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REVIEW

Molecular analysis to study invasions by forest pathogens: examples from Mediterranean ecosystems

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Summary. Biological invasions by plants and animals have been the subject of several review papers, but invasions by plant pathogens have only occasionally been described and reviewed. The present paper discusses exotic plant diseases whose epidemiology has been clarified using molecular analysis. Because the list of all exotic plant diseases is quite large, this review focuses on forest diseases caused by exotic microbes in Mediterranean ecosystems. In particular, the contribution of molecular studies on exotic forest diseases in favor of or against general invasion biology theory is highlighted. The review follows different phases of the invasion process, giving examples of particular diseases/pathogens for which characteristics have been analyzed.

Key words: exotic diseases, founder events, population structure, disequilibrium, emergent forest pathogens.

Exotic emergent diseases are, by definition, represented either by new host-pathogen combinations or by the range expansion of known diseases (Woolhouse *et al.*, 2005; Desprez-Lousteau *et al.*, 2007). In this review, focus is on the first type of emergent diseases. Three components are needed for the occurrence of an emergent exotic disease: a) the pathogen must be transported into a new range; b) the exotic pathogen must find a new suitable susceptible host, and c) ecological conditions must be favorable to the spread of the disease (Parker and Gilbert, 2004; Woolhouse *et al.*, 2005). Although natural spread of plant pathogens may lead to emergent diseases, the vast majority of forest epidemics can be traced back to the introduction of an exotic pathogen caused by human activities (Wingfield *et al.*, 2001).

Indeed, there are three major modes for the introduction of pathogens: 1. direct introduction, through which a pathogen is directly transported from its native source to a foreign environment; 2. short term indirect introduction that relies on the introduction of the pathogen in a restricted habitat from which it is then released into the broader environment; and finally, 3. long-term indirect introduction, through which an exotic plant pathogen is routinely introduced into a specific habitat, e.g. a production orchard, from where it then escapes into the wild.

Each one of these three modes of introduction will result in distinct epidemiological and genetic patterns. Exotic disease agents directly introduced into the environment are normally

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characterized by a distinctive genetic bottleneck in their populations, due to the limited number of founder individuals (Goodwin *et al*. 1994; Milgroom *et al.*, 1996; Ivors *et al.*, 2004; Gonthier *et al.*, 2007). These introductions are also characterized by ranges that gradually, although not always symmetrically, expand around the original introduction site (Milgroom *et al.*, 1996; Gonthier *et al.*, 2007). Indirect short term introductions normally result in multiple introductions of genetically similar individuals (if they arrive through a common pathway of introduction). As a result, distinct isolated foci of infection will more or less simultaneously start the epidemic, with the potential for such foci to coalesce and create a larger zone of infestation (Harrington *et al.*, 2006; Mascheretti *et al.*, 2008; Milgroom *et al.*, 2008). Long-term indirect introductions are normally the result of pathogens being potentially accepted in the agricultural or horticultural setting. Because of this "pathogen acceptance", often derived by the possibility of controlling agricultural diseases through management practices and chemical treatments, multiple and distinct strains of the pathogen may be introduced in these artificial settings, from where an escape into neighboring natural environments is inevitable. The outcome of this third mode of introduction is extremely variable, but often results in extremely distant infection foci, each characterized by genetically distinct genotypes, with a clear local relationship between those activities that constitute a source of the exotic pathogen and wildland infestations, while no clear genetic relationship may exist among infection foci within the same type of natural habitat, but in clearly distinct regions (Oudemans and Coffey, 1991; Dobrowolski *et al.*, 2003).

Introduction pathways

One of the pressing issues of emergent diseases is obviously that regarding the possible pathways of introductions of these exotic microbes. The majority, if not the entirety, of introductions of exotic pathogens presumably have to do with human activities (Wingfield *et al.*, 2001). In general, the intercontinental movement of live plants has been identified as a major avenue for such introductions. The list of diseases introduced directly into Mediterranean regions, or that have expanded into Mediterranean regions from a point of introduction because of the documented movement of infected plants include chestnut blight (Milgroom *et al.*, 1994), white pine blister rust (Kinloch *et al.*, 1998), Phytophthora root canker (Dobrowolski *et al.*, 2003), sudden oak death (Ivors *et al.*, 2006; Mascheretti *et al.*, 2008) and presumably the establishment of Armillaria root rot in an orchard in South Africa (Coetzee *et al.*, 2001, 2003). Other diseases such as cypress canker or pine pitch canker have presumably been transferred through the movement of infected plants or seeds, but evidence is still lacking. In at least two cases, namely annosus root rot and stain canker of plane trees, military activity during World War II has been suggested as the source of introduction of aggressive plant pathogens from North America into Italy (Engelbrecht *et al.*, 2004; Gonthier *et al.*, 2004). Additionally, the trade of alder seedlings used in riparian restoration projects has been identified as a major route of dispersion for an emergent European disease of alders, caused by a new interspecific hybrid. This hybrid pathogen has potentially arisen multiple times in nurseries where parental species, not necessarily just two (see Ioos *et al.*, 2006), were and possibly still are in sympatry (Brasier *et al.*, 2004c). A list of exotic diseases, including the relevance of molecular studies for each one of them is presented in Table 1.

Introductions of annosus root rot in Italy, of white pine blister rust in Western and Eastern North America, and of chestnut blight on the East Coast of North America have resulted in discrete and contiguous ranges of expansion. In some respects, all these diseases can be regarded as direct introductions into the environment, even if it is known that the original establishment of most of these introduced pathogens was in commercial plant nurseries or in landscaped gardens. Sudden oak death (SOD) in California is probably the best-documented example of multiple indirect introductions linked to the sale of infected ornamental plants (Garbelotto *et al.*, 2001; Rizzo *et al.*, 2002) (Fig. 1). In the case of SOD, it is estimated that the connection between nursery environments and natural forests allowing for the escape of pathogen genotypes was the original cause of the forest epidemic (Ivors *et al.*, 2006; Mascheretti *et al.*, 2008). Host jumping between ornamental plants and native hosts was eventually controlled by the regulations and quar-

Table 1. Forest diseases presented in this review and some of the related findings achieved by means of molecular analyses.

Invasive pathogen	Disease	Zones of invasion	Findings by molecular analyses
Armillaria spp.	Root rot	South Africa	Source, area of introduction, introduction route, transmission strategy
Ceratocystis platani	Canker stain of sycamore	Italy, Modesto California (USA)	Area of origin
Cronartium ribicola	White pine blister rust	Eastern and western North America	Differentiation between eastern and western introductions, dispersal ability, founder effects
Cryphonectria parasitica	Chestnut blight	North America and Europe	Area of origin, areas of introduction, age of local infestations, reproductive strategy, dispersal ability, disequilibrium among populations, transmission pattern of dsRNA viruses
Fusarium circinatum	Pine pitch canker	California (USA), South Africa, Japan, Spain	Area of origin, reproductive strategy
Heterobasidion annosum P ISG	Root and butt rot of conifers	Italy	Source, area of introduction, transmission strategy, hybridization with native sister taxon, mode of introduction
Ophiostoma ulmi / *novo-ulmi*	Dutch elm disease	Europe and North America	Evolution by interspecific hybridization, horizontal transfer of positively selected genes in, genetic diversity typical of post epidemic phase
Phytophthora alni	Alder dieback	Europe	Hybrid swarm, possible identification of parents, multiple and different hybridization events
Phytophthora cinnamomi	Phytophthora root rot	California (USA), Australia, South Africa	Area of origin, routes of introduction, reproductive strategy, evolutionary strategies
Phytophthora pseudosyringae	Moderately aggressive pathogen, unnamed disease	California (USA)	Source, reproductive strategy, exotic nature
Phytophthora ramorum	Sudden oak death	California, Oregon (USA)	Mode and area of introduction, source of introduction, reproductive strategy, dispersal ability, localized evolution
Phytophthora nemorosa	Mild pathogen, unnamed disease	California, Oregon (USA)	Reproductive strategy, exotic nature

antines imposed by US regulatory agencies (Anonymous, 2007). Because of the awareness of SOD, long-term repeated introduction events have been avoided and consequently the original infestation that started the SOD epidemic of California can be ascribed to very similar pathogen genotypes being introduced in a "relatively" short period of time (10–20 years) in different locations of California (Garbelotto and Rizzo, 2005). A much more complex pattern of introduction is that resulting from repeated escapes from agricultural or horticultural situations of unregulated pathogens. *Phytophthora cinnamomi* is probably the best example of such repeated introductions (see below).

Fig. 1. Sudden oak death, an exotic disease introduced in California through ornamental plants is reshaping this forest by killing the majority of tanoaks (*Lithocarpus densiflorus*).

Molecular studies unraveling pathways of introduction

The basic assumption behind all studies attempting to identify the source of a pathogen introduction is that the founder individuals responsible for an invasion will be genetically most similar to individuals belonging to a source population. The ease with which this assumption can be tested, depends on several factors including: 1. the availability of genetic markers with enough variability to allow for the distinction among genotypes of the invasive species; 2. having a collection of samples that includes a significant representation of genotypes worldwide and that also includes individuals from or related to the source population(s); and, 3. the availability of genetic analyses that will unambiguously determine the genetic linkage between source and invasive populations.

The origin of microbial invasions is often more arduous to determine than that of invasive plants or animals. One of the main reasons is that the size and the abundance of many microbial populations makes it extremely unlikely that the source population will actually be sampled and included in a comparative genetic analysis. For this reason, extremely powerful approaches such as assignment or paternity tests have almost never been used to identify source populations of a microbial invasion. Similarly, coalescent or nested clade analyses capable of reconstructing the geographical patterns of pathogen movement have yet to be widely used to study invasions of forest ecosystem, while they have been applied in some agricultural situations, where larger sample sizes of pathogen populations can be more easily obtained (Carbone and Kohn, 2001), or where natural range expansions of microbes have a longer history (Bergemann *et al.*, 2008). Most studies of invasive forest pathogens have in general been based on smaller sample sizes and have used a phylogeographic approach. This approach has emerged as the obvious one since it has been determined that, worldwide, undifferentiated fungal populations are the exception rather than the rule. A strong phylogenetic signal thus allows for the clear distinction of fungal populations from different regions of the world based on DNA sequence variation. When such phylogenetic signals may be too weak, due to the lack of significant genetic divergence among populations belonging to the same species, the approach has been a population genetic one. Through this approach, allele frequencies at different loci are compared among populations and linkage between a source and an invasive population may be determined by their genetic similarity, the level of migration between the two, and the level of genetic substructuring between them. A visual representation of either genetic distance or genetic structure among all populations studied may help to identify the source from which an invasive population is derived in a way that is not dissimilar from the phylogeographic approach. Despite the power of the approaches mentioned above, the lack of the source population(s) in the sampling scheme may not allow unraveling of the origin of the invasive organism. For a glossary of population genetic terms used in this review, see Table 2.

Phylogeographic approaches to unravel the origins of invasions

Two examples of a phylogeographic approach to unravel the origin of introduced forest pathogens are provided by the cases of *Armillaria mellea* and *A. gallica* introduced into South African gardens from Europe, presumably by early European settlers transporting rootstock of European plants into the new continent, and by that of *Heterobasidion*

Table 2. A glossary of population genetics terms and techniques named in the review.

Term	Description	Relevant reference
AFLP (Amplified fragment length polymorphism)	A DNA fingerprinting technique that is a combination of RFLP and arbitrary primer PCR; does not require prior sequence knowledge. Normally targets entire nuclear genomes	Vos P. *et al.,* 1995..
AMOVA (Analysis of Molecular Variance)	Analysis used to split the total genetic variation into that occurring within populations, among populations within regions, and between regions	Excoffier L. *et al.*, 1992.
Bayesian phylogenetic inference	Bayesian inference of phylogeny utilizes Markov chain Monte Carlo (MCMC) simulation in combination with the chosen model and data to produce a posterior probability distribution of trees.	Yang, Z. and B. Rannala, 1997.
F_{ST} (Fixation index)	A measure of population differentiation based on genetic polymorphisms	Wright S.,1951.
Genomic RFLP (Restriction fragment length polymorphisms)	These markers detect polymorphic sequences using a labeled probe hybridized against digested genomic DNA.	Milgroom M.G. *et al.*, 1992.
G_{ST}	The proportion of the total allelic diversity found among populations	Nei M.,1987.
H_{TR}	A measure of the mean regional genetic diversity adjusted for sample size	Nei M. and R.K. Chesser, 1983.
Ia (Index of association)	A statistical test that measures the extent of linkage equilibrium within a population by quantifying the amount of recombination among a set of sequences and detecting association between alleles at different loci	Smith J.M. *et al.*, 1993.
Microsatellites or SSR (Simple sequence repeats)	A polymorphic sequence of DNA consisting of tandemly repeated units of DNA where the repeat unit is usually 1–4 nucleotides long. Normally they are neutral and codominant	Balloux F. and N. Lugon-Moulin, 2002.
Multilocus genotype (Software that employs multilocus data)	The allelic composition of an individual, based on multiple loci	Agapow P.M. and A. Burt, 2000.
Nm (Number of migrants)	A measure of gene flow among populations, or of genetically effective migration rate $Nm=(1/F_{ST})-1/4$	Wright S., 1969.
SCAR (Sequence characterized amplified region)	Markers that will amplify a specific locus; normally consisting of locus-specific PCR primers	Davis J.E. *et al.*, 2005.
UPGMA (Unweighted pair group method with arithmetic mean)	A clustering procedure that uses a genetic distance matrix to draw an evolutionary dendrogram. The major assumption of this method is the equal rate of mutation along all dendrogram branches	Sneath P.H.A. and R.R. Sokal, 1973.
Φ_{ST}	The percent of variation partitioned among populations in AMOVA. Comparable to the Fixation Index	Excoffier L. *et al.*, 1992.

annosum from the Eastern USA into Italy, presumably by the US Army during the liberation of Italy in 1944 (Fig. 2). In two papers, Coetzee *et al.* (2001, 2003) reconstructed the phylogeny of a set of worldwide isolates of *Armillaria* species using parsimony analyses of two distinct gene genealogies of nuclear ribosomal loci, namely the ITS and IGS. Both confirmed the presence of *A. mellea* of European origin on planted oaks in a garden in Cape Town. In the second paper, using a similar approach, Coetzee *et al.*, were able to show that both *A. mellea* and *A. gallica* (also of European origin) were present on native South African plant hosts again in a garden in Cape Town.

Fig. 2. Italian stone pine forms closed canopy forests. This opening has been caused by the root pathogen *Heterobasidion annosum* from North America, unwittingly introduced in Italy by US troops during World War II.

The introduction of *Heterobasidion annosum* from the USA into Italy was discovered by Gonthier *et al.* (2004) and Linzer *et al.* (2008), using a multilocus Bayesian phylogenetic reconstruction approach. Both studies identified European and North American populations of *H. annosum* as belonging to two reciprocal monophyletic sister clades, and determined that fungal genotypes associated with significant Italian stone pine mortality in the zone of infestation near Rome belong to the Eastern North American and not to the European clade. Based on one highly variable locus, Linzer *et al.* (2008) suggest the source population for this invasion may be located in the Southeastern USA. Because pine mortality was being observed in a large hunting estate comprising almost exclusively native Mediterranean plants and closed to the general public for over 4 centuries, it was difficult to identify the potential mode of transport of the pathogen between the two continents. The discovery that the walls around the fortified estate had been breached by the 85th division of the US Army, which camped inside the pine forest for 6 weeks, provided a likely link between the two regions, and is the first strong evidence identifying military operations as a source of microbial introductions. Although Gonthier *et al.* (2007) determined the exotic pathogen is now present in pine stands along 100 km of coastline, the severity of symptoms in the walled estate as opposed to less extensive symptoms away from the estate, corroborates the hypothesis of an army-linked introduction in an area between Anzio and Rome.

Although the approaches to identify introductions of *Armillaria* and *Heterobasidion* were similar, the actual pathways of introduction were quite distinct, as different were the ecological and evolutionary consequences of these introductions. *Armillaria* is a root pathogen that may persist symptomless on the root boles of infected plants; furthermore, the pathogen produces extremely resilient rhizomorphs that may remain viable in the soil, even if detached from hosts. Hence, its long distance movement associated with the movement of rootstock is extremely plausible. On the other hand, movement of *Heterobasidion*-infected plant material is totally undocumented, and the lack of rhizomorphs or any other resting structures make transport of this root pathogen through infested soil an extremely unlikely event. Short distance movement of this pathogen through infected wood material has been reported, and it has been suggested that crates made of untreated lumber and used by the US Army may have been the source of introduction for this pathogen (Gonthier *et al.*, 2004). Although the ecological and evolutionary consequences of introductions are not the focus of the present review, the two invasions may be progressing very differently. *Armillaria* in South Africa is slowly taking ground, mostly by short distance sec-

ondary "vegetative" growth in the soil as indicated by the clonal structure of introduced populations (Coetzee *et al.*, 2001), while airborne meiospores have been identified as the main method of spread for the exotic sexually reproducing *Heterobasidion*, resulting in populations comprising large number of distinct genotypes (Gonthier *et al.*, 2007).

Population genetics approaches to unravel the origin of exotic pathogens

One of the earliest examples of introduction pathways for forest pathogens elucidated by a population genetics approach is that of the chestnut blight pathogen, *Cryphonectria parasitica*. Milgroom *et al. (*1996), using polymorphic genomic RFLP markers shown to segregate in Mendelian ratios, identified the North American population of the pathogen as most likely derived from Japan rather than China. Both UPGMA similarity reconstruction and estimates of gene flow based on number of migrants (Nm) identified the North American population as more closely related to Japan than to China. Eight subpopulations from North America clustered together in a sister clade to the monophyletic clade inclusive of all seven Japanese subpopulations sampled. The relatedness between Japan and North America was further supported by the placement of one North American subpopulation within the Japanese clade, and by the fact that in pairwise estimates, Nm values were large for the Japan-North America comparison. All evidence presented in this paper suggested that European populations descend from North American populations: the two studied European subpopulations were nested within the North American clade and Nm values between North America and Europe were the highest recorded in this study. Despite the evidence pointing to a North American origin of the European population of *C. parasitica,* the authors warned that limited sampling in Japan and Europe may be affecting the outcome of the analysis, and that further sampling in these regions may unveil a direct connection between them. It should be noted that Nm values are affected both by recurrent gene flow and by historical events such as the long distance movements of genotypes normally involved in the introduction of exotic microbes. If recurrent gene flow can be excluded, for instance because of large geographic distance among populations and the lack of an explainable way for genotypes to cover such distance frequently, then Nm (or G_{ST} from which it is derived) can be used to infer an historical linkage between populations characterized by low G_{ST} and high Nm values.

The question of whether European *C. parasitica* may derive from an Eastern Asian or a North American source is still an open one. The chestnut blight pathogen is famous for the presence of dsRNA hypoviruses affecting its virulence. Studies on dsRNA have found little overlap in viral strains between North America and Europe and have highlighted a close relatedness of Italian strains with those from Southern China and Japan (Peever *et al.*, 1998; Liu *et al.*, 2007). Again, what were the specific pathways of introduction of the pathogen in the two continents? Asian chestnuts, possibly carrying the infection, were introduced multiple times in the Eastern USA both from China and Japan, while it has been suggested that the introduction into Europe may be linked to the movement into the continent of chestnut hybrids resistant to ink disease caused by *P. cinnamomi* (Heineger and Rigling, 1994)

A further example of population genetic analyses highlighting the source of an infestation by an exotic fungus, is that of the pathogen responsible for stain canker of plane trees, the ascomycete *Ceratocystis. platani*. This pathogen is known to be present in Eastern North America (Walter *et al.*, 1952), but its virulence on native *Platanus occidentalis* seems low, and only occasionally there have been reports of epidemic outbreaks. In the early 1900s and in the 1980s two outbreaks of the disease were reported respectively around Naples, Italy (Panconesi, 1999), and in Modesto, California (Perry and Mc Cain, 1988). In both cases, the epidemic was serious, and in Italy, thanks to the abundance of both susceptible hybrid planes and possibly insect vectors, the range of the disease increased to encompass most of the country. Elgenbrecht *et al. (*2004) used a range of nuclear and mitochondrial markers to investigate the relationships between the Eastern North American, California, and Italian populations. High genetic variability detected in Eastern North America suggests that the species may be native to that region, while low nuclear and mitochondrial diversity suggests the Italian and California populations were the result of an

introduction of one (Italy) and a few closely related genotypes (California). Although the appearance of the disease around Naples after the World War II may indicate it was transported through infected crates used by the US military, this hypothesis, although appealing, remains unsubstantiated.

Fusarium circinatum is the causal agent of pine pitch canker, a disease that represents a serious threat to Monterey pine (*Pinus radiata*) both in California where the species is native (McCain *et al.*, 1987), as well as in regions including Spain (Landeras *et al.*, 2005), Chile (Wingfield *et al.*, 2002), and South Africa (Viljoen and Wingfield, 1994), where Monterey pine is the most common species employed in plantation forestry. The disease, present also in Italy (Carlucci *et al.,* 2007), can kill both adults and seedlings, and the pathogen is known to be present on seeds and asymptomatic cones. It is hypothesized that introductions of the disease throughout the world may have been accomplished through this seed-mediated dispersal (Gordon *et al.*, 2001). The disease was first described in 1946 in the Southeastern US where it appeared to be endemic (Hepting and Roth, 1946). Since then, it has also been determined that *F. circinatum* occurs frequently in Mexico, although serious disease problems are only reported in large pine plantations and never in the wild (Guerra-Santos, 1999). Wikler and Gordon (2000) used a population genetic approach to unravel the origin of this pathogen: using eight polymorphic loci, they identified Mexico as the potential area of origin for this pathogen because of the high genetic and genotypic diversity measured there. However, based on low genetic distance measurements and sharing of at least two genotypes between California and Southeastern US, the authors identified the latter as a possible source for the California invasion.

Understanding routes of single or multiple introductions for oomycete plant pathogens

Several aerial *Phytophthora* species (i.e. species affecting aerial portions of hosts, rather than the roots) have recently been discovered in California and Oregon. At least three of them represent new species (*P. ramorum*, *P. nemorosa*, and *P. pseudosyringae*), and two of them (*P. ramorum* and *P. pseudosyringae*) have also been reported in Europe, although it should be noted that the European population of *P. pseudosyringae* has been described from roots and basal stem cankers of tree hosts rather than from branches and leaves (Linzer *et al.*, 2008, and references within). These taxonomically relatively distant species have similar host ranges and cause similar symptoms, but in North America, they are characterized by strikingly different virulence and by different geographic ranges. *Phytophthora ramorum* is extremely virulent on oaks and related species and is the cause of the disease known as sudden oak death (SOD). Although present in an area spanning hundreds of kilometers, SOD has a relatively limited geographic distribution when compared to that of its hosts. *P. pseudosyringae* and *P. nemorosa* instead, have low to medium virulence on oaks and are present throughout most of California and Southern Oregon, including the interior mountains from which *P. ramorum* is absent. These differences between the three species led to the hypothesis that while *P. ramorum* may be an introduced species, the other less virulent species may be native to North America (reviewed in Garbelotto and Rizzo, 2005). Molecular analyses employing a range of markers such as AFLPs, microsatellites, and even genomic analyses were employed to infer the nature (exotic *vs*. native) and the origin of these three species. In particular, the origin and nature of *P. ramorum* are of great interest, as the disease it causes is one of the most devastating forest epidemics of our time.

Phytophthora ramorum was originally known to be found in three distinct "environments": California and Southern Oregon forests, North American commercial plant nurseries, and European commercial plant nurseries (Rizzo and Garbelotto, 2003). Only more recently, there have been isolated reports of this pathogen in European gardens and wildlands (Brasier *et al.*, 2004a, 2004b). This pathogen is believed to be exotic to all four environments, but does the genetic data support this notion? In particular, it had been hypothesized that the California forest strain may have been acquired from Europe through the nursery trade. The first study tackling the origin of *P. ramorum* using AFLPs identified Californian wild populations as basically clonal, with AFLP similarity higher than 95%, but also determined that California wild isolates and European nursery isolates all belonged to completely different evolutionary lineages, making it impossible for European nurseries to be the

source. Interestingly, diversity within the European nursery population was larger than that observed in California, suggesting possible repeated introductions of genotypes belonging to the same evolutionary lineage (Ivors *et al.*, 2004). The partitioning of European nursery genotypes and of Californian wild genotypes in two distinct lineages was confirmed by the discovery that each belonged to a distinct mitochondrial type (Kroon *et al.*, 2004), and that all California wild genotypes were of mating type A2, while all European nursery genotypes with a single exception were of mating type A1 (Brasier, 2003). Because of the spatial segregation of the two mating types, all reproduction both in Californian forests and in European nurseries was predicted to be asexual. A microsatellite study by Ivors *et al*. (2006) quantitatively proved through the index of association test (Ia) that reproduction in California was strictly asexual, and further confirmed the partitioning of the two distinct lineages in the two continents. The study also determined that US nurseries harbored both lineages and a third new and yet undescribed lineage. US nurseries thus became the obvious medium through which *P. ramorum* had been brought into US forests, as wild US genotypes were present in nursery populations, but US nursery populations also included genotypes belonging to two other lineages, absent from wild populations.

Although reproduction in the wild US *P. ramorum* population is strictly asexual, new microsatellite alleles are supposed to arise because of either mutation or somatic recombination (Dobrowoloski *et al.*, 2003). It is also expected that the number of new alleles should be proportional to the effective population size (i.e. size of the population that undergoes asexual reproduction). Prospero *et al. (*2007) reported on the presence of distinct genotypes arisen through such mutational events, and identified dominant genotypes that persist year after year, and rare genotypes that arise and disappear frequently. In this study, no gene flow was detected between wild Oregon populations and nurseries, as indicated by high F_{ST} values between the two, suggesting that nursery genotypes were not being currently released in the wild. Using a similar array of microsatellite markers, Mascheretti *et al. (*2008) were able to reconstruct the history of the SOD epidemic in California. The asexual nature of the California wild population had been previously demonstrated, hence this study avoided the pitfalls of using population genetics approaches designed for sexually reproducing populations. Instead, examination of the molecular data revealed that each locus is evolving simply: with either only one allele varying and the other allele remaining fixed, or with both alleles varying in a concerted (always homozygous) manner. From the point of view of molecular variation then, one allele at each locus was effectively redundant. The data were therefore reduced to a haploid state. This permitted a maximally efficient analysis of the data by allowing Φ_{ST} to incorporate the 'evolutionary' distance between all loci simultaneously ('haplotype-like') rather than being calculated as an average across loci. Results indicated that no gene flow was occurring between nurseries and wildland populations, as demonstrated by high Φ_{ST} values (hence low migration) between them. Nonetheless, when population sampled in 2002 were compared with nursery populations, high levels of gene flow were detected, and in two cases, allele frequency between two sites and the nurseries were identical (Φ_{ST} =0). Placed in the broader context, these two populations appeared as ancestral to the 16 Californian populations sampled in this study, and were therefore identified as the two populations that started the SOD epidemic. Mascheretti *et al. (*2008) thus were able to prove that the SOD epidemic in California forest most likely originated from nursery escapes at two precise locations in California. Because extremely low Φ_{ST} values can be determined both by current and historical gene flow among populations, how were the authors able to state that the infestation started in the same period at both sites? Could the very low Φ_{ST} value be determined by gene flow between the two sites? The presumption of multiple introductions from the same source population (e.g. the nursery population) was corroborated by the observation that the two presumed introduction sites are over 100 km apart, separated by the San Francisco Bay. Based on the known biology of the organism and on spatial autocorrelation analyses (see next section), it is virtually impossible to have the large level of migration needed to obtain a Φ_{ST} approaching the 0.

Phytophthora ramorum is a recently described organism with a poorly known range of distribution: all published studies have failed to identify the origin of this pathogen because the source population has not yet been sampled. A thorough analysis of

the entire genome has, however, highlighted rates of heterozygosity typical of a sexually outcrossing species: hence, somewhere in the world, native *P. ramorum* populations with genotypes bearing both mating types coexist and reproduce sexually (Tyler *et al.*, 2006). However, the current genetic structure of known populations of the pathogen is characterized by widespread excess heterozygosity, which has been explained by occasional mating between genotypes from genetically divergent lineages, followed predominantly by clonal reproduction (Ivors *et al.*, 2006).

Genetic information, limited geographic range, and extremely high virulence on some Californian hosts all point to the exotic nature of *P. ramorum*. In contrast, the recently described *P. nemorosa* and *P. pseudosyringae*, also characterized by aerial biology, are less virulent and occupy larger geographic ranges. Two studies have recently tried to elucidate the origins of these two species. Monahan *et al. (*2008) use *Phytophthora*-specific PCR primers to amplify ribosomal DNA from symptomatic herbarium specimens conserved at the Jepson's Museum of Natural History (Berkeley, CA, USA) and failed to identify any *Phytophthora* across the entire museum collection. Using AFLPs, Linzer *et al. (*2008) showed that the genetic structure of these two species is extremely similar to that of *P. ramorum*, and in the case of *P. pseudosyringae*, all Californian isolates are nested within the European population, strongly indicating a European origin for the Californian population of *P. pseudosyringae*. The myth of native co-evolved aerial *Phytophthora* spp. is thus potentially debunked, and evidence was presented regarding invasions by microbes whose effects are not immediately noticeable. Although a clear pathway of introduction for these *Phytophthora* spp. is not clear, at least one of them (*P. nemorosa*) has been increasingly reported in commercial nurseries.

One of the best examples of repeated multiple introductions of unrelated genotypes in natural ecosystems, is that provided by the oomycete *Phytophthora cinnamomi*. Originally from either Papua New Guinea or Sumatra, where studies reported the presence of high genotypic diversity and both A1 and A2 mating types (Old *et al.*, 1984; Linde *et al.*, 1999). This pathogen has long been introduced in the agricultural and horticultural environments, from which - thanks to its broad host range - it has repeatedly escaped on different wild hosts around the globe (Shearer and Tippet, 1989; Brasier, 1996; Tainter *et al.*, 1997, 2000; Swiecki *et al.*, 2003; Garbelotto *et al.*, 2006). While the genetic diversity of introduced *P. cinnamomi* populations tends to be limited locally, and reproduction appears to be mostly asexual (Linde *et al.*, 1999, 1997; Dobrowolski *et al.*, 2003), very different genotypes have been found to be responsible for emergent diseases of varying severity on agricultural and wild hosts at the regional or global scale (Oudemans and Coffey, 1991). Oudemans and Coffey (1991), using isozyme markers, were able to show that this species contained several distinct groups of genotypes, and suggested that each group represented a separate introduction into a commodity. *Phytophthora cinnamomi* is heterothallic, and the fact that each group comprised a single mating type explained both why individual introductions were characterized by extremely simplified and clonally reproducing populations, and why the same genotype would appear in different parts of the world on the same host. The escape from agriculture into wildlands has been inevitable. Dobrowolski *et al. (*2003) used microsatellites to determine that multiple introductions were responsible for the epidemic in their study sites, and further determined that local diversification occurred in the absence of sex, through mutations and somatic recombination. In California, *P. cinnamomi* has been recently described in coast live oak woodlands (Garbelotto *et al.*, 2006) and in the foothills of the Sierra Nevada (Swiecki *et al.*, 2003). In the first case, the pathogen acts as a secondary pathogen mostly pruning the oak root systems, and by doing so makes infected trees subject to decline during years of drought. In the second case, the pathogen is extremely aggressive on two species of local manzanita (*Arctostaphylos* spp.; Family Ericaceae): one of the two, the Ione manzanita, is an endangered species brought to the brink of extinction by mining activities and by the introduction of this pathogen (Fig. 3). Molecular analyses using the microsatellites described by Dobrolowski *et al. (*2003) have determined that at least three introductions are responsible for high manzanita mortality. Genotypes found in manzanita are very diverse belonging to at least three distinct groups and are identical to those found on some ornamental plants and in Christmas tree

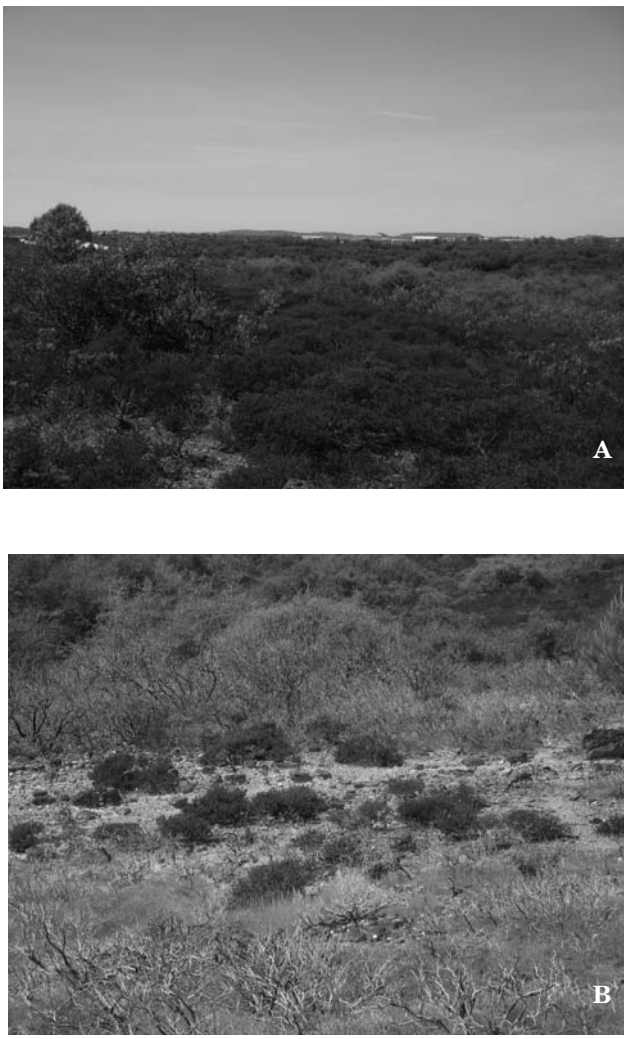

Fig. 3. A. An healthy stand of Ione manzanita (*Arctostaphylos myrtifolia*) in the Sierra Nevada of California. B. A stand of Ione manzanita (*Arctostaphylos myrtifolia*) killed by the exotic *Phytophthora cinnamomi*.

farms, suggesting pathways of introduction. On oak woodlands, however, genotypes are all closely related and match genotypes found in local avocado orchards. In both cases, infestations can be explained by local escapes of the pathogen from local agricultural or horticultural situations (data presented at the 2007 IUFRO *Phytophthora* in Forest Ecosystems conference in Asilomar, CA, USA). While the reported range of severity is probably determined by different host-pathogen combinations and by different ecological and climatic conditions, significant differences among genotypes are known to exist (Huberli *et al.*, 2001). Research needs to address the role played by this intraspecific variability, especially as recent reports have indicated the local occurrence of multiple introductions of multiple distinct genotypes, originated from distinct evolutionary lines or lineages (Dobrowolski *et al.*, 2003).

Pathogen establishment, host shifts, and first phase of dispersal

Once an exotic pathogen has been introduced into a new environment, it will need to successfully infect a host for its establishment to occur. It has been shown that host shifts are more likely when the original host and the new host are more closely phylogenetically related (Gilbert and Webb, 2006), but there are many examples of generalist pathogens that may be pre-adapted to infect a large number of hosts. This has been the case for *Phytophthora ramorum*, a generalist pathogen that can infect over 100 species of plants across several higher plant families and ferns. The analysis of the genome of *P. ramorum* (Tyler *et al.*, 2006) has demonstrated the presence of hundreds of genes involved in plant-pathogen interactions, indicating that the generalist habit of this introduced microbe can in part be explained by its evolutionary interaction with a diverse group of hosts. However, infection alone is not sufficient to determine an epidemic. Reproductive rates must be high to ensure high infectivity: high rates of reproduction may be driven either by the overall low presence of resistance to the exotic pathogen because of the lack of co-evolution, or because the environmental/ecological conditions may be extremely favorable to the introduced pathogen (Parker and Gilbert, 2004).

Three important traits of early invasions: genetic bottlenecks, disequilibrium, and strong genetic structuring of invasive pathogen populations

Independently of the mechanisms underlying the outbreak of an emergent infectious disease, one of the most common traits of introduced exotic pathogens is the low level of genetic diversity amongst its populations. Thus, in general, limited genetic diversity has been taken as the trademark of an exotic organism. Notable exceptions are repre-

sented by those epidemics that are caused by multiple distinct introductions from different sources such as is the case of *P. cinnamomi* (Hardham, 2005), or of the hybrid species *Phytophthora alni*, an emergent pathogen originating from a swarm of diversified interspecific hybrids, thought to have been generated, or at least propagated, in plant nursery environments (Brasier *et al.*, 1999, 2004c; Ioos *et al.*, 2006). It should be pointed out that many fungal and oomycete pathogens may either reproduce exclusively clonally or self-fertilize. Thus, lack of genetic diversity *per se* should not be taken as an absolute marker of an introduced organism, unless such uniformity is in contrast with more marked variability of conspecific populations worldwide. For instance, the exotic nature of *P. ramorum* in Californian forests was supported by multiple lines of evidence including overall low genetic variability using AFLPs and microsatellite markers, a much narrower genetic variability when compared to worldwide or Californian nursery populations, the presence of a single mating type, and association among alleles higher than expected in an outcrossing population (Brasier, 2003; Ivors *et al.*, 2004; Ivors *et al.*, 2006; Mascheretti *et al.*, 2008).

In spite of the overall low genetic variability of introduced pathogen populations, one of the trademarks of invasive pathogens is a first phase of range expansion characterized by the founding of populations that are genetically distinct from one another. This may become evident, for instance, when comparing genetic differentiation among subpopulations in the native range of an invasive organism with differentiation among populations in the invasion area. In the case of the chestnut blight pathogen, *C. parasitica*, Milgroom *et al.* (1994) identified larger population differentiation within North America than in native China. One of the reasons for this phenomenon may lie in the lack of equilibrium of recently established organisms. Consider a source population and a new population created by migration from the source population. While a source population has already undergone an increase in size and reproductive potential, resulting in migrants leaving such a population, it will take a certain amount of time for the new population to undergo the same process. During that time, gene flow will be unidirectional from the source into the new population. As a result, the new population will be characterized by a) fewer alleles than the source population, b) potential losses of alleles due to genetic drift more likely to occur in newer and smaller populations, and c) potential localized dominance of some positively selected alleles. Thus, even if it the new population derived from the source, the two populations may be genetically different during the initial phase of invasion. The disequilibrium phase is lengthened by long reproductive cycles and/or short dispersal potential of the invasive species, unfavorable environmental conditions including the presence of competitors or predators, and by complex topographic features resulting in fragmented habitats.

An interesting example of differences in the early phases of invasion is provided by the two independent introductions of the same pathogen, *Cronartium ribicola*, the cause of white pine blister rust, on the Eastern and Western Coasts of North America. Several studies have determined that there is abundant gene flow among populations in Eastern North America and limited genetic structuring for the invasive species within the entire region (Et touil *et al.*, 1999; Hamelin *et al.*, 2000, 2005). In contrast, genetic difference is high between Eastern and Western populations, a result of the fact that the two invasions were caused by distinct introductions, probably from different sources (Hamelin *et al.*, 2000). As opposed to the genetically homogeneous situation of the East, populations in the West seem to differ at the regional level. In western Canada, where the pathogen has been introduced for a longer period of time, there appears to be gene flow among different populations (Hamelin *et al.*, 2005). Conversely, analyses of other western populations, including several from California, have highlighted the presence of strong differentiation among populations, without any correlation between geographic and genetic distance (Kinloch *et al.*, 1998). These results were interpreted as determined by the fact that the invasive population is not in equilibrium and genetic structure is strongly driven by founder events and local allelic drift. Although *C. ribicola* has been present in both regions a comparable amount of time, the rugged topography of the West and the presence of mosaicism, both in terms of types of habitats and genetic background and susceptibility of hosts, has created an heterogeneity in part

responsible for the prolonged lack of equilibrium in populations of this invasive pathogen in the region. The presence of resistance genes in some of the western pine hosts appears to be positively selected upon invasion by the pathogen (Richardson *et al.*, 2008), and it is foreseeable that changes in the host genetic structure is going to have an impact on the genetic structure of the host pathogen.

Even in the case of clonally reproducing organisms, there is evidence that invasive species are initially characterized by strong genetic structuring. In the case of the clonally reproducing *P. ramorum* in California forests, the use of five polymorphic microsatellite markers allowed for the detection of 35 genotypes originated through mutations or somatic recombination. Study sites at 15 km or more from one another were characterized by high Φ_{ST} values, indicating that founding events represented by migration of genotypes from source populations to new areas were not followed by abundant migration at that geographic scale (Mascheretti *et al.*, 2008).

Spatial autocorrelation analyses to study the dispersal ability of invasive pathogens

The demographic aspect of early invasions is inevitably correlated with the reproductive potential and with the dispersal ability of the invasive pathogen (Woolhouse *et al.*, 2005). As stated above, understanding the dispersal ability of a pathogen is essential for predicting the rate at which it will colonize its newfound range, and is one of the most important types of information necessary to design disease control strategies. Unfortunately from a control perspective, forest pathogens mostly spread through microscopic airborne spores. While it is possible to identify infectious propagules, e.g. spores, as belonging to a specific forest pathogen, this analysis is often cumbersome, complicated, and will not allow for the determination of their paternity. Population genetics analyses provide alternative ways to study dispersal rates of microbial organisms, by studying the patterns of dispersal of specific alleles or specific genotypes, rather than that of the dispersal propagules themselves. Spatial autocorrelation studies expressed as correlations between distances and genetic similarity indices or as variograms depicting the expected *vs*. the observed levels of genetic diversity for different space intervals, can thus be used to identify patterns of pathogen dispersal. Using this approach, different patterns may emerge for different pathogens: for instance while clustering of genotypes was observed at the small spatial scale (genotypes from different cankers on the same tree or from adjacent trees) for *C. parasitica* (Milgroom and Lipari, 1995), no such clustering was seen for *C. ribicola* in eastern Canada (Et touil *et al.*, 1999; Hamelin *et al.*, 2005).

An interesting case is once again offered by the clonally reproducing *P. ramorum*. Mascheretti *et al. (*2008) identified that genetic similarity dropped rapidly between 0 and 200 m suggesting natural dispersal is normally at a rather short distance, but also identified a bimodal distribution with similarity increasing between 1 and 3 km. This observation was justified by the large size of the infectious propagules of *P. ramorum*. The pathogen produces large sporangia (50–80 μm in length), which are not easily airborne except for the presence of strong winds. The limited range of dispersal inferred by these genetic analyses provides a justification for the high genetic structure observed among Californian populations of this pathogen. Furthermore, the presence of a long tail in the autocorrelation analyses may explain the rare cases of new infestations at rather great distances. It should be noted that these analyses have to be read in terms of inoculum dilution, so that a long tail suggesting long distance movement of a small percentage of spores, may become relevant when population sizes are extremely high, but may be irrelevant when populations crash.

The duration of the initial establishment phase of invasion by a pathogen is variable and depends on traits such as the pathogen's virulence levels, availability and distribution of susceptible hosts, reproductive potential and ecological conditions. This phase is also referred to as "lag phase" due to the fact that normally there is a lag between introduction of the pathogen and discovery of such introduction. Short lag phases can be expected for aggressive pathogens with a large availability of new hosts and favorable environmental conditions, while long lag phases may be expected of pathogens with lower virulence, scarce availability of hosts, and in areas with environmental conditions that are only partially supporting their reproduction. Long or short, by the time a lag phase is over it is normally too late to eradicate the pathogen: short lag phases in fact correspond to highly infectious and fast reproducing

pathogens that may be hard to control even a few months after they have been introduced.

Invasion

Once an exotic pathogen has been successfully introduced and has established itself in a primary zone of infestation, the processes leading to its movement into a broader geographic range may start. In this invasive phase, exotic pathogens have to deal with adaptation to new environments and to the problems related to their low genetic differentiation in the presence of increasing disequilibrium over increasing geographic distances. This situation may lead to even further genetic bottlenecks because of the loss of alleles by genetic drift in small new populations. Two elements, combined or individually, may be required for a successful invasion: population sizes need to increase in order to limit the effects of genetic drift, and new genetic variability needs to arise, either through mutation, recombination, or interspecific gene flow.

Four different genetic scenarios may be identified in this phase:

1. Invasion by a supervirulent strain of an asexual organism: high fitness may be determined by high pathogenicity of pathogen, high susceptibility of host, or both. Because of high fitness, lack of genetic variability may not be a problem, at least for the short term, in this still relatively initial invasion phase. Example: *Fusarium circinatum* in California.
2. Invasion by exotic is dispersal-limited and dispersal occurs exclusively during occasional favorable environmental conditions. Invasive will spread significantly only when conditions allow for it: this will lead to range increase and rapid demographic expansion in the new range. Example: *Phytophthora ramorum* in California.
3. Invasive pathogen has a relative ease of dispersal, but there is isolation by distance. Populations will undergo a local lag phase in every new location. Newer infestations characterized by lower generic variability than older infestations. Example: *Cryphonectria parasitica* in Europe.
4. Invasive pathogen will increase its genetic variability by hybridization with a related organism, native or previously naturalized in the zone of infestation. This often leads to unidirectional or unbalanced gene flow from one species into the other. This process may either favor the invasion of a new species, or it may lead to invasion of alien genes into the established native or naturalized species. Examples: *Ophiostoma novo-ulmi* worldwide, North American *Heterobasidion annosum* in Italy.

Different case studies exemplify different successful modes of invasion, with or without an increase in genetic variability of the invasive pathogen

Californian populations of *F. circinatum* represent a good example of a pathogen well into its invasive phase. In California the pathogen was first described in 1987 (Mc Cain and Perry, 1987), and since then has been continuously expanding its range, causing significant amount of mortality especially where *Pinus radiata*, one of its most susceptible hosts, has been planted outside of its natural range (Gordon *et al.*, 2001). All evidence accumulated so far indicates that the pathogen in California is exclusively reproducing asexually. Despite its documented presence in California for about 20 years, genetic differentiation of this pathogen remains very low regionally (H_{TR}=0.26) as opposed to high genetic differentiation in Mexico, one of the presumed centers of origin for this pathogen (H_{TR}=0.46) (Wikler and Gordon, 2000). High susceptibility of the host (Gordon *et al.*, 2001) and long periods of favorable climatic conditions (Garbelotto *et al.*, 2008) have facilitated the spread of this exotic pathogen without the need for significant genetic differentiation.

Although a recent discovery, the sudden oak death pathogen, *P. ramorum* has been intensively studied. It is exclusively reproducing asexually in California forests (Ivors *et al.*, 2006), and three closely related genotypes have been identified as the founders of the forest epidemic (Mascheretti *et al.*, 2008). In 2008, at least 36 genotypes were reported in Californian populations of *P. ramorum*, with the majority of them present in wildlands, as opposed to nurseries. Genotypes were shown to be all closely related and derived from one progenitor, as shown by the fact that they differ at one or two loci only, and that allelic differences at such variable loci are limited to one or two microsatellite repeats. Because of the lack of sex, all new alleles have been hypothesized to be the result of mutations. As discussed above, there is a strong structure among populations of *P. ramorum*, in part explained by its limited movement range and its recent arrival in California. Nonetheless, when comparing genotypic diversity in new infestations and old

infestations, no significant differences were detected. This can be explained by the fact that reproduction of *P. ramorum* mostly occurs when there is abundant rainfall in the warm April and May spring months. When conditions are favorable, population explosions have been witnessed: these demographic explosions allow for mid range dispersal from source populations, but also for local population build-up in newly infested areas. These expansions are driven by the huge reproductive potential of the pathogen, which is known to be able to complete its asexual reproductive cycle 48 hours after infection, if climatic conditions remain favorable (Rizzo and Garbelotto, 2003). Large local demographic expansions are thus climate-driven rather than age-driven and, as a result, in favorable years, there is an overall demographic expansion throughout the range of the pathogen. Assuming a constant mutation rate for the species, the number of new genotypes will be proportional to the size of each population. Increase in size of all populations driven by fast reproductive potential and favorable environmental conditions will lead to comparable genotypic diversity across sites, independent of the age of each infestation. Mascheretti *et al. (*2008) also elegantly showed that evolution of new genotypes is a site-specific process, i.e. the same new genotypes do not arise with equal likelihood everywhere, but different mutations arise and/or survive in different sites, thus starting the creation of distinct lineages of local evolution. This process combined with limited gene flow among sites contributes to the strong genetic differentiation among sites. The minimum spanning network used to exemplify the frequency and relatedness of each genotype (Mascheretti *et al.*, 2008) also indicates we are probably at the end of the initial establishment phase for this pathogen, as the ancestral genotypes in the center of the network are overall still the most abundant ones. However, local dominance of novel genotypes at newer infestation sites points to a transition into a second-phase of the invasion. *Phytophthora ramorum* provides an example of increasing diversity attained through mutations in a clonal species. It should be highlighted, however, that genotypic diversity and genetic diversity are not necessarily synonymous. Nonetheless, increased genetic diversity among new and old genotypes has been reported, as exemplified by the fact that Φ_{ST} values among new infestations are significantly larger than Φ_{ST} values measured among old infestations. Furthermore, novel genotypes are not the results of reassortment of old alleles but rather the result of the rise of new alleles (Mascheretti *et al.*, 2008).

Genetic diversity among populations of the chestnut blight fungus, *C. parasitica*, is more limited in areas where it has been introduced such as North America and Europe, than that measured in areas such as China and Japan, where the pathogen is native. This limited diversity is not explained solely by lack of sexual reproduction, as recombination has been shown to occur in North America and in some European sites, but mostly by a reduced allelic diversity, normally attributed to the genetic bottleneck associated with the introduction of the pathogen into these continents. Genetic diversity for this pathogen has been estimated for populations worldwide using vegetative compatibility (vc). This homogenic system ensures stable cytoplasmic fusion is attained only by genotypes carrying the same vc alleles at multiple loci. Because dsRNA-determined hypovirulence spreads across fully or partially compatible vc groups, this type of analysis has been commonly performed in light of its biological and management significance. Populations within Europe, Northern Italy and Southern France have been reported as having the highest vc diversity, while areas that have been infested more recently such as Spain, Portugal, Greece, Turkey and Bulgaria have been shown to be characterized by decreased diversity (Milgroom *et al.*, 2008, and references within). Because the number of vc loci is limited, genetic differences exist among isolates belonging to the same vc group. Milgroom *et al. (*2008) performed an analysis of genetic variability of well-established populations in Italy and compared it with that of more recent populations in Bulgaria, Greece, Turkey and Sicily. In order to get a definitive picture of the genetic structure across European populations, instead of vc groups alone, the authors used a combination of markers such as vc groups, SCAR genotyping, and MAT alleles determination to define multilocus genotypes. When comparing fringe areas of the invasion with putative older source areas, it was determined that lower diversity of multilocus genotypes was encountered in new infestations, with the distinct dominance of one or two genotypes and the presence of a single mating type. Formal analysis confirmed that loci were in linkage disequilibrium, a trait indicative of asexually reproducing populations.

Furthermore, the retrieval of identical multilocus genotypes across different sites was also congruent with a clonal reproduction scenario for these areas. In contrast, two sites in Italy showed high diversity of multilocus genotypes and evidence of sexual recombination. A further study using gene genealogies and coalescent analyses on the viral genome in the same two Italian populations of the fungus confirmed viral ds-RNA molecules are being exchanged locally across vc types more than predicted though laboratory matings, but indicated limited gene flow between these two distant sites, suggesting that overall in Europe, pathogen populations are still in disequilibrium (Carbone *et al.*, 2004). Similar results of differential genetic diversity were obtained when comparing vc diversity and mating type frequency in three new infestations in Switzerland as opposed to older infestations (Hoegger *et al.*, 2000). Mutations and recombination events potentially starting with the appearance of both mating types at these sites will lead to further diversification of new invasive populations.

A final example of a different way of increasing diversification is that of invasive pathogens hybridizing with closely related sympatric species. Results of these interspecific hybridization events range from the creation of hybrid forest pathogens with new host ranges, such as in the case of *P. alni* (Brasier *et al.*, 1999), to hybrids that may be limited in their ecological fitness, but that may take advantage of disturbed habitats such as stumps, as reported for *Heterobasidion* hybrids in North America (Garbelotto *et al.*, 1996; Garbelotto *et al.*, 2007). In general, hybridization allows for gene transfers in between species, as exemplified by the case of the two Dutch elm disease pathogens *O. ulmi* and *O. novo-ulmi*. The two species were strikingly different in the levels of genetic diversity: *O. novo-ulmi* was originally characterized by very low vc diversity, while *O. ulmi* is consistently characterized by the presence of many vc groups, supposedly to slow the spread of cytoplasmic viral deleterious d-elements (Brasier, 2001). Paoletti *et al. (*2006) proved with genetic analyses that the recent diversification of *O. novo-ulmi* in Europe has been driven by hybridization with *O. ulmi* and the acquisition of vc and MAT-1 alleles from this species. Both types of loci increase the chances of slowing down transmission of the viral elements, because such elements are not transmitted through sexual progeny and are not infectious among genotypes characterized by different vc types. There is good supporting evidence that this gene flow is selected for by the presence of cytoplasmic d-factors: in Europe, where viral presence is high, so is vc diversification of *O. novo-ulmi*, while in North America where viral presence is minimal, so is vc diversity of the pathogen. In New Zealand, where d-elements and *O. ulmi* are both absent, populations of *O. novo-ulmi* are characterized by a single mating type and vc uniformity. Hybridization thus has been shown to allow for the interspecific transfer of genes with important functions, allowing the mostly clonal *O. novo-ulmi* to protect itself from newly acquired viruses through an increase of its vc diversity and increased sexual reproduction. In areas such as Italy and Spain, high diversity of genotypes is reported for *O. novo-ulmi* while *O. ulmi* is rare. Nonetheless, many individuals are being characterized as interspecific hybrids between the two, suggesting again that hybridization may be widespread and a driving force associated with increased genetic diversity of *O. novo-ulmi*. High diversity is also thought to be characteristic of post-epidemic phases, while epidemic phases are generally characterized by the rapid spread of one or a few fit genotypes (Santini *et al.*, 2004; Solla *et al.*, 2008).

The introduction of *Heterobasidion annosum* from North America into Italy has been recently reported (Gonthier *et al.*, 2004, 2007). This introduction, attributed to infected wood brought into Italy by US troops (Gonthier *et al.*, 2004) has resulted in an invasion extending over 100 km of the Mediterranean coast near Rome, with associated significant mortality of Italian stone pine. Invasion of coastal woodlands has been complete in the zone of invasion, and apparently the native *H. annosum* is rarely present in these dry pine forests. In a marshy forest in the southern edge of the zone of invasion, the North American species has encountered the native relative and it is hybridizing with it, as proven by incongruent placement of putatively hybrid genotypes in gene genealogies of three loci. The ability to detect hybrids may be linked to the relative short time the pathogen has been in this southern edge of its zone of infestation. Areas where the pathogen may have stayed longer may be characterized by the presence of

gene introgression requiring screening of a larger number of loci in order to be detected. Detection of gene introgression may be an indicator of areas where the invasive pathogen has been established for a longer period of time.

The consequences of hybridization are not always clear: it may be too early to tell in the *Heterobasidion* example, but in the *O. ulmi* / *novo-ulmi* system, hybridization has allowed for the transfer of functional genes allowing for increased sexual reproduction and increased vegetative incompatibility. These traits have conferred a clear advantage to the invasive species, by making it more resistant to viral infection. In other cases such as that of *P. alni*, multiple hybridization events may result in multiple variants with distinctive genetic and phenotypic traits, not all equally aggressive and uniform. Nonetheless, the notable shift in host range associated with the rise of hybrid species, although not the topic of the present review, is of great interest, and comparable to invasion events by exotic pathogens.

Conclusions

Population genetics has proven a highly valuable tool in the hands of forest pathologists interested in understanding the origins of exotic invasions and the mechanisms driving spread of exotic microbes. It has provided insights on the spread potential of these pathogens, on their reproductive and transmission strategies, and on their ability to evolve either through mutations or recombination. Molecular genetic information has been used to prove whether the same pathogen found on different host species really belongs to the same gene pool, thus allowing for an understanding of infection processes. Determining levels of genetic diversity has allowed inferences to be made on the demographic expansion of diseases and to determine whether a disease outbreak may be at the beginning or at the end of an epidemic phase. Through coalescent analysis and phylogeographic approaches, population geneticists have been able to indicate routes of movement of these microbes, greatly assisting regulators in curtailing similar introduction through similar entry avenues. Finally, population genetics has allowed identification of hybridization, a little understood mechanism in fungi, but with extremely far-reaching consequences, because of its effects on host range determinants and overall pathogenicity. In a departure from traditional population genetics approaches that hinge on the use of neutral genetic markers, forest pathologists are starting to investigate patterns of flow of positively or negatively selected alleles (or infectious elements) using modern robust analytical approaches. By doing so, they are at the forefront of this new area of investigation among invasion biologists. Results from the case studies presented in this review paint different and complex scenarios for invasions by plant pathogens that enrich the varied scenarios known for biological invasions in general.

The cases reviewed in this paper provide evidence of introduction pathways for pathogens, but also provide evidence for the general theory of evolution. For instance, the insular nature of invasive pathogen populations has provided proof that microsatellite markers evolve in a stepwise manner and that the genetic structure of a pathogen is closely correlated with its dispersal potential. The relevance of molecular work is not only theoretical, however (Fig. 4). Regulators and legislators are now increasingly looking to molecular data to identify pathogens and understand how they are moved and introduced into new habitats or new regions of the world.

Fig 4. Population genetics information helps elucidate the biology of pathogens and the epidemiology of exotic forest diseases and can lead to policies aimed at mitigating effects of such diseases. In this case, a road closure is aimed at preventing the movement of soil infested with *P. cinnamomi* in an area free of the disease.

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