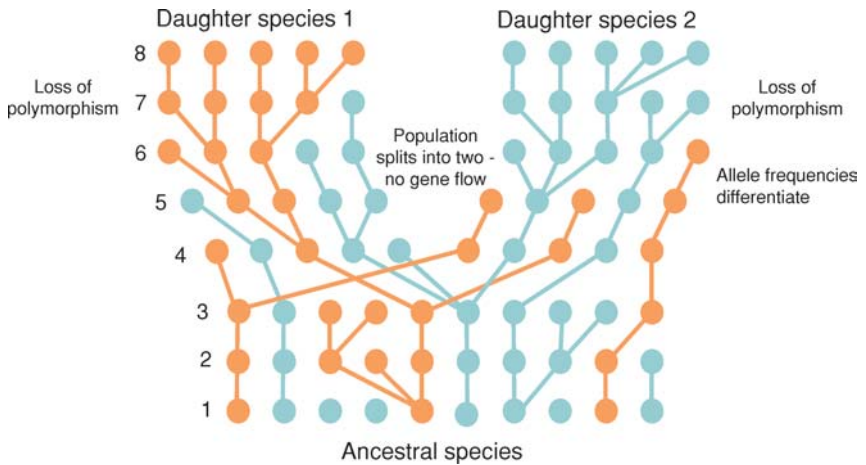
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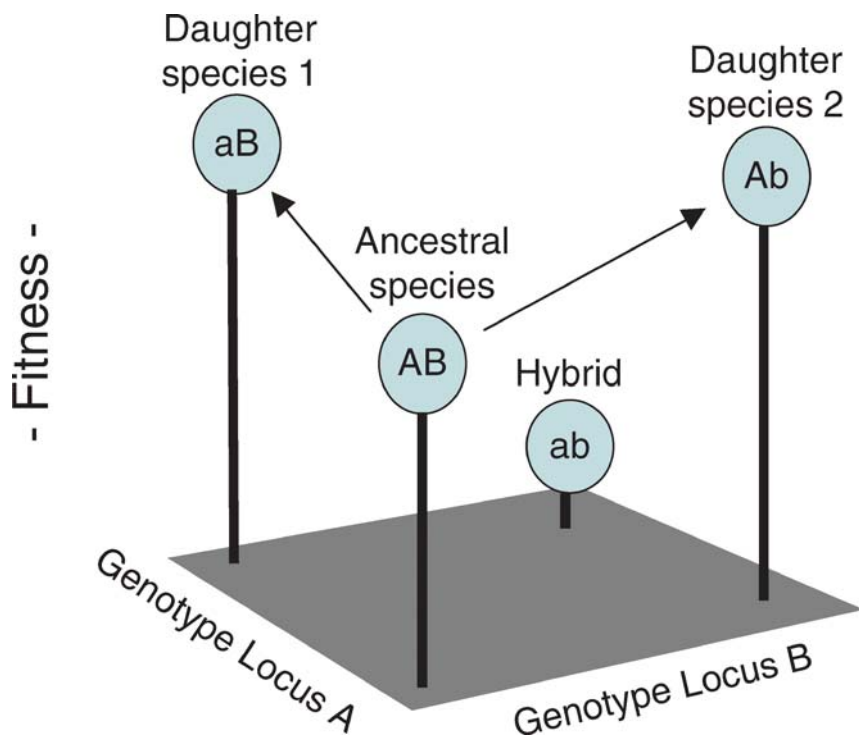
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**Figure 1** Accumulation of genetic differences and speciation. Circles represent a single gene locus with two alleles, orange and blue. The numbers represent the generations. Lines between generations represent descent. Population size is arbitrary; natural populations are usually much larger. New allelic variation may appear at any time due to mutation or immigration. Fixation of alleles in the incipient daughter species may be random, due to the effects of genetic drift, or may be deterministic, driven by natural selection either on the gene itself or on a gene located nearby on the same chromosome (hitchhiking). With either drift or selection, genome-wide differences accumulate between the daughter species.



**Figure 2** Dobzhansky-Muller antagonistic gene interaction associated with postzygotic isolation. Ancestral species of genotype  $AB$  undergoes a split. In one incipient daughter species, allele  $a$  becomes fixed as a consequence of adaptation to its environment. In the other incipient daughter species, allele  $b$  becomes fixed as a consequence of adaptation to its environment. The ancestral species evolves into the two daughter species without any loss of fitness. In the hybrid, however, alleles  $a$  and  $b$  of the two loci interact antagonistically (negative epistasis) and the hybrid shows a severe loss of fitness. This type of interaction is not restricted to pairs of loci; alleles at multiple loci may interact in this manner, producing a “snowball” effect of even greater postzygotic isolation as the negative interactions accumulate much faster than the number of genes involved. The example above is adapted for haploid organisms, i.e., most fungi.



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