

# THE ORIGIN OF SPECIES IN FUNGI

**Tatiana Giraud\*, Pierre Gladieux  
and Michael Hood**

*\*Corresponding author:*

Departement Genetique  
et Ecologie Evolutives  
Laboratoire Ecologie, Systématique  
et Evolution  
UMR 8079 CNRS-UPS-AgroParisTech  
Bâtiment 360, Université de Paris-Sud  
91405 Orsay cedex France  
Tatiana.giraud@u-psud.fr

## The problem of speciation

The origin of new species is one of the most central and persistent challenges in biology, being the process by which the great diversity of life is generated. Understanding how 1.5 million fungal species have arisen over the past 500,000,000 years is of fundamental importance, yet there are also important consequences regarding agricultural pathogens, emerging human diseases, or fungi used for agriculture, industry, and biotechnology. Although tremendous progress on the origin of species has been made since the book of Darwin's book (1859), the subject remains heavily debated and with many recent realizations about the genetics that underlie speciation processes.

To study speciation (i.e. the origin of new species), it seems necessary to first define what a species is. All biologists more or less agree that species represent different lineages that evolve independently, without freely exchanging genes (De Queiroz, 2007). This means that matings occurs within species, and mating between species is absent or very exceptional. This is highly applicable to fungi because many, although not all, species indeed mate and undergo regular sexual reproduction in nature. In fact, the structures we enjoy eating are the result of sexual development of basidiomycete fungi, and even of some ascomycete fungi (e.g. morels and truffles).

A major obstacle, particularly for fungi, has been to recognize and delimit species in nature. Classically,

fungal species, just like most plants and animals, have been recognized using morphological characters. Since the last century, experiments with mating crosses have also been used to assess which groups are reproductively compatible with each other. These experiments revealed that crosses between some fungal strains were impossible even though they were thought to belong

that many cryptic species exist in groups of fungi where morphological characteristics provide little or no ability to distinguish them. For instance, genealogies based upon DNA sequences have discovered eight cryptic species within a single morphological species in the genus *Neurospora* (Dettman et al., 2003a). In contrast to the situation in Ascomycetes, DNA analyses have



Figure 1. *Armillaria* species.

to the same species, indicating the existence of several hidden or “cryptic” species within a group known by a single taxonomic name. This was the case, for instance, in the mushrooms that were once thought to be the species *Armillaria mellea*, but where the strains from North America could not mate with strains from Europe (Anderson et al., 1980; Anderson and Ullrich, 1978). More recently, technological advances have allowed copying and sequencing DNA from regions of fungal genomes, which yield insights directly into the amount of genetic exchange between groups of organisms; in modern terms, this measure of genetic exchange is central to the very definition of “species.” Such DNA technologies have revealed

not revealed many cryptic species in mushroom-forming basidiomycete fungi, maybe because mating tests and morphological characteristics of the mushroom cap were already sufficient to correctly delimitate most species (Le Gac and Giraud, 2008).

Our modern DNA techniques have nevertheless revealed a much richer diversity of fungal species than was previously recognized, particularly among the smaller and less conspicuous forms (Taylor et al., 2000). Consequently, the question of how such new species arise in nature has become a highly active field of research, and fungi provide a most tractable model for addressing speciation more broadly across all

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eukaryotic kingdoms (Giraud et al., 2008; Kohn, 2005).

It has long been believed that species originate mostly through their isolation in different geographic areas (i.e. “allopatric divergence”) because extrinsic geographic barriers seemed obvious impediments to genetic exchange. In fact, among the numerous recently discovered complexes of cryptic species, many appear consistent with such allopatric divergence, where genetically isolated species occupy non-overlapping geographic ranges. This is the case for instance in what was known as *Armillaria mellea* (Fig. 1), as mentioned above, where North American and European strains have been shown to belong to different species (Anderson et al., 1980). Similarly, the genus *Lentinula*, which contains the shiitake mushrooms cultivated for centuries in China and Japan, was shown to encompass seven cryptic species using genealogies based on comparing DNA sequences. The most ancient divergence in the *Lentinula* corresponded to species in the Old World versus the New World, and age of this divergence could correspond to the fragmentation of the Laurasian supercontinent (Hibbett, 2001).

In contrast to the wide acceptance of allopatric divergence, the possibility of speciation occurring without geographic separation (i.e. “sympatric divergence”) had long been dismissed, particularly for sexual organisms. If populations live in the same area and can meet and exchange genes, it is indeed difficult to understand how they can become sufficiently isolated and diverge to become new species. Even relatively rare matings at each generation should cause the genetics of the two populations to become homogenized, which would impede adaptation to different resources or habitats (Maynard Smith, 1966). Theoretical models have shown, however, that sympatric divergence could be possible under certain conditions, in particular for pathogenic fungi that mate on their host plants, or saprophyte fungi that mate within their specialized substrate (Giraud et al., 2006), because gene exchange can then occur only between fungi adapted to the same host or substrate. Cases of speciation in sympatry by adaptation to different host plants have been suggested for instance in *Mycosphaerella*

*graminicola*. This wheat pathogen arose recently, most probably during wheat domestication in the Fertile Crescent, by sympatric differentiation from *Mycosphaerella* pathogens of natural grasses (Stukenbrock et al., 2007).

### Nature of reproductive isolation in fungi

As seen above, an essential character of speciation in sexually reproducing organisms is the emergence of barriers to gene flow, i.e. mechanisms that prevent matings from spreading genes from one group of organisms into another. Two types of reproductive barriers are usually distinguished, pre-mating and post-mating, depending on their time of occurrence, before or after fertilization.

Assortative mating is the most common pre-mating barrier, which occurs when individuals or gametes are able to bias mating partners to those that are similar to themselves. Assortative mating seems to be especially important in the reproductive isolation of mushrooms, where the clamp connections between the fungal cells that reflect the onset of mating and basidiocarp fructification are almost exclusively observed when the tested mycelia belong to the same species (Le Gac and Giraud, 2008). Such assortative mating has been observed for instance between very close species of the saprophyte mushrooms in the genera *Serpula*, *Hyphoderma*, *Flammulina* and *Polyporus*, or between related species of the ectomycorrhizal mushrooms *Laccaria*, *Sistotrema* and *Hebeloma*. However, these pre-mating barriers are often weaker when the species do not live in the same geographic area, for instance on different continents. Presumably geographic isolation prevents selective pressure to prevent crosses between incipient species (Le Gac and Giraud, 2008), whereas when species live in sympatry there may be selection to avoid the cost of producing hybrids that would likely be unviable or sterile (i.e. post-mating barriers as described below). In the saprophyte mushroom *Amylostereum* for instance, pre-mating barriers are always complete between species from the same continent but species from different continents can be crossed experimentally (Boidin and Lanquetin, 1984). These examples

of pre-mating barriers in basidiomycete mushrooms are contrasted by many ascomycete fungi, including numerous plant pathogens, where there are often no pre-mating barriers dependent upon geographic provenance. For example, closely related ascomycete species are able to mate freely when experimentally crossed (Le Gac and Giraud, 2008), but at least for many plant pathogenic Ascomycetes hybrids are not formed in nature because the species are adapted to different host plants and mating occurs on or inside the plants where they grow. As mentioned above, such adaptation to the host plant can itself constitute a type of pre-mating barrier: fungi do not mate with each other because they cannot grow together on the same host plant (Giraud et al., 2006).

Post-mating barriers refer to the inviability and sterility that is often observed in hybrids. In such cases, crosses between evolutionary lineages occur and lead to the production of hybrids, but they exhibit little ability to grow and develop or to further produce functional offspring. For instance, crosses among *Neurospora* species led to the production of few and abnormal fruiting structures (perithecia) and ascospores viability was low (Dettman et al., 2003b). Relatively few studies have reported hybrid inviability or sterility in mushrooms because the experimental induction of basidiocarps is often very difficult (unfortunately for mushroom eaters). It is therefore difficult to analyse experimentally the progeny of hybrids in mushroom-forming fungi.

An interesting and different type of reproductive isolation is when a new species originates as the result of hybridization between two extant species. This phenomenon is not uncommon in fungi (Olson and Stenlid, 2002). A remarkable example is that of the rust fungus *Melampsora × columbiana* that emerged from hybridization of *M. medusa*, a parasite of the poplar tree *Populus deltoides*, and *M. occidentalis* parasite of the tree *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, and the hybrid rust fungus was able to infect the newly cultivated hybrid poplar. Several other cases have been reported of hybrid speciation in

fungi, mostly in Ascomycetes, such as in the fungal endophytes of grasses *Neotyphodium* (Moon et al., 2004), but no cases of hybrid speciation are known in mushrooms, possibly because most of the close species living in the same area exhibit complete pre-mating isolation or intersterility, impeding the production of viable hybrids.

### Asexual fungi

Many fungi appear exclusively clonal, without any ability to undergo sexual reproduction. In asexual fungi, the theoretical issues of species formation are completely different from those in sexual organisms. There is no mating to homogenize the gene pools or prevent divergence between populations. Any new form of a gene (an allele) allowing adaptation to a new environment can thus give rise to a new and isolated lineage, essentially a new “species.” The difficulty in asexual organisms is rather to understand why discrete entities exist that we can recognize as species instead of continuous distributions of characters. A possible explanation for this is that asexual species seem to emerge regularly from sexual species, but do not last for very long in ecological times. For instance, in the fungus *Penicillium*, there seem to be many recent asexual species deriving from sexual species in the genus *Talaromyces* (LoBuglio et al., 1993).

Another explanation for the existence of discrete asexual species could be the adaptation to particular environments, or for pathogens to different hosts. The clonal fungus *Penicillium marneffei* for instance, the causal agent of disease in immuno-compromised humans, exhibits geographic endemicity despite long-distance migration via aerially dispersed spores. Different clones of the fungus seem to be associated with different environmental conditions, which suggested that adaptation to these environments is constraining the organism’s ability to successfully disperse in nature (Fisher et al., 2005).

### *Microbotryum violaceum*, the anther-smut pathogen

Basidiomycete fungi in the genus *Microbotryum* are found as pathogens



Figure 2. Teliospores of *Microbotryum* in the anthers of the plant *Silene uniflora*.

on wild plant species in the carnation family (Caryophyllaceae), and these fungi have a remarkable life history; they

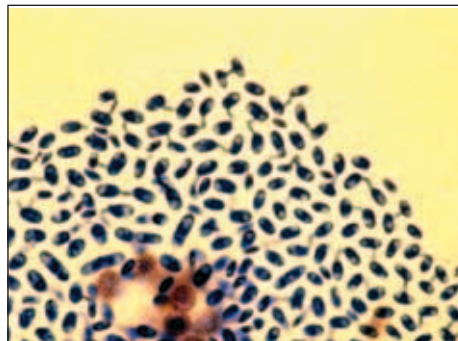


Figure 3. Sporidia of *Microbotryum* mating on a medium.

produce fungal spores in place of the plant’s pollen and are then spread to new hosts by the normal insect pollinators, resulting in a disease called “anther smut” (Fig. 2). The conspicuous disease symptoms and the ease of culturing *Microbotryum* in the lab (Fig. 3) caught the attention of early mycologists and naturalists, who used anther-smut fungi to address questions about specialization, Mendelian inheritance of mating factors, and barriers to reproduction (Goldschmidt, 1928; Kniep, 1919; Liro, 1924). Recently, applications of molecular approaches and DNA sequencing have provided many new insights into the dynamics of speciation in these fungi.

Anther-smut fungi were originally

grouped under the species name *Ustilago violacea*, in the genus of smut fungi from grasses because their similar appearance. However, with the advent of new techniques for DNA sequencing, anther-smut fungi were reassigned to the class of fungi related to the rusts (as distant a reassignment as between rust fungi and the familiar mushroom-forming Agaricomycetes), illustrating how the new molecular paradigm now drives classification of fungal species (Swann et al., 1999).

More recently, DNA sequencing has revealed little to no exchange of genes between anther-smut fungi found on different hosts, even when they occur in sympatry, and new species are now being named based upon this modern molecular criterion (Le Gac et al., 2007a). Curiously, diverse anther-smut fungi are able to recognize and mate with each other, and there appears to be no negative effect based upon how distant the species are (Le Gac et al., 2007b). It may be that the mating signals (pheromones and receptors) are highly conserved, and in fact the pair of two alternate mating receptors in *Microbotryum* has an origin nearly as old as the fungal kingdom (Devier et al., 2009). However, the viability of hybrids, and even more so their fertility, are greatly reduced in comparison to non-hybrids (De Vienne et al., 2009; Le Gac et al., 2007b; Sloan et al., 2008). Like many fungi, there is a high rate of change in the chromosome structure among *Microbotryum* (Zolan 1995; Perlin 1997), and this can inhibit the cell division that would normally produce the haploid gametes (De Vienne et al., 2009). Nevertheless, there is evidence of rare and quite ancient genetic exchange between *Microbotryum* species that may have given rise to a new species (Devier et al., 2010), and the conditions necessary for this mode of speciation are currently being pursued.

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## *Heterobasidion annosum*

The basidiomycete *Heterobasidion annosum* is responsible for root- and butt-rot on conifers and has long been considered as a single species with a wide host range. However, experimental crosses have revealed that not all strains would mate one with each other (Stenlid and Karlsson, 1991). Three groups have been defined based on intersterility, and they showed different ecological preferences: The S group (renamed *H. parviporum*) is a pathogen almost exclusively of Norway spruce (*Picea abies*) in most parts of Europe and of Siberian fir (*Abies sibirica*) in northeastern Europe, while the P group (*H. annosum*) is found mostly on pine, and the F group (renamed *H. abietum*) is more saprophytic, although sometimes also pathogenic on spruce species. Experimental crosses revealed that five genes were involved in intersterility (Chase and Ullrich, 1990). In agreement with the expectation that premating isolation should be selected for in sympatry, intersterility appeared lower among allopatric strains of different groups (from North America and Europe) than between sympatric strains. The differentiation of these *Heterobasidion* species was suggested to have occurred following the migration of the main spruce and fir hosts, into Europe from eastern Asia (Johannesson and Stenlid, 2003).

## *Venturia inaequalis*, the apple scab

*Venturia inaequalis* is an ascomycete responsible for the scab disease on apples (*Malus* spp.) (Fig. 4), and appears to be a good model for sympatric speciation in fungal pathogens. Genes conferring resistance to the pathogen have been identified in apple trees and used in orchard production. Most of the resistance genes are however no longer efficient as novel virulences having evolved in the fungal populations. One interesting exception is the *Vf* resistance gene. This resistance gene has long been considered as a unique example of durable plant resistance, conferring complete field immunity to *V. inaequalis* during more than 40



Figure 4. Symptoms of *Venturia inaequalis* on apples.

years. The first breakdowns of the *Vf* resistance were reported in 1988 from Germany, in 1989 from England, and then in several other European countries. In France, the first isolates breaching *Vf* resistance have been observed in 1995 from a single orchard of cider apples in Normandy. The subsequent spread of the disease on this cider apple variety in Northwest France was monitored over 10 years. Genetic analysis showed that a single mutation overcame *Vf* resistance and subsequently spread in fungal populations. However, the virulent and avirulent fungal populations have remained genetically separated despite their co-occurrence in orchards containing both resistant and susceptible trees (Guérin et al., 2007; Guérin and Le Cam, 2004). What could explain their isolation in the face of a life cycle with regular sexual reproduction? The hypothesis of inter-sterility was not supported by the observation that viable progeny could be produced between them experimentally (Benaouf and Parisi, 2000). Reproductive isolation could be due to a mere host specialization to *Vf* and non-*Vf* host varieties (Giraud et al., 2006) since this fungus mates within the leaves of its host tree after having grown an infectious mycelium.

Consistent with this idea, measures of the infection success of fungal isolates of *Vf* and non-*Vf* host varieties on the reciprocal host type in fact showed a strict host specificity of the two fungal populations. This host specificity barrier may thus *alone* be responsible for the observed reproductive isolation between sympatric populations of *V. inaequalis*.

## Conclusion

In conclusion, major advances on speciation in fungi are currently being made, and their broad distribution, biological importance, and easy of experimental manipulations have led fungi to be among the most tractable models for the general study of speciation across all of eukaryotes (Giraud et al., 2008; Kohn, 2005). Fungi also exhibit some specific and interesting modes of speciation, and many open questions remain which will be fascinating to explore.

## Acknowledgements

We acknowledge the grants ANR 06-BLAN-0201 and ANR 07-BDIV-003. We

thank Bruno Le Cam for the picture of the apple scab.

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