**Microorganisms Incited Speciation in Plant Pathogens and Emergence of New Plant Diseases**

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**Introduction**

Speciation, the splitting of one species into two, is one of the most fundamental problems of biology, being the process by which biodiversity is generated. Understanding how the 1.5 millions of fungal species (Hawksworth, 1991) have arisen is of fundamental interest and has tremendous applied consequences in the cases of agricultural pathogens, emerging human diseases, or fungal species used in industry and biotechnology. Although much progress on the origin of species has been made since the book of Darwin (1859), the subject remains heavily debated. Fungi are excellent models for the study of eukaryotic speciation in general, although they are still rarely included in general reviews on this subject (Coyne and Orr, 2004). First, many fungi can be cultured and crossed under laboratory conditions, and mycologists have long reported numerous mating experiments among fungal species. Second, fungi display a huge variety of life cycles and geographical distributions, allowing the study of which parameters most significantly influence the speciation processes. Third, numerous species complexes are known in fungi, encompassing multiple recently diverged sibling species (Dettman*et al*., 2003), which allows investigations on the early stages of speciation. Understanding the demographic and ecological factors that lead to the evolution of plant-pathogen species can greatly advance our knowledge of how they adapt to agricultural systems and provide new insights for plant disease management. In addition to these potential applications, fungi and are ideal models for understanding the biological features that lead to new species in general. Fungi comprise a large number of species (About 70,000 species) (Hawksworth, 1997), of which many can cause disease in a wide variety of plant hosts. These factors make them appealing models for evolutionary biologists because pathogen biology provides an extraordinary window into how species interactions shape divergence patterns in different organisms and allows for clear hypotheses formulation. For example, given the dependence of plant pathogens on their host species, it is expected that some of the major mechanisms underlying plant-pathogen species emergence include host-range expansion and host jumps (Broders*et al*., 2012). We first address the question of species definitions and species criteria and then review the patterns of speciation in fungi, situating them in the general theory as applied to eukaryotes. We focus particularly on the aspects that have seen recent and significant developments, such as sympatric speciation, cospeciation, hosts shifts, reproductive character displacement, and the time course of speciation.

**Species definition vs species criteria**

To study speciation, it seems necessary to first define species. The continual proposal of new species concepts may lead one to think that there is no general agreement about what species are. To the contrary, it has been argued that all modern biologists agree that species correspond to segments of evolutionary lineages that evolve independently from one another (De Queiroz, 1998). The apparently endless dispute about species concepts stems from the confusion between a species definition (describing the kind of entity that is a species) and species criteria (standard for judging or recognizing whether individuals should be considered members of the same species). Many so called ‘‘species concepts” actually correspond to species criteria, i.e., practical means to recognize and delimit species (De Queiroz, 2007). The Biological Species Concept (BSC) for instance emphasizes reproductive isolation, the Morphological Species Concept (MSC) emphasizes morphological divergence, the Ecological Species Concept (ESC) emphasizes adaptation to a particular ecological niche, and the Phylogenetic Species Concept (PSC) emphasizes nucleotide divergence. These species criteria correspond to the different events that occur during lineage separation and divergence, rather than to fundamental differences in what is considered to represent a species. One may wonder why there are conflicts over which species criterion we adopt. There are three main reasons why such criteria cannot be universal: (i) speciation is a temporally extended process, but one which varies tremendously in its pace among different types of organisms, (ii) several modes of speciation can occur, during which the phenomena used for species recognition do not necessarily appear in the same chronological order, (iii) characteristics of certain organisms render some criteria difficult to apply. Let us take as example the most popular yet the most challenged species criterion, the BSC. For proponents of the BSC, the capacity to interbreed delimits the infraspecies level, and ‘‘Biological Species” are intersterile groups (Mayr, 1942). This criterion is based on reproductive isolation, but this is only one of the many stages of speciation. Depending on the mode of speciation, intersterility can occur at early or late stages of speciation, and can constitute the critical stage (in sympatric speciation), or it may be only a by-product of genetic divergence (in allopatric speciation). Obviously, the BSC will be most useful in the first case (sympatric speciation), whereas species criteria based on evidence for lack of gene flow using molecular markers will be more discriminating in the latter case. Intersterility is the stage at which the process has become irreversible, but this stage may take very long to reach. Until quite recently, the most commonly used species criterion for fungi has been the MSC. However, many cryptic species have been discovered within morphological species, using the BSC (Anderson and Ullrich, 1978), or the GCPSR (Genealogical Concordance Phylogenetic Species Recognition), an extension of the PSC. This latter species criterion uses the phylogenetic concordance of multiple unlinked genes to indicate a lack of genetic exchange and thus evolutionary independence of lineages. Species can thus be identified that cannot be recognized using other species criteria due to the lack of morphological characters or incomplete prezygotic isolation. The GCPSR criterion has proved immensely useful in fungi, because it is more finely discriminating than the other criteria in many cases, or more convenient (e.g. for species that we are not able to cross), and is currently the most widely used within the fungal kingdom (Dettman*et al*., 2003; Johnson*et al*., 2005).

**How to study speciation in plant pathogens**

The completion of speciation is signaled by the existence of distinct RIMs. Thus, understanding how species originate necessarily focuses on understanding what biological features prevent gene flow. Although research in plant pathology is usually not characterized as speciation research, a series of approaches has aimed at dissecting the biological basis of reproductive isolation between plant-pathogen species. We divide these studies into two categories and refer to the studies that have identified RIMs between species as the classical studies. The second category, genomic studies, involves genome-scale studies that identify genomic features that are involved in reproductive isolation or in interspecies differences. Classical studies include allopatric speciation, sympatric speciation and ecological speciation while as genomic studies include speciation by hybridization and chromosomal speciation. Each of these studies are discussed below (Restrepo*etal* .,2014).

**Allopatric speciation**

How new species arise in nature is still a highly active field of research. It has long been believed that species originate mostly through allopatric divergence (Mayr, 1963), because extrinsic geographic barriers seemed obvious impediments to gene flow. Fungi could appear as exceptions because eukaryotic micro-organisms have long been considered to have global geographic ranges (ubiquitous dispersal hypothesis; Finlay, 2002), at least for those not dependent on a host having a restricted range. This was in particular true for airborne fungal pathogens because their spores can be dispersed over very long distance. Among the numerous complexes of sibling species recently uncovered using the GCPSR criterion, many however appear consistent with allopatric divergence, because the cryptic species occupy non-overlapping areas separated by geographic barriers (Taylor *et al*. 2006). Among the recent examples, a multiple gene genealogies approach revealed the existence of cryptic species among the morphological species *Neurosporacrassa* (Dettman*et al*., 2003). They had non-overlapping geographical ranges, suggesting allopatric speciation: one phylogenetic species was located in the Congo, another in the Caribbean and Africa (but not Congo), and a third one was restricted to India. In yeasts, Kuehne*et al*. (2007) showed, also using a multiple gene genealogies approach, that *Saccharomyces paradoxus*, a close relative of *Saccharomyces cerevisiae* present in temperate woodlands in the northern hemisphere, was composed of two distinct genetic groups, A and B. The majority of isolates from group A were from Eurasia whereas all isolates from group B had been collected in North America, suggesting a differentiation of these incipient species in separate continents. Another example comes from *Fusariumgraminearum*, a fungus responsible for scab on wheat and barley, which had long been considered as a panmictic species with a broad distribution. Recent studies however identified at least nine phylogenetically distinct and geographically separated species (O’Donnell *et al*., 2004). Four of them were clearly endemic to South America, one was found only in Central America, one in India and one in Australia (O’Donnell *et al*., 2004). Examples (Table 1.) can also be found among basidiomycetes, for instance in *Armillariamellea*, where North American and European strains have been shown to belong to different species(Anderson *et al*., 1989).

**Table-1.Speciation cases reported to be allopatric.**

|  |  |  |  |
| --- | --- | --- | --- |
| **S. No.** | **Species** | **Speciation mode** | **Comments** |
| 1 | *Ceratocystisfimbriata* | Allopatric | Fungi and hosts show disjoint ranges |
| 2 | *Phytophthoranicotiana* | Allopatric | Speciation occurred after an allopatric period |
| 3 | *Venturiainaequalis*ecotypes | Allopatric | Wide geographic divergence |
| 4 | *Fusariumnepalensis* | Allopatric | Geographic range of all species does not overlap |
| 5 | *Fusariumgramineciarum* | Allopatric | Geographic range of all species does not overlap |
| 6 | *Pyrenophoratritici-repentis* | Allopatric | Glacial refuges may have provided conditions for speciation |
| 7 | *Microbotrymlychnidis-dioicae* | Allopatric | Very little or no recent gene flow was detected |

5. **Sympatric speciation**

Compelling evidence for the sympatric divergence is extremely difficult to provide, because excluding a past period of allopatry is almost always impossible (Coyne and Orr, 2004). Evidence consistent with sympatric divergence of fungal populations driven by parasitic adaptation to different hosts has however been reported. An example is provided by *Ascochyta* pathogens, where recent multilocus phylogenetic analyses of a worldwide sample of *Ascochyta* fungi causing blights of chickpea, faba bean, lentil, and pea have revealed that fungi causing disease on each of these hosts form distinct species (Peever, 2007). Experimental inoculations demonstrated that infection was highly host-specific, yet in vitro crosses showed that the species were completely interfertile. The host specificity of these fungi may therefore constitute a strong reproductive barrier, and the sole one (Peever, 2007), following a mechanism of sympatric divergence by host usage. The coexistence in sympatry of interfertile populations specialized on different hosts that remain reproductively isolated cannot indeed be explained currently by models other than the reduced viability of immigrants. This mechanism seems to be able to maintain the species differentiated in sympatry and could similarly have created the divergence in sympatry. It is however difficult to exclude a period of allopatry in the past that would have facilitated specialization, i.e., the accumulation of different alleles beneficial on alternate hosts. An elegant way to demonstrate the sympatric occurrence of speciation is to show that gene flow has occurred after initial divergence (Wu and Ting, 2004). This approach is very promising and has been used so far in fungi only on *Mycosphaerellagraminicola*, showing that this wheat pathogen arose recently, most probably during wheat domestication in the fertile crescent, by sympatric differentiation from*Mycosphaerella* species pathogens of natural grasses. Table 2 shows some more examples.

**Table-2.**Speciation cases reported to be sympatric.

|  |  |  |  |
| --- | --- | --- | --- |
| **S. No.** | **Species** | **Speciation mode** | **Comments** |
| 1 | *Botrytis cineria* | Sympatric | Ecological factors favoured speciation |
| 2 | *Didymellarabiei* | Sympatric | Speciation occurred geographic proximity |
| 3 | *Mycosphaerellagraminicola* | Sympatric | Coalescent analysis show little or no gene flow during the divergence process |
| 4 | *Phytophthorainfestans* | Sympatric | No change in geographic range since speciation |
| 5 | *Botrytis cineria ecotypes* | Sympatric | No geographic barriers separated pathogen populations |
| 6 | *Rhizoctoniasolani* | Sympatric | Species are currently sympatric in their geographic range |
| 7 | *Phytophthora mirabilis* | Sympatric | No geographic barriers separated pathogen populations |

**Nature of reproductive isolation**

As seen above, a sine qua non of speciation in sexually reproducing organisms is the decrease of gene flow between incipient species due to the development of reproductive barriers. Two types of reproductive barriers are usually distinguished, prezygotic and postzygotic, depending on their time of occurrence, before or after fertilization. In fungi having a long dikaryotic stage, nuclear fusion occurs long after individual or gamete fusion, which may render the term postzygotic ambiguous. We will therefore here use the terms pre- and postmating for fungi, which qualifies time before or after cell fusion. Premating isolation may include different kinds of barriers: for organisms depending on biotic vectors, specialization of these vectors can prevent contact between two populations even if they lie close to one another, yielding ecological isolation. For example the complex *Microbotryumviolaceum*, where the insect vectors are different to some extent between host species, leading to a reduction in mating opportunities among strains from different plants, although the barrier is not complete. Specialization may also allow for ecological premating isolation if mating occurs within habitats (hosts for parasites), as discussed above (Giraud *et al*., 2006). Allochrony, i.e., differences in the time of reproduction, may also be efficient to promote premating isolation. The sister species *Saccharomyces cerevisiae* and *S. paradoxus* exhibit for instance different cell growth kinetics; this allows most individuals of one species to undergo homospecific crosses before or after reproduction of the individuals of the other species. Proportion of interspecific matings can therefore be significantly reduced without the need of incompatibility factors. As has been invoked in plants, a high rate of selfing may be efficient in limiting interspecific matings. Selfing has been suggested to act as a reproductive barrier in the anther smut fungus *M. violaceum*. Assortative mating due to mate recognition occurs if individuals or gametes are able to discriminate between conspecifics and heterospecifics. Assortative mating seems to be especially important in the reproductive isolation of *Homobasidiomycota*, where clamp connections between mycelia of opposite types are almost exclusively observed when the tested mycelia belong to the same species. Postmating isolation refers to barriers associated with hybrid inviability and sterility and is expected to arise as a result of the divergence of incipient species. In the case of postmating isolation, heterospecific crosses occur and lead to the production of unfit offspring. Hybrids may be inviable or sterile due to genetic incompatibilities if mutations fixed independently in the diverging lineages display negative epistatic interactions when brought together in the same individual, a phenomenon known as Dobzansky–Muller incompatibilities (Orr and Turelli, 2001). This kind of intrinsic postmating reproductive isolation is responsible for the numerous reported cases in fungi of crosses that initiate and subsequently abort during in vitro experiments. For instance, heterospecific crosses among *Microbotryum* species produce in vitro fewer viable mycelia than conspecific ones, and crosses among *Neurospora* species lead to few or abnormal perithecia or to few viable ascospores (Dettman et al., 2003). Postmating isolation may also be linked to ecological factors. Hybrids are then perfectly viable and fertile in a benign environment, such as in vitro conditions, but unfit in a natural environment. This can be the case if hybrids display intermediate traits between parental phenotypes and, as a result, are poor competitors in either parental environment. Despite its potential importance to reduce gene flow, such ecological, postmating barriers have rarely been investigated in fungi. In the species complex *M. violaceum*, hybrids between two close species were inoculated onto both parental host species. In one of the host species, hybrids performed as well as the parental species specialized for this host, indicating that there are no genetic incompatibilities in hybrids. However, when inoculated in the reciprocal host, hybrids did not perform as well as the parental species specialized for this host, showing that in other environmental conditions the same hybrids had a lower viability (Le Gac*et al*., 2007). Mycologists have extensively studied the pre and postmating reproductive barriers that are accessible via in vitro crosses, namely intrinsic premating mate recognition and intrinsic postmating barriers. Despite the potential importance of ecological barriers to gene flow, they are still understudied in fungi. Using fungal systems to investigate reproductive isolation both in the lab and in nature would be a great approach to the virtually unexplored question of the relative contributions of the various reproductive barriers to the decrease of gene flow between sibling species (Ramsey *et al*., 2003) and to understand which barriers arise first during speciation.

**Speciation by hybridization**

Many fungal species do not exhibit complete intersterility which gives the opportunity for hybridization. Hybrid speciation is classified according to the ploidy level of the resulting individuals: when hybrids have a chromosomal number that sums that of the parental species, the process is called allopolyploid speciation, whereas hybrids with ploidy identical to that of the parents are referred to as allodiploids or homoploids. Allopolyploids have a higher ploidy level than the parental lines, but their karyotype is interestingly often not the exact addition of the two parental genomes, due to losses of chromosomes (Le Gac*et al*., 2007). As a consequence, many ancient polyploidy speciation events may have been overlooked. Recent allopolyploid hybrids have however, been identified in diverse genera: *Botrytis allii*, the agent of gray mold neck rot of onion and garlic. The presence of multiple hybrids in some taxa suggests that hybrids could have selective advantages over parental species, at least in some cases. Alloploidy would provide simultaneously instant reproductive isolation, due to triploidy in backcrosses, and a new ecological niche. Evidence for homoploid speciation comes from a ploidy level identical to that of its parents and a broad heterozygosity. Contrary to allopolyploids that are reproductively isolated from their parents, homoploid hybrids are in competition not only with their parents but also with backcrossed individuals, which renders stable allodiploid species much more unlikely than polyploid ones. A well described case of homoploid speciation is that of the rust *Melampsoracolumbiana* that emerged from hybridization of *M. medusa*, parasite of *Populusdeltoides*, and *M. occidentalis*, parasite of *P. trichocarpa* (Newcombe*et al*., 2000). This hybrid emerged in 1997 when a poplar hybrid resistant to the two parental rust species was widely grown in California, the hybrid rust being able to infect the hybrid poplar. In this case, the homoploid hybrid clearly had a novel ecological niche, a new host. Another question is why many loci actually stay heterozygous despite potential recombination among F1 hybrids? This may be due to a selective advantage of simultaneous heterozygosity at many loci. Interestingly, recent focus on the gene expression in hybrids provides a potential mechanism for such an advantage. Hence, hybridization may allow exploring fitness landscapes outside that of the parental species. This would facilitate the maintenance of hybrids in a new niche and thereby their persistence as a new species.

**Chromosomal speciation**

Another mechanism allowing instant speciation is chromosomal speciation. The first model of chromosomal speciation (speciation due to chromosomal rearrangements) considered that if two isolated populations had fixed karyotypic differences, and that recombination between rearranged chromosomes were generating unbalanced gametes that lowered fitness, between-population gene flow could be prevented upon secondary contact (White, 1978). This model was then dismissed on the rationale that rearrangements that cause a sufficient reduction in fitness in heterozygotes could not be fixed in a population precisely because of this reduction in fitness. When at low frequency, the rearrangement will indeed always be in a heterozygous state, and should not be able to increase in frequency. Fungi may however be some of the rare organisms where this speciation scenario could occur because of asexual reproduction and selfing that allow mutants with karyotypic rearrangements to reproduce without loss of fitness. New models of chromosomal speciation consider that the effects of chromosomal rearrangements on recombination rates are more important than those on fitness to explain speciation: a chromosomal rearrangements creates a large region of suppressed recombination where one or more specialization genes can accumulate and lead to the localized restriction of gene flow, which could eventually drive the populations to speciation (Le Gac*et al*., 2007). In fungi, the small size of chromosomes has long been a barrier to the study of chromosomal rearrangements and chromosomal speciation. The invention of the pulsedfield-gradient-gel-electrophoresis allowed the separation of intact fungal chromosomes and revealed that an extremely high proportion of fungal species exhibited chromosome- length-polymorphism (CLP). Chromosomal rearrangements leading to CLP reported in fungi include deletions, reciprocal and insertional translocations, chromosome breakage and fusion or complete chromosome loss, which may in large part be due to transposable elements and other dispersed repetitive sequences (Zolan*,* 1995). In the ascomycete*Sordariamacrospora* and in the basidiomycete*Coprinuscinereus*, intraspecific sexual crossing of strains harboring different karyotypes resulted in low fertility in the progeny, concordant with the idea that chromosomal rearrangement can play a role in the speciation process (Zolan*et al*., 1994). However, these karyotypically differentiated strains may also have differed in their genic content. In order to isolate the effect of karyotypic rearrangement, Delneri*et al*. (2003) elegantly constructed strains of *Saccharomyces cerevisiae*differing uniquely by the presence of reciprocal translocations but otherwise completely isogenic. They showed that crosses between such strains had lowered spore fertility and proposed that chromosomal rearrangements, for yeasts at least, are able to provide partial isolation. Chromosomal rearrangements can thus theoretically have a role in speciation in fungi, but showing that the rearrangements were a cause of the divergence, and not only its consequence, remains a challenging task.

**Asexual fungi**

In asexual fungi, the theoretical issues of species formation are completely different from those in sexual organisms. There is no recombination to break down combinations of multiple alleles adapted to a given habitat, and the selective pressure on one gene has an effect on the whole genome. Any new allele allowing adaptation on a new niche can thus give rise to a new ‘‘species”. The difficulty in asexual organisms is rather to understand if, and why, discrete entities exist that we can recognize as species, instead of continuous distributions of phenotypes/genotypes. Asexual organisms in fact seem to form discrete species, and the hypotheses invoked to explain their existence despite lack of homogeneizing gene flow are the existence of discrete ecological niches, random processes of extinctions of intermediate genotypes/phenotypes (Coyne and Orr, 2004), or the recurrent apparition of asexual species from sexual ones. The example is *Magnaporthegrisea* complex, many species of which are strictly asexual and host-specific. One of the species of this complex, *M. oryzae*, an important fungal pathogen of rice, has been shown to have arisen recently, possibly in association with rice domestication (Newcombe*et al*., 2000). Isolates from rice, millet, cutgrass, and torpedo grass appeared also strictly asexual, and to constitute recent host-specific lineages. These patterns in the *M. grisea* complex appear consistent with the idea that acquisition of abilities to infect new hosts in asexual parasitic fungi can readily form new species because recombination will not prevent the differentiation from the ancestral populations. Fungi, with their enormous diversity of modes of reproduction, seem ideal subjects to test the different hypotheses on the nature of species in non-recombining organisms. In some asexual fungi however, recombination can still occur between individuals via somatic recombination (Bos, 1996), which can be considered as equivalent to sex as regards the speciation issue. Hyphal fusions between genetically different individuals is controlled by elaborate vegetative compatibility systems (Bos, 1996), resulting in a condition of heterokaryosis. The exchange of nuclei and organelles can lead to parasexuality via highly transient nuclear fusion and subsequent chromosomal segregation and/or ameiotic recombination. In fungi undergoing such somatic recombinations, vegetative compatibility groups (VCG) could be considered as reproductively isolated from each other and therefore as distinct species. This has been suggested in *Aspergillusflavus*, where the different VCGs indeed formed genetically distinct lineages (Newcombe*et al*., 2000).

**Which factors usually restrict the possibility of speciation?**

The existing theory of ecological speciation (Rundle and Nosil, 2005)can be used to better understand factors constraining or promoting the adaptation of pathogens to a new host. According to this theory, alleles providing an advantage on a new host need to greatly increase in frequencies in the local population on that new host. This increase is accomplished by strong selection for local adaptation. The theory also tells us that locally advantageous combinations of alleles need to be protected from being ‘diluted’ by ancestral alleles brought by immigrants. Such a dilution is expected if mating is random and immigration is recurrent. Therefore, protection of locally adapted allele combinations is required through the evolution of assortative mating (which can be achieved by mate choice, or habitat choice if mating occurs within habitats) or by strongly reduced viability of immigrants (Nosil*et al*., 2005)The evolution of assortative mating and speciation can be prevented by several factors, including a lack of genetic variation, immigration of ancestral genes, or the costs of being choosy in the selection of mate and/or habitat. Moreover, recombination and segregation will work against the establishment of locally advantageous allele and trait combinations by continuously destroying them. Therefore the overall success of ecological speciation in the presence of gene flow is dependent upon a delicate balance of several factors, as discussed above.

**Features of fungal pathogens of plants promoting ecological speciation**

Several features of life-history traits in fungal pathogens are conducive to ecological speciation by reducing the constraints that usually impair speciation. We detail these features below and examine their consequences for the possibility of ecological speciation.

**Very large numbers of spores;** population persistence and large mutational input: Pathogenic fungi can produce thousands of spores per lesion per day (Andrivon*et al*., 2007)and multiple asexual cycles on the same individual plant can yield hundreds of separate infections. This means that billions of spores can be released from a given plant during an infection by a single fungal genotype. Such large numbers of spores can allow the population to persist on a new host even if selection against allele combinations adapted to infection of the ancestral host is extremely strong and the initial degree of adaptation to a new host is very low. Moreover, such large numbers of spores allow the rapid and recurrent creation of genetic variation by mutations. Empirical examples support this logic. For instance, consider evolution of virulent strains able to overcome resistance in crops. New cultivars of plants with resistance genes conferring complete resistance often become susceptible in just a few years if deployed over large areas due to the rapid appearance of new fungal genotypes by mutations that can infect the hitherto resistant plants. Producing a very large number of spores represents an alternative reproductive strategy which can make the evolution of the mechanisms for the choice of host and mate unnecessary.

**Mating within hosts and outside hosts**; pleiotropy between host adaptation and assortative mating: Many pathogenic fungi can disperse over large distances after mating or by asexual spores, but they cannot disperse between infection of the host and mating. This is the case for the many ascomycete fungal pathogens (Figure 1 ) responsible for most of the devastating crop diseases. In cases of obligate biotrophs undergoing sex within their host plant, mating occurs only between individuals able to grow on the same host. This means that mutations providing adaptation to a new host will pleiotropically affect local adaptation and mating patterns. This ‘magic trait’ scenario is one of the most favourable for ecological speciation (Gavrilets*et al.,* 2004). A theoretical model has shown that, because of this characteristic of the lifecycle of some fungal pathogens, adaptation to a new host can significantly restrict gene flow even in sympatry without the need for mate choice or host choice. In this model, the barrier to gene flow is the reduced viability of immigrants With very strong selection this can completely prevent neutral gene flow. Several studies provide empirical support for the generality of this mechanism in natural fungal plant pathogens (Nosil*et al*., 2005).



**Figure-1**.Lifecycle of a pathogen mating within its host and the possibility of ecological speciation by host shift.

Whether a plant pathogen mates within or outside its host has important consequences for the level of gene flow between populations adapted to different hosts. Consider two populations of an obligate biotroph fungal pathogen adapted to two different hosts. Assume there exists a single diallelic haploid locus involved in a gene-for-gene relationship, with allele A1 preventing infection of host 2 (because it codes for an effector recognized by the plant and inducing a defence reaction), and allele A2 preventing infection of host 1. Consider also a neutral locus with alleles B1 and B2. Figures 1 and 2 depict different types of barriers that could act to restrict neutral gene flow between the pathogen populations adapted to the alternative hosts, i.e. host choice, host adaptation, geographic barriers between the two hosts, mate choice (i.e. assortative mating between the host races), and post-zygotic barriers. The arrows show potential gene flow. The red crosses indicate the steps in the lifecycle where the barriers to gene flow act to prevent gene exchange at the neutral locus. The figures illustrate that, for obligate biotrophs mating within their hosts, host adaptation alone can be a barrier to gene flow (even at neutral loci) because only individuals able to grow on the same host can eventually mate. In contrast, host adaptation cannot substantially reduce gene flow at neutral loci if mating occurs outside the host. A lifecycle of a pathogen mating outside of its host is represented in Figure 1. Geographic barriers, mate choice, and postzygotic barriers can prevent gene flow at a neutral B locus (B1 and B2 alleles). Host choice and host adaptation can decrease the frequencies of allele A1 on host 2 and allele A2 on host 1, but cannot prevent gene flow at the neutral locus B. This is a typical lifecycle of some basidiomycete fungal pathogens such as rusts and smuts. A lifecycle of an obligate biotroph mating within its host is represented in Figure 2. Host choice and host adaptation can prevent gene flow at the host choice or specialization loci (A1 and A2 alleles), but also at the neutral locus (B1 and B2 alleles), as can geographic barriers, mate choice, and post-zygotic barriers. Host adaptation can therefore pleiotropically cause specialization and reproductive isolation if mating occurs within hosts, which is not the case if mating occurs outside the host (Figure 1). This is a typical lifecycle of ascomycete plant pathogens.



**Figure-2**.Lifecycle of a pathogen mating outside its host and the possibility of ecological speciation by host shift.

**SPECIES RECOGNITION IN PLANT PATHOGENS**

Because reproductive isolation is often not easily assessed, fungal and oomycete species have traditionally been defined with morphological and phenotypic traits. Contemporary species description integrates these classical approaches with sequence-based phylogenetic approaches that attempt to find evidence of reproductive isolation (Baum and Donoghue, 1995.). The premise of such approaches is that different gene genealogies of two groups that have ceased interchanging genes should reflect concordant topologies among loci. The most common practice involves the use of gene genealogies to detect long branches, which in turn can be interpreted as discontinuities in population structure that might signal lack of gene exchange. Such studies have revealed that the number of species in fungi and oomycetes tends to be underestimated by a factor of two (Hibbett and Taylor, 2013). In an attempt to unify molecular taxonomy, some general guidelines have been proposed as to what levels of divergence are required to designate species in fungi (Dettman*et al*., 2003), although how these correlate with reproductive isolation is unknown. Accordingly, species description should rely on multiple, unlinked loci from neutrally evolving genes with strong phylogenetic signals (Avise and Wollenberg,1997). Phylogenetic species recognition practices and the application of other species concepts are extensively reviewed elsewhere. Identifying species is a challenging task for asexual populations. The majority of species concepts have been developed for individuals that reproduce sexually and recombine with individuals from the same gene pool. Thus, a rigorous framework is required to study species formation in those groups of organisms that do not reproduce sexually or for which no sexual stage is known. Population genetics and evolutionary theory for asexual taxa provide some guidance and several quantitative extensions of the gene genealogies approach have been proposed. Two of the most comprehensive proposals are the K/θ method (or 4X rule) and the application of branching rates in phylogenetic trees. The K/θ method (Birky and Barraclough, 2009) uses DNA polymorphism and basic coalescent theory to identify clusters of organisms that are diverged enough to be considered separate species. The average number of differences between individuals of two species (after correcting for multiple hits) equals, on average, 8Neμ, where Ne is the effective population size and μ is the mutation rate (Barracloughet*et al*., 2003). This quantity is known as K. Intraspecific polymorphism, θ, is roughly equivalent to 2Neμ. The K/θ metric reflects divergence scaled by the effective population size and is thought to reveal different species if greater than 4, hence the reference to the 4X rule. This approach identifies putative species only on the basis of reciprocal monophyly using rooted trees and ascribes a metric to delimitate populations from true species (Rosenberg, 2003). An alternative framework for considering whether a clade has diversified into discrete genetic clusters is to consider branching models in phylogenetic trees (Pons *et al*., 2006).This approach is more complex than the K/θ method but allows for a global test of the relative rates of divergence within a clade. Under a null model, the entire sample derives from a single asexual population, i.e., without divergence into independently evolving or ecologically distinct species. Under this null hypothesis, the pattern of branching is expected to conform to a standard coalescent model for a single population. However, if speciation has taken place, the branching pattern of a phylogenetic tree deviates from the coalescent null hypothesis and has longer branches in the deeper regions of the phylogenies. These long branches are thus a diagnostic that reveals species boundaries. A large body of theoretical work is available to specify the likelihood of a given pattern of branching under a particularmodel (Kaplan *et al*., 1991), ranging from a neutral coalescent in a population of constant size to populations that increased through time or experienced different forms of selection (Rosenberg and Nordborg, 2002). To our knowledge, this approach has not been applied to plant pathogens but, along with the K/θ method, has the potential for application to the identification of species boundaries and determination of rates of speciation.

**Speciation and the emergence of new diseases**

Understanding the appearance of new pathogenic species can inform control methods and prevention plans (Ahmed *et al*., 2012, Stukenbrock and McDonald, 2008.). Population genetics inferences and the study of evolutionary processes in fungi and oomycetes can reveal how pathogens emerge to cause new diseases. Emergence proceeds by one of three processes. The simplest scenario does not involve the evolution of new species and occurs when a pathogen colonizes a new geographical area in which it finds the host species that it infected in its ancestral location. *Mycosphaerellafijiensis*is an example of a recent worldwide epidemic that affects banana plantations. Molecular genealogies indicate that the range expansion of this pathogen has occurred through bottlenecks (i.e., severe population reductions) and that the spread of the disease might be caused by movement of plant material infected with ascospores (Halkett*et al*., 2010). A similar pattern is observed with the sudden oak death pathogen *Phytophthoraramorum*. This pathogen consists of several distinct genetic lineages that appear to have diverged a long time ago, possibly before modern agriculture (Goss *et al*., 2009). A second process of disease emergence occurs when a pathogen colonizes a new geographic area and develops the capability of infecting new hosts. This process, mediated by adaptation (via host shifts or expansion of the host range), enables the pathogen to thrive in hosts different from its ancestral plants. If the new adaptation leads to reproductive isolation from the parental species, then speciation is correlated with the emergence of the new pathogen and of the new pathogenic syndrome. *Rhynchosporium*, the causal agent of scald disease, seems to be composed of three cryptic pathogen species that have evolved by ecological divergence and host specialization to barley, rye, and *Agropyron*(Zaffarano*et al*., 2008). Gene genealogies also suggest that *Colletotrichumkahawae* emerged as a pathogen that specializes in green coffee berries (Silva *et al*., 2012). The recent emergence of these pathogens is correlated with host shifts, suggesting that agriculture has played an important role in the emergence of diseases. A final possibility is that emergent pathogens and diseases originate after the hybridization between two species; the hybrid then hows a new suite of traits not observed in the parents (a type of inheritance known as transgressive segregation), allowing it to infect new hosts. Hybridization and admixture after bringing closely related pathogens into contact could favor cross-host-species disease transmission (Gladieux *et al*., 2011). The demographic inference of gene exchange between plant-pathogen species in an explicitly spatial context can lead to a deeper understanding of the range constraints of plant-pathogen species.

**Some implications of linking emerging diseases and ecological speciation**

Recognizing that emerging diseases caused by fungal plant pathogens often result from host shift speciation, and that several characteristics of fungal plant pathogens render them conducive to this type of ecological speciation, will improve our knowledge of the mechanisms responsible for disease emergence and the biodiversity of fungi. In addition, this recognition has several important consequences for our understanding of disease dynamics and evolution, as well as for designing more efficient and sustainable control programs. First, if host adaptation alone can be sufficient for speciation, then intersterility, one of the most commonly applied criteria for delimiting species, will not be an appropriate criterion. Interfertility can be retained long after gene flow has ceased between plant pathogens species if the sole reproductive barrier is host adaptation. Failure to recognize that host adaptation can be an efficient barrier facilitating speciation by host shifts means that two distinct pathogenic species could be considered as one. This can lead to the development of control measures that overlook the specificities of each species, such as specific fungicide resistance (Giraud *et al*., 1997).Accurate delimitation and identification of species is also fundamental for making sound quarantine decisions and policies, and for the implementation of strategies specifically designed to target the right taxa. Furthermore, host adaptation alone can allow for very rapid speciation via host shift, so rapidly evolving markers would be needed to delimit the species. Second, it is important to link emerging diseases with ecological speciation to assess if new diseases are due to spillover, host range expansion, or host shift speciation. These different scenarios affect the control measures to be taken (e.g. whether one or multiple hosts should be targeted by fungicides). Also, the dynamics and evolution of the disease will be different if the pathogen is adapted to a single versus multiple hosts (Gandon*et al.,* 2004). or if the newly attacked host is only a reservoir for the second host. Finally, if host adaptation is sufficient for speciation onto a new host via host shifts, then disease emergence can be relatively rapid. This should be taken into account in theoretical models aiming to understand and predict disease emergence. Furthermore, models of the evolution of fungal pathogens characteristics (e.g. virulence) should take into account the specificities of the pathogen lifecycle. For instance, the software package Quantinemo (Giraud *et al*., 1997).in which no dispersal between selection and mating is allowed, can be used for modeling pathogens mating within their hosts. Also, the lifecycle should be considered when predicting which pathogens are the most serious threats: plant fungal pathogens mating within their hosts are expected to cause disease emergence on novel hosts more readily. This feature should be accounted for in quarantine policies, control design, and plant breeding programs.

**Conclusion**

In conclusion, important advances have been made recently on the speciation in fungi, and they have proved tractable biological models for the general study of speciation. Fungi also exhibit some specific and interesting modes of speciation, and many open questions remain which will be fascinating to explore. Recently developed analytical methods for studying past gene flow and differentiation should be useful to determine in which cases fungal speciation by specialization onto novel hosts has occurred in sympatry. Evolutionary biology provides a powerful framework for understanding key aspects of the natural history of pathogens (Xhaard*et al*., 2011). The study of evolutionary processes in plant pathogens is a nascent field that promises to deliver new model systems and a comprehensive view of mechanisms involved in speciation and adaptation. On a more practical scale, the development of multilocus databases will allow for the identification of pathogenic lineages in a phylogeographic framework for additional comparative studies to understand the population dynamics of plant pathogens. One of the ultimate goals of plant pathology is to be able to predict the emergence of new pathogens, and this can be done using an evolutionary perspective. The combination of classical approaches and high-resolution genomic studies will reveal demographic and genetic characteristics of how speciation occurs in oomycetes and fungal plant pathogens. The addition of new technological developments will bolster classical approaches and questions. Studying the evolutionary and ecological dynamics of plant pathogens can lead to a better understanding of how these organisms affect agricultural processes over time. The study of recently diverged pathogenic species can also address key questions in evolutionary theory. What is the level of gene flow required to override divergence by selection? What is the role of hybridization in the generation of more virulent pathogens? Do hybrids show new traits or do they always show reduced fitness compared with their parents? Does hybridization (and admixture) lead to the collapse of species boundaries? These questions, among others, are at the very core of evolutionary biology research but also have direct implications in the day-to-day management of plant diseases, as they essentially resolve the origins and mechanisms of emergence of new pathogens.

**Literature cited:**

S. Ahmed, D.T. Labrouhe and F.Delmotte (2012). Emerging virulence arising from hybridisation facilitated by multiple introductions of the sunflower downy mildew pathogen *Plasmoparahalstedii*. *Fungal Genetics and Biology,* **49**:847–855.

J.B. Anderson and R.C. Ullrich(1978). Biological species of *Armillariamellea* in North America.*Mycologia,* **71**: 402–414.

J.B. Anderson, S.S. Bailey and P.J. Pukkila (1989).Variation in ribosomal DNA among biological species of *Armillaria*, a genus of root-infecting fungi.*Evolution,* **43**:1652–1662.

D. Andrivon (2007). Adaptation of *Phytophthorainfestans*to partial resistance in potato: evidence from French and Moroccan populations. *Phytopathology,* **97**: 338–343.

J.C. Avise and K. Wollenberg(1997). Phylogenetics and the origin of species.*Proc. Natl. Acad. Sci. USA,***94**:7748–7755.

T.G. Barraclough,C.W. Birky and A. Burt(2003).Diversification in sexual and asexual organisms.*Evolution,***57**:2166–2172.

D.A. Baum and M.J. Donoghue (1995). Choosing among alternative “phylogenetic” species concepts.*Syst. Bot.,***20**:560–573.

C.W. Birky and T.G. Barraclough (2009). Asexual Speciation.**In***Lost Sex: The Evolutionary Biology of Parthenogenesis*, ed. I. Schron, K. Martens and P. Van Dijk, pp. 201–216. Dordrecht, Neth. Springer.

C.J. Bos(1996). Somatic recombination.**In**: Bos, C.J. (Ed.), Fungal Genetics, Principles and Practice. Marcel Dekker, New York, pp. 73–95.

K.D. Broders,A. Boraks,A.M. Sanchez and G.J. Boland (2012). Population structure of the butternut canker fungus, *Ophiognomoniaclavigignenti-juglandacearum*, in North American forests. *Ecology and Evolution,***2**:2114–2127.

J.A. Coyne and H.A. Orr(2004).Speciation.Sinauer Associates, Sunderland, MA.

C. Darwin(1859). On the Origin of Species. Murray, London.

K. De Queiroz(1998). The general lineage concept of species, and the process of speciation.**In**: Endless Forms: Species and Speciation. Eds., D.J. Howard and S.H. Belocher, Oxford University Press, pp. 57–75.

K. De Queiroz(2007). Species Concepts and Species Delimitation.*Taylor & Francis*,**56**: 879–888.

D. Delneri, I. Colson, S. Grammenoudi, I.N. Roberts,E.J. Louis and S.G. Oliver(2003). Engineering evolution to study speciation in yeasts.*Nature,* **422**: 68–72.

Dettman, J.R., Jacobson, D.J. and Taylor, J.W. 2003.Amultilocus genealogical approach to phylogenetic species recognition in the model eukaryote *Neurospora*.*Evolution***57**:2703–20.

Felsensein, J. 1981. Skepticism towards Santa Rosalia, or why are there so few kinds of animals?*Evolution***35**: 124–138.

Finlay, B., 2002. Global dispersal of free-living microbial eukaryote species.*Science***296**: 1061–1063.

Gandon, S. 2004. Evolution of multihost parasites.*Evolution***58**: 455–469.

Gavrilets, S. 2004. Fitness Landscapes and the Origin of Species, Princeton University Press

Giraud, T. 1997. RFLP markers show genetic recombination in *Botrytis cinerea* and transposable elements reveal two sympatric species. *Mol. Biol. Evol.***14**: 1177–1185.

Giraud, T. 2006. Selection against migrant pathogens: the immigrant inviability barrier in pathogens. *Heredity***97**: 316–318.

Giraud, T., Gladieux, P. and Gavrilets,S. 20010. Linking the emergence of fungal plantdiseases with ecological speciation.*Trends in Ecology and Evolution***25**: 387–395.

Gladieux, P., Vercken, E., Fontaine, M.C., Hood, M.E. and Jonot. 2011. Maintenance of fungal pathogen species that are specialized to different hosts: allopatric divergence and introgression through secondary contact. *Mol. Biol. Evol.* **28**:459–71.

Goss, E.M., Carbone, I. and Grunwald, N.J. 2009.Ancient isolation and independent evolution of the three clonal lineages of the exotic sudden oak death pathogen *Phytophthoraramorum*.*Mol. Ecol.* **18**:1161–74.

Halkett, F., Coste, D., Platero, G.G., Zapater, M.F, Abadie, C. and Carlier, J. 2010. Genetic discontinuities and disequilibria in recently established populations of the plant pathogenic fungus *Mycosphaerellafijiensis*. *Mol. Ecol.* **19**: 3909–23.

Hawksworth, D.L. 1991. The fungal dimension of biodiversity: magnitude, significance, and conservation. *Mycol. Res.***95**: 641–655.

Hawksworth, D.L. 1997. Fungi and biodiversity: international incentives. *Microbiologia***13**: 221–26.

Hibbett, D.S. and Taylor, J.W. 2013. Fungal systematics: Is a new age of enlightenment at hand. *Nat. Rev. Microbiol.* **11**:129–33.

Johnson, J.A., Harrington, T.C. and Engelbrecht, C.J.B. 2005.Phylogeny and taxonomy of the North American clade of the *Ceratocystisfimbriata* complex.*Mycologia***97**: 1067–1092.

Johnson, P.A., Hoppensteadt, F.C., Smith, J.J. and Bush, G.L., 1996. Conditions for sympatric speciation: a diploid model incorporating habitat fidelity and non-habitat assortative mating. *Evol. Ecol.***10**: 187–205.

Kaplan, N., Hudson, R.R. and Iizuka, M. 1991. The coalescent process in models with selection, recombination and geographic subdivision.*Genet. Res.* **57**:83–91.

Kawecki, T.J. 1998. Red Queen meets Santa Rosalia: arms races and the evolution of host specialization in organisms with parasitic lifestyles.  *Nature***420**: 635–651.

Kondrashov, A. 1986. Sympatric speciation: when is it possible? *Biol. J. Linn. Soc.***27**: 201–223.

Kuehne, H.A., Murphy, H.A., Francis, C.A. and Sniegowski, P.D. 2007.Allopatric divergence, secondary contact and genetic isolation in wild yeast populations.*Curr. Biol*. **17**: 407–411.

Le Gac, M., Hood, M.E., Fournier, E. and Giraud, T. 2007. Phylogenetic evidence of host-specific cryptic species in the anther smut fungus. *Evolution***61**: 15–26.

Mayr, E., 1942. Systematics and the Origin of Species.Columbia University Press.

Mayr, E., 1963. Animal Species and Evolution.Harvard University Press, Cambridge, MA.

Newcombe, G., Stirling, B., McDonald, S. and Vradshaw J. 2000.*Melampsora x columbiana*, a natural hybrid of *M. medusae* and *M. occidentalis*.*Mycol. Res.***104**: 261–274.

Nosil, P. 2005. Perspective: reproductive isolation caused by natural selection against immigrants from divergent habitats. *Evolution***59**: 705–719.

O’Donnell, K., Ward, T.J., Geiser, D.M., Kistler, H.C. and Aoki, T. 2004. Genealogical concordance between the mating type locus and seven other nuclear genes supports formal recognition of nine phylogenetically distinct species within the*Fusariumgraminearum*clade. *Fung.Genet. Biol*. **41**: 600–623.

Orr, H.A. and Turelli, M. 2001. The evolution of postzygotic isolation: accumulating Dobzhansky–Muller incompatibilities. *Evolution***55**: 1085–1094.

Peever, T. 2007.Role of host specificity in the speciation of Ascochyta pathogens of cool season food legumes.*Eur. J. Plant Pathol.***119**: 119– 126.

Pons, J.D., Barraclough, T.G., Gomez-Zurita, J., Cardoso, A. and Duran, D.P. 2006.Sequence-based species delimitation for the DNA taxonomy of undescribedinsects.*Syst. Biol.* **55**:1–15.

Ramsey, J., Bradshaw, H.D. and Schemske, D.W. 2003.Components of reproductive isolation between the monkeyflowersMimuluslewisii and M. caridinalis (Phyrmaceae).*Evolution***57**: 1520–1534.

Restrepo, S., Tabima, J.F., Mideros, M.F., Grunwald, N.J. and Matute, D.R. 2014.Speciation in fungal and oomycete plant pathogens.*Annu. Rev. Phytopathol.***46**:75–100.

Rice, W.R., 1984. Disruptive selection on habitat preference and the evolution of reproductive isolation: a simulation study. *Evolution***38**: 1251–1260.

Rosenberg, N.A. 2003. The shapes of neutral gene genealogies in two species: probabilities ofmonophyly, paraphyly, and polyphyly in a coalescent model. *Evolution* **57**:1465–77.

Rosenberg, N.A. and Nordborg, M. 2002. Genealogical trees, coalescent theory and the analysis of genetic polymorphisms. *Nat. Rev. Genet.* **3**:380–90.

Rundle, H.D. and Nosil, P. 2005. Ecological speciation.*Ecol. Lett.***8**: 336–352.

Silva, D.N., Talhinhas, P., Cai, L., Manuel, L. and Gichuru, E.K. 2012. Host-jump drives rapid and recent ecological speciation of the emergent fungal pathogen *Colletotrichumkahawae*. *Mol. Ecol.* **21**: 2655–70.

Singh, R.P. 2008. Will stem rust destroy the world’s wheat crop? *Adv. Agronomy***98**: 271–309.

Slatkin, M., 1987.Gene flow and the geographic structure of natural populations.*Science***236**: 787–792.

Stukenbrock, E.H. and McDonald, B.A. 2008. The origins of plant pathogens in agro-ecosystems.*Annu. Rev. Phytopathol.* **46**:75–100.

Taylor, J.W., Turner, E., Townsend, J.P., Dettman, J.R. and Jacobson, D., 2006. Eukaryotic microbes, species recognition and the geographic limits of species: examples from the kingdom fungi. *Philos. Trans. R. Soc. B.***361**: 1947–1963.

White, M.J.D. 1978. Modes of speciation. Freeman, San Francisco.

Wu, C. and Ting, C., 2004. Genes and speciation.*Nat. Rev. Genet.***5**: 114–122.

Xhaard, C., Fabre, B., Andrieux, A., Gladieux, P. and Barres, B. 2011. The genetic structure of the plant pathogenic fungus *Melampsoralarici-populina*on its wild host is extensively impacted by host domestication. *Mol. Ecol.* **20**: 2739–55.

Zaffarano, P.L., McDonald, B.A. and Linde, C.C. 2008. Rapid speciation following recent host shifts in the plant pathogenic fungus *Rhynchosporium*. *Evolution* **62**: 1418–36.

Zolan, M.E. 1995.Chromosome-length polymorphism in fungi.*Microbiol.Rev*.**59**: 686–698.

Zolan, M.E., Heyler, N.K. and Stassen, N.Y. 1994.Inheritance of chromosome-length polymorphisms in Coprinuscinereus.*Genetics***137**: 87–94.