# Population genetics of fungal diseases of plants

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# POPULATION GENETICS OF FUNGAL DISEASES OF PLANTS

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**Summary:**

Although parasitism is one of the most common lifestyles among eukaryotes, population genetics on parasites lag far behind those on free-living organisms. Yet, the advent of molecular markers offers great tools for studying important processes, such as dispersal, mating systems, adaptation to host and speciation. Here we highlight some studies that used molecular markers to address questions about the population genetics of fungal (including oomycetes) plant pathogens. We conclude that population genetics approaches have provided tremendous insights into the biology of a few fungal parasites and warrant more wide use in phytopathology. However, theoretical advances are badly needed to best apply the existing methods. Fungi are of prime interest not only because they are major parasites of plants and animals, but they also constitute tractable and highly useful models for understanding evolutionary processes. We hope that the emerging field of fungal evolution will attract more evolutionary biologists in the near future.

**KEY WORDS :** isolation by distance, population structure, $F_{ST}$, selfing, outcrossing, clonality, cryptic species.

Although parasitism is one of the most common lifestyles among eukaryotes, population genetics on parasites lag far behind those on free-living organisms, probably because they are rarely conspicuous in the environment, do not posses the visible morphological or behavioural variation used in the early studies of population genetics, and are less charismatic than the macrofauna. However the advent of molecular markers offers great tools for studying key processes of parasite biology, such as dispersal, mating systems, host adaptation and patterns of speciation. Population genetics studies have also valuable practical applications, for instance for studying the evolution of drug resistance or new virulence. Another reason to study parasites is that they display a huge diversity of life cycles and lifestyles, thus providing great opportunity for comparative studies to test pathogen-specific questions or general issues about evolution. Nevertheless, the field of parasitology has yet to attract more evolutionary biologists. This is even truer for the field of phytopathology, despite the importance of crop diseases for human activities. Furthermore, there are few connections so far between scientists in the fields of parasitology and phytopathology despite their obvious common interests. Here we highlight some studies that used molecular markers to address questions about the population genetics of fungal plant pathogens, fungi being taken *sensus lato, i.e.* including oomycetes. We do not aim providing an exhaustive review, but instead we use selected examples, mainly from our own works, to illustrate peculiarities of the phytopathogenic lifestyle. We focus on the study of mating systems (*i.e.* selfing, outcrossing, clonality), between and within population dispersal and population structure due to host adaptation.

## MATING SYSTEMS

Knowledge about the reproduction modes is of fundamental importance to the evolutionary biology of parasites. Outcrossing promotes gene exchange and can hence speed up the spread of new mutations in combinations with other beneficial alleles, which is critical in the context of arms race between hosts and pathogens (Gandon *et al.*, 1996). However, selfing or clonality provides a gene transmission advantage (Charlesworth, 1980), the fast multiplication of the most fit individuals in a population, and the insurance of reproduction for organisms having a low probability of finding a mating partner, such as parasites frequently ending up alone in a host individual. Fungi are micro-organisms presenting extremely contrasted biological cycles and studying their mating system is a challenging task. The most central issues about mating

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systems in fungi are: 1) the importance of clonal *versus* sexual reproduction, as most species are able to produce both sexual and asexual spores; 2) the importance of selfing versus outcrossing when sexual reproduction occurs, and the existence of particular modes of selfing, such as automixis; 3) the importance of parasexuality, some fungi being able to recombine genetic material without a sexual cycle.

Among the complex life cycles of fungi, obligate sexuality is rarely the rule, although some species require a sexual event before infecting their hosts, as *Microbotryum violaceum,* the basidiomycete responsible for the anther smut of Caryophyllaceae. Most fungal species are capable of both sexual and asexual reproduction, that can occur synchronously, as in the important wheat leaf pathogen *Mycosphaerella graminicola* (Cowger *et al.*, 2002), or sequentially, as in the rust *Puccinia graminis* (Leonard & Szabo, 2005), where sexual spores are produced only at the end of a seasonal epidemic development. The lack of obvious sexual structures can be a poor indicator as to the real importance of mating in natural populations (Taylor *et al.*, 1999). Molecular markers, in particular microsatellites, provide a very effective way to assess the real importance of clonality and sex in natural pathogen populations. For example, in the grey mould *Botrytis cinerea* sexual forms have never been observed on grapes, whereas molecular markers revealed pervasive recombination (Fournier & Giraud, 2008; Giraud *et al.*, 1997). In contrast, sexual forms have been observed in the wheat leaf rust *Puccinia triticina* (Wahl *et al.*, 1984 ), while genetic analysis of French populations revealed a strong clonal structure, with no or very little evidence of genetic recombination (Goyeau *et al.*, 2007). In the yellow rust of wheat *Puccinia striiformis* f.sp. *tritici*, studies measuring linkage disequilibrium in European populations similarly indicated a strongly clonal structure (Hovmoller *et al.*, 2002). Moreover, the high degrees of heterozygosity observed at microsatellite markers (Enjalbert *et al.*, 2002) and in IGS sequences (Roose-Amsaleg *et al.*, 2002) provided evidence for a Meselson Effect, where ancient asexual lineages exhibit high divergence between their homologous chromosomes due to the accumulation of independent mutations on different alleles (Halkett *et al.*, 2005).

The evolution of selfing from outcrossing has received much attention for hermaphroditic plants, but relatively little work is available on sexual fungi that are capable of both selfing and outcrossing fertilisation. Control over mating systems in fungi is largely influenced by the genetic composition of haploid gametic stage and the life history traits determining whether these haploids are widely dispersed. Haploid mating characteristics can be classified as either heterothallic or homothallic, where in heterothallic fungi mating must involve fertilization between distinct and compatible haploid strains. Homothallic species produce haploids that are self compatible, leading instantly to genome-wide homozygosity. Yet, even where mating must occur between distinct haploid strains, diploid selfing among the gametes from one individual remains highly possible due to the segregation of alternate mating types during meiosis. In *M. violaceum* for instance, one of the first fungi in which heterothallism was demonstrated (Kniep, 1919), population genetics studies have shown strong heterozygote deficits (Delmotte *et al.*, 1999; Giraud, 2004), indicating high rates of diploid selfing. Selfing may be common in natural populations because of a lack of available mating partners or because of a preference for selfing compared with outcrossing. In *M. violaceum*, diploid spores are dispersed and only one may arrive at a time on a given flower, and because further dispersal of the haploid products of spore germination and meiosis is limited, selfing may often be the only option available. Some data however also indicate that a preference for selfing versus outcrossing exists (Giraud *et al.*, 2005; Hood & Antonovics, 2000), that is mediated through developmental patterns that favour intra-tetrad mating (Hood & Antonovics, 2000). At the opposite extreme, the ascomycete pathogen *Cryphonectria parasitica,* responsible for the chestnut blight, provides an example of a predominantly outcrossing mating system (Marra *et al.*, 2004). In fact many fungi disperse primarily as haploid ascospores or basidiospores, which greatly favours outcrossing. An example of contrasted mating systems in two closely related species is given by *Plasmopara viticola* and *P. halstedii,* oomycetes responsible respectively for the downy mildews of grapevine and sunflower. Molecular markers showed that the two species displayed similar levels of genetic diversity (Chen *et al.*, 2007; Delmotte *et al.*, 2006). The homothallic species *P. halstedii* showed considerable heterozygote deficit ($F_{IS}$ = 0.95), probably due to limited dispersal ability before mating and thus lack of available sexual partners in the field (Giresse *et al.*, 2007), while the populations of *P. viticola* only slightly deviated from Hardy-Weinberg proportions (Chen *et al.*, 2007; Delmotte *et al.*, 2006).

Fungi exhibit a variety of other ways to recombine genetic information than sexuality, gathered under the terms of parasexuality or somatic recombination (Bos, 1996). The filamentous network of fungal hyphae continuously provides opportunities for the fusion of cells that efficiently transports nutrient and signalling molecules throughout the fungal body, the thallus. Such fusions between genetically different individuals is controlled by elaborate vegetative compatibility systems (Bos, 1996), resulting in a condition of heterokaryosis, *i.e.* the coexistence of distinct nuclei within the same cells. The exchange of nuclei and organelles can lead to parasexuality via the highly transient nuclear fusion (*i.e.*

karyogamy) and subsequent chromosomal segregation and/or ameiotic recombination (Fincham *et al.*, 1979). The cellular and molecular processes that contribute to parasexuality remain poorly understood and the genetic recombination produced can be difficult to be distinguished from the products of a true meiosis. Deciphering the relative importance of clonal *vs* selfing *vs* outcrossing *vs* parasexual events in fungal populations is thus an intricate task, where molecular markers are indispensable tools, in particular microsatellites. As proposed by Halkett *et al.* (2005), a sequential analysis is necessary to infer the mating behaviour of a population, first identifying clones, then partitioning polymorphism within and between clones to test for Hardy-Weinberg equilibrium, linkage disequilibrium between loci and recombination signal. Analysis of genetic information for a given species will also depend heavily on its ploidy and on its life cycle: tests based on intra-individual heterozygosity of loci will be only available in diploids/dikaryons species, while haploids will allow more accurate studies of linkage disequilibrium, with access to the gametic phase. Better knowledge of mating systems can have very direct implication for the control of diseases caused by fungal parasites. For examples, in the textbook case of two-host disease cycle of wheat rust *P. graminis*, the eradication of *Berberis*, the host harbouring the sexual cycle, greatly lowered the occurrence of the disease epidemics and reduced fungal population genetic variation by avoiding the recombination of virulence factors (Roelfs, 1982).

## DISPERSAL AND GENE FLOW

Parasite dispersal is another one of the key factors affecting the co-evolutionary dynamics of host-parasite interactions (Thompson, 1994). The extent of dispersal among populations has for instance a major influence on local adaptation (Gandon & Van Zandt, 1998) and the evolution of host resistance (Barrett & Harder, 1996). It has long been claimed that eukaryotic micro-organisms have global geographic ranges (ubiquitous dispersal hypothesis; Finlay, 2002), in particular airborne fungal pathogens because their spores can be dispersed over very long distances either by wind or thought the transport of infected plants. However, few empirical studies have attempted to measure actual parasite dispersal using molecular markers. In the framework of population genetics, dispersal capacities of organisms can be assessed using F-statistics (Wright, 1951), and the well-known relationships $F_{ST} = 1/(1+4Nm)$ for diploids, $F_{ST}$ being the genetic differentiation among populations, $N$ the effective population size, and $m$ the migration rate between populations. Based on a non-exhaustive survey of 26 fungal pathogen species for which the genetic structure has been analysed, we found a mean $F_{ST}$ of 0.2 +/– 0.05, which was similar to a previous mean value reported for 19 fungal species, including pathogens, mycorhizes and saprobes (Morjan & Rieseberg, 2004). This suggests a certain restriction in gene flow in fungal pathogens, at odds with the ubiquitous hypothesis. The range of $F_{ST}$-values was large, suggesting substantial differences in dispersal abilities or effective population sizes in these organisms. Yet, very high genetic differentiation was reported between close populations for some fungal pathogens, such as *M. violaceum* ($F_{ST}$ = 0.9; (Delmotte *et al.*, 1999), and *Aphanomyces euteiches,* a soilborne pathogen causing root rot of several legumes ($F_{ST}$ = 0.7; Grunwald & Hoheisel, 2006). Conversely, low genetic differentiation was reported for other fungal pathogens, for instance among populations of *Armillaria gallica*, a saprophyte associated with various tree species, able to cause a root rot under some conditions (Saville *et al.*, 1996), and *P. viticola* (Gobbin *et al.*, 2006), with $F_{ST}$ values of 0.02 in both cases.

Several authors have however pointed out the limitations of $F_{ST}$ computation for estimating gene flow (*e.g.* Whitlock & McCauley, 1999). Several further limitations arise from the life history specificities of fungal pathogens. First, many studies focused on recently introduced populations because of the interest to study invasive fungi that threaten new hosts. It may then be difficult to disentangle the effect of gene flow and of demographic non-equilibrium. For *C. parasitica* for instance, the chestnut blight disease introduced in USA during the 19th century, genetic differentiation was different in populations for the native and introduced ranges sampled at a similar spatial scale ($F_{ST}$ = 0.11 *vs* 0.19 respectively; Milgroom *et al.*, 1996). A similar trend for a higher genetic differentiation in the most recent introduced area was found for *M. graminicola*, an important wheat leaf pathogen (Zhan *et al.*, 2003). Second, cryptic species or host races are commonly described in fungi (*e.g.* Fournier *et al.*, 2005; Le Gac *et al.*, 2007). When one is not aware of extant cryptic species, recent genetic divergence of species or subspecies can be mistaken as evidence of restricted gene flow within species.

Another approach for assessing dispersal process is to test for the isolation-by-distance model (IBD model; Wright, 1943). Theoretical studies have shown that, under a constant and isotrope gene flow in two dimensions that decreases with spatial distance, either $Nm$ or $F_{ST}/(1 - F_{ST})$ estimated between pairs of populations decrease linearly with the logarithm of the geographic distance separating these populations (Rousset, 1997). In the few studies that have used this approach in pathogenic fungi, an isolation by distance pattern was generally detected at the intra-continental scale. This was the case for *Plasmopara viticola* in Europe (Gob-

bin *et al.*, 2006), *C. parasitica* in China, its native area (Milgroom *et al.*, 1996), and *Gibberella zeae*, causing *Fusarium* head blight on wheat and barley in the USA (Zeller *et al.*, 2004). At the global scale, some patterns of isolation were also detected, for example in *M. graminicola* (Linde *et al.*, 2002) and in *Rhynchosporium secalis* (Zaffarano *et al.*, 2006), causing a scald disease on various *Poaceae* species. In contrast, in *Cronartium ribicola*, causing the white pine blister rust, no IBD pattern was detected among nine populations separated by a maximum distance of 2,000 km in Canada (Et-touil *et al.*, 1999). The authors interpreted this latter result as the signature of long distance migration with frequent extinction-recolonization events creating high differentiation among close populations.

Overall, the estimation of dispersal abilities of fungal pathogens using genetic markers is still disappointing because the assumptions underlying the models are often biologically unrealistic, and because estimations are dependant on the effective population size, which is rarely known in fungi. Methods based on coalescence approaches may be good alternatives because they do not necessarily rely on the assumptions of equilibrium and constant gene flow among populations, and because effective population size can be estimated independently of dispersal rate (*e.g.* Stukenbrock *et al.*, 2006). Other approaches, based on landscape genetics (*e.g.*, Guillot *et al.*, 2005; Manel *et al.*, 2003), should also be considered because rather than estimating a dispersal rate, these methods attempt to detect barriers to gene flow and to integrate the results for predicting the spread of advantageous alleles, the role of the fragmentation of host population, and the delimitation of spatial units where gene flow is less restricted.

## HOST ADAPTATION AND CRYPTIC SPECIES

Populations of fungal parasites face a variety of different hosts in natural and cultivated environments. In particular, different host species exert strong disruptive selection, and the systems are continually engaged in co-evolutionary dynamics. Under certain conditions, disruptive selection exerted by host variation can drive the differentiation of parasite populations, even in sympatry, to yield new host races or specialized sibling species (Kawecki, 1998). This is quite straightforward in asexual species, where the selective pressure on one gene has an effect on the whole genome in the absence of recombination. The clonal fungus *Penicillium marneffei* for instance, the causal agent of penicilliosis disease in immuno-compromised humans, exhibits geographic endemicity despite long-distance migration *via* aerially dispersed spores. Fisher *et al.* (2005) used DNA typing to show that different clones of the fungus are associated with different environments and suggested that adaptation to these environments is constraining the organism's ability to successfully disperse in nature.

In sexual organisms, although members of the population may also be most fit on different resources or micro-habitats, genetic exchange is a strong force that homogenises variation at all loci not under disruptive selection (Rice, 1984). This is why sympatric divergence may be difficult to achieve in sympatry in sexual organisms. However, because in many of fungal parasites sex must occur on the host, adaptation to a new host plant may be sufficient to restrict gene flow in sympatry (Giraud, 2006; Giraud *et al.*, 2006). Some evidence consistent with process of sympatric divergence of fungal pathogen populations driven by adaptation to different hosts has been reported. Fournier & Giraud (2008) analyzed populations of *B. cinerea* isolated from sympatric populations of grapevines and brambles in different French regions. Their results showed that gene flow was extremely low between parasites isolated from the two different host species even though these hosts were in sympatry. Another example is the ascomycete *Venturia inaequalis*, responsible for scab, a major apple disease in most areas of the world; *V. inaequalis* also attacks crab apple, hawthorn, various ornamentals of the genus *Malus*, loquat and other plants (Le Cam *et al.*, 2002). *Formae speciales* have recently been described in this pathogen that are highly specialized on two different host plants, apple and pyracantha. Further, molecular genetic data show that the formae speciales exchange no genes in sympatry (Le Cam *et al.*, 2002).

## CONCLUSION

Molecular methods have much to offer to the study of fungal parasites, allowing elucidation of ecological and microevolutionary processes in parasites. Population genetic approaches have provided important insight for a few phytopathogens on their mating systems, dispersal, and population structure due to host adaptation. However, much wider employment of these techniques in phytopathology is warranted, where it is still too restricted, although much progress has been made recently. Microsatellite markers in particular are very powerful tools (Jarne & Lagoda, 1996) and should be more widely used for population studies in fungi, despite the technical challenges of their isolation in this Kingdom (Dutech *et al.*, 2007). Further, new methods to analyze data are being developed at a rapid pace, using for instance the Bayesian or the coalescence frameworks, or coupling geography and genetics to unravel migration and speciation histories, which should allow even more power-

ful inferences on the evolutionary processes. However, further theoretical development is badly needed to apply the extant molecular methods to the variety and specificities of the fungal life cycles, such as pervasive clonality and alternation between haplo- and di-ploid phases (Balloux & Lugon-Moulin, 2002; Halkett *et al.*, 2005). Fungi are of prime interest not only because they are major parasites of plants and animals, but they also constitute tractable and highly useful models for understanding evolutionary processes. We hope that the study of fungal evolution will attract more evolutionary biologists in the near future.

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