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Genetic structure of the poplar rust fungus *Melampsora larici-populina*: Evidence for isolation by distance in Europe and recent founder effects overseas

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ABSTRACT

Dispersal has a great impact on the genetic structure of populations, but remains difficult to estimate by direct measures. In particular, gradual and stochastic dispersal are often difficult to assess and to distinguish, although they have different evolutionary consequences. Plant pathogens, especially rust fungi, are suspected to display both dispersal modes, though on different spatial scales. In this study, we inferred dispersal capacities of the poplar rust fungus *Melampsora larici-populina* by examining the genetic diversity and structure of 13 populations from eight European and two overseas countries in the Northern hemisphere. *M. larici-populina* was sampled from both cultivated hybrid poplars and on the wild host, *Populus nigra*. The populations were analyzed with 11 microsatellite and 8 virulence markers. Although isolates displayed different virulence profiles according to the host plant, neutral markers revealed little population differentiation with respect to the type of host. This suggests an absence of reproductive isolation between populations sampled from cultivated and wild poplars. Conversely, studying the relationship between geographic and genetic structure allowed us to distinguish between isolation by distance (IBD) patterns and long distance dispersal (LDD) events. The European populations exhibited a significant IBD pattern, suggesting a regular and gradual dispersal of the pathogen over this spatial scale. Nonetheless, the genetic differentiation between these populations was low, suggesting an important gene flow on a continental scale. The two overseas populations from Iceland and Canada were shown to result from rare LDD events, and exhibited signatures of strong founder effects. Furthermore, the high genetic differentiation between both populations suggested that these two recent introductions were independent. This study illustrated how the proper use of population genetics methods can enable contrasted dispersal modes to be revealed.

1. Introduction

Dispersal is a broad source of genetic variation in plant pathogen populations. It leads to the foundation of new populations and can have a strong and rapid impact on population genetic structure, hence influencing evolutionary processes. The aerial spread of plant pathogens can occur in one of two dispersal modes, each with very different epidemiological and evolutionary consequences (Brown and Hovmøller, 2002). The first, often regarded as the natural process of dispersal, results from the gradual spread of the disease from an original source of inoculum. Often wind-mediated, this gradual dispersal process is characterized by a rapid decrease of the probability of dispersal with distance to the source. It leads to a particular spatial genetic structure, called the isolation by distance (IBD) pattern. Indirect estimates of dispersal, based on population genetics, provide several means to test for IBD (Rousset, 1997; Smouse and Peakall, 1999; Hardy and Vekemans, 1999; reviewed in Fenster et al., 2003), namely by testing the null hypothesis of increasing population genetic differentiation with geographic distance (Epperson et al., 1999). The second dispersal mode involves the transport of spores over very long distances (even between continents), often in a single step. These long distance dispersal (LDD) events could result in devastating disease outbreaks (Aylor, 2003). LDD events have had drastic consequences for human well-beings because of the worldwide spread of plant diseases (Brown and Hovmøller, 2002) such as the potato late blight, caused by *Phytophthora infestans* (Fry et al., 1992). LDD events are rare and highly stochastic, which makes them difficult

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to study (Nathan et al., 2003). In this attempt, it is of primary importance to disentangle LDD events that result in the foundation of new populations, from an underlying IBD pattern. In the absence of selection, departure from the IBD pattern may indicate stochastic dispersal processes resulting either in a panmictic unit, where intense random gene flow tends to erase population differentiation (Zeller et al., 2004), or in distinct populations founded by LDD events. In the latter case, strong founder effects can be expected (Slatkin, 1977), leading to larger population differentiation, reduced genetic diversity, and genetic disequilibrium. This is exemplified in populations of the banana leaf streak fungus, *Mycosphaerella fijiensis*, which are located out of its original distribution area (Carlier et al., 1996; Rivas et al., 2004). To our knowledge, no population genetics analysis has yet assessed the extent of gradual vs. stochastic dispersal processes in a plant pathogenic fungus.

Rust fungi are ideal models to tackle these questions, because in most species both gradual and stochastic dispersal processes likely occur (Nagarajan and Singh, 1990; Brown and Hovmøller, 2002). Indeed, rust fungi are more likely than other pathogens to be wind-dispersed over very long distances because their spores are comparatively resistant to environmental damage (Rotem et al., 1985). The Eurasian poplar rust fungus *Melampsora larici-populina* causes severe damage and economic losses in poplar cultivation (Frey et al., 2005). This heteroecious macrocyclic rust fungus, alternating on larches (*Larix* spp.), produces five spore stages during its life cycle, three of them (basidiospores, aeciospores and urediniospores) being wind-dispersed (Frey and Pinon, 2004). The high dispersal capacities of the asexual spores (urediniospores) are illustrated by the spread of a new virulent lineage, which covered Western Europe in less than 5 years (Pinon and Frey, 2005). Previous studies of *M. larici-populina* populations using neutral markers showed frequent occurrence of recombination (Gérard et al., 2006), but did not demonstrate a relationship between geographic and genetic distances, either in France (Gérard et al., 2006) or in the United Kingdom (Pei et al., 2007). This was probably due to the insufficient scale of sampling compared to the dispersal potential of this pathogen.

M. larici-populina is native to Eurasia and its distribution range is supposed to encompass that of its natural host *Populus nigra*. In the past century, the distribution range of the rust has expanded greatly, now covering most poplar-growing regions worldwide. Outbreaks of *M. larici-populina* on other continents were reported from South America (Spegazzini, 1918; Fresa, 1936; Kern and Thurston, 1954), Southern Africa (Lloyd, 1971; Gibson and Waller, 1972), and Australia (Walker et al., 1974). In 1973, *M. larici-populina* was introduced to New Zealand from Australia, likely via trans-Tasman air-currents over a 2000 km distance (Wilkinson and Spiers, 1976). In North America, *M. larici-populina* was first detected in 1991 in the US Pacific Northwest (Washington State and Oregon) (Newcombe and Chastagner, 1993), and subsequently in California (Pinon et al., 1994) and in Eastern Canada in 2002 (Innes et al., 2004). In 1999, *M. larici-populina* was also discovered in Southern Iceland, infecting *Populus trichocarpa* clones originally from Alaska (H. Sverrisson, personal communication).

In some areas, where the alternate host (*Larix* sp.) is absent, *M. larici-populina* can survive only asexually (Walker et al., 1974). In contrast, if larch is present, the fungus can reproduce sexually by producing aeciospores on larch needles. For instance, following its outbreaks on poplars, aeciospores of *M. larici-populina* were found on larch needles (*Larix* spp.) in New Zealand (Wilkinson and Spiers, 1976), Canada (Grondin et al., 2005) and Iceland (H. Sverrisson, personal communication). This implies that *M. larici-populina* has completed its life cycle, proceeded to sexual recombination, and has potentially established durably in these new areas. There is actually no evidence that the recent populations still display the hallmark of founder effects, such as a larger population differentiation, a reduced genetic diversity and a genetic disequilibrium.

In the present study, our aim was to apply population genetics methods to decipher the population structure of *M. larici-populina* on both continental and global scales. The first goal was to use recently developed microsatellite markers (Barrès et al., 2006) to characterize the genetic diversity and structure of *M. larici-populina* populations on the European continental scale. Because sampling both wild and cultivated poplars could bias the estimation of the genetic structure by selecting host-adapted individuals, additional typing for virulence characters was performed. This allowed for comparison with results obtained using selected markers (Pinon and Frey, 2005). We paid particular attention to the relationship between geographic and genetic distances in order to determine whether or not there was an IBD pattern. The second aspect of this study was to document the population genetic structure and characteristics of two recently founded populations in Canada and Iceland. In particular, we assessed whether or not (i) they display evidence for founder effects and (ii) they display an IBD pattern with the European populations. This sampling design, which includes isolates from both the pathogen's native range and from recently colonized areas, will help assess whether population genetics tools can help to distinguish between the different modes of dispersal.

2. Materials and methods

2.1. Sampling strategy

Poplar leaves infected with *M. larici-populina* were collected by the authors and collaborative researchers in 10 countries of the Northern hemisphere (Fig. 1, Table 1). Eleven populations were sampled from European countries: Bosnia and Herzegovina (BIH), Czech Republic (CZE), France (FRA-1, FRA-2, FRA-3, and FRA-4), Germany (DEU), Italy (ITA), the Netherlands (NLD), Poland (POL), and the United Kingdom (GBR). Two overseas populations were sampled from Iceland (ISL) and Canada (CAN). Samples were collected in the summer and autumn of 2003, except for FRA-2, FRA-3 and FRA-4 populations which were sampled in autumn of 2004. In each country, approximately 30–100 rust-infected leaves were harvested from poplar trees, over a total area ranging from 50 to 400 m^2. Whenever possible, leaves were sampled from distinct poplar trees, in order to minimize the effect of clonality. Seven populations were sampled from natural *P. nigra* stands, whereas the Icelandic population was sampled from *P. trichocarpa*. The five remaining populations were sampled from different hybrid poplars, mainly *P.* × *euramericana* and *P.* × *interamericana* (Table 1).

2.2. Pathotype identification

One single uredinium (sporulating lesion producing urediniospores) per leaf was randomly selected and grown on fresh leaf discs of *P.* × *euramericana* 'Robusta' in controlled conditions as described by Gérard et al. (2006). Briefly, a 10-μl droplet of water agar (0.1 g l^{-1}) was deposited onto each selected uredinium with a micropipette and urediniospores were dispersed within the droplet with the micropipette. The resulting spore suspension was applied as 1-μl droplets on 12-mm-diameter leaf disks of 'Robusta'. Leaf disks were incubated floating on deionized water in 24-well polycarbonate cell culture plates, abaxial surface uppermost, at 20 °C under continuous illumination (fluorescent light, 25 mmol s^{-1} m^{-2}). After an incubation period of 8–10 days, the sporulating disks were harvested and the urediniospores were

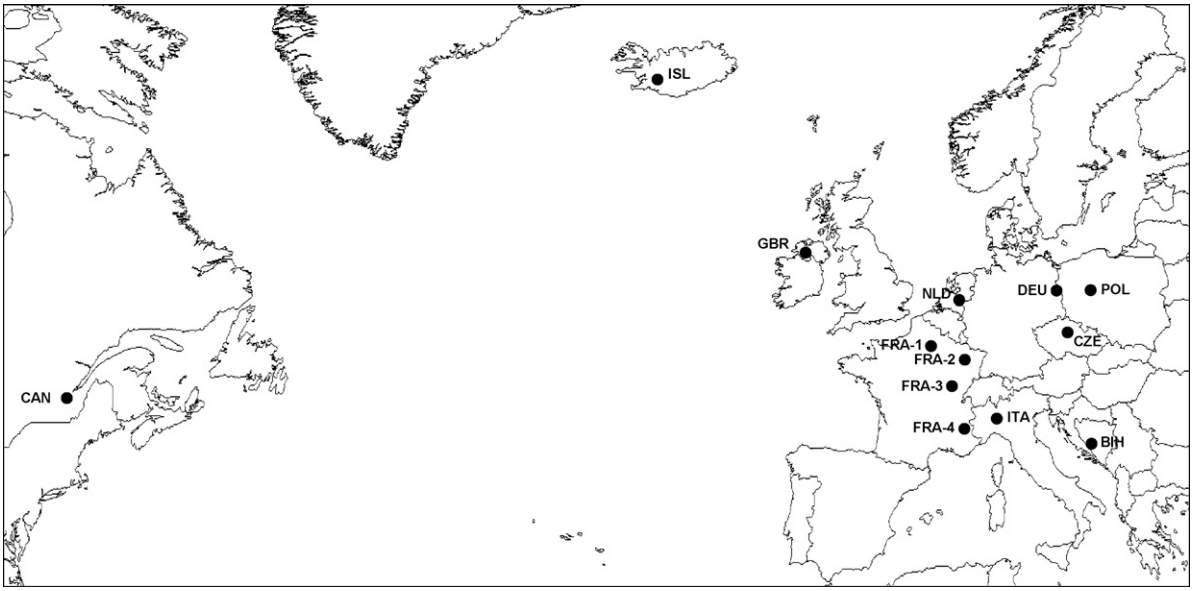

Fig. 1. Origin of the 13 *Melampsora larici-populina* populations collected for the study.

suspended in 100 μl of water agar (0.1 g l^{-1}). Each mono-uredinial isolate then was inoculated as 1-μl droplets onto 12-mm-diameter leaf disks of a differential set containing eight poplar clones with different qualitative resistance genes and incubated as described above. This differential set allowed us to detect the presence of eight virulence factors in the pathogen (Pinon and Frey, 2005). In this gene-for-gene system (Flor, 1971), a virulence factor is defined as the ability for the pathogen to cause disease on a given poplar clone, and a pathotype as a combination of virulence factors. The poplar differential set contained: *P.* × *euramericana* 'Ogy' (Vir1), *P.* × *jackii* 'Aurora' (Vir2), *P.* × *euramericana* 'Brabantica' (Vir3), *P.* × *interamericana* 'Unal' (Vir4), *P.* × *interamericana* 'Rap' (Vir5), *P. deltoides* '87B12' (Vir6), *P.* × *interamericana* 'Beaupré' (Vir7), and *P.* × *interamericana* 'Hoogvorst' (Vir8). The formation of uredinia on the leaf disks was checked daily between the 7th and the 14th day postinoculation, and the resulting pathotype (combination of virulences) was determined for each isolate.

2.3. DNA analysis

After pathotype identification, approximately 2 mg of urediniospores were collected for each isolate and stored at −20 °C in Eppendorf tubes until DNA extraction. DNA was extracted using DNeasy® 96 Plant Kit (Qiagen). We followed the Fresh Leaves protocol (DNeasy® 96 Plant Handbook, September 2002), except that samples were disrupted with one tungsten carbide bead and suspended in 200 μl of extraction buffer during 2 × 1 min, instead of 2 × 1.5 min, at 30 Hz. DNA was eluted in a final volume of 200 μl. Individuals were genotyped using 11 microsatellite markers (Barrès et al., 2006). PCRs were performed individually in a PTC-200 Peltier thermal cycler (MJ Research) using conditions previously described (Barrès et al., 2006). An exception was locus μMLP31, where the PCR mix was modified as follows: 15 ng template DNA, 2 μl of 10× reaction buffer, 3 mM $MgCl_2$, 0.7 μg/μl BSA (Sigma), 0.2 mM dNTP, 0.5 U *Taq* polymerase (Sigma), and 0.2 μM forward and reverse primers in a 20 μl final reaction volume. To allow size and dye multiplexing, forward primers were labeled with three different dyes (Proligo): D2 for μMLP13, μMLP22, μMLP27, and μMLP37; D3 for μMLP20, μMLP28, and μMLP30; and D4 for μMLP09, μMLP12, μMLP31, and μMLP36. PCR products were separated, sized, and analyzed on a CEQ™ 8000 Genetic Analysis System (Beckman Coulter). In order to reduce the number of analyses, PCR products were pooled in two sets of loci. *Set A* was made up of μMLP09, μMLP13, μMLP27, μMLP30, and μMLP36 loci with volumes of 2, 3, 4, 4, and 4 μl, respectively, in a 34 μl final volume. *Set B* contained μMLP12, μMLP20, μMLP22, μMLP28, μMLP31, and μMLP37 loci, with volumes of 3, 4, 7, 4, 2, and 8 μl, respectively, in a 61 μl final volume. Internal size

Table 1
Characteristics of the collection sites of *M. larici-populina* populations

Country	Population ID	Location	Host	Latitude	Longitude	Collector
Bosnia and Herzegovina	BIH	Ovcarevo, Konjic	*Populus nigra*	43°41′N	17°59′E	D. Ballian
Czech Republic	CZE	Veltruby, Kolin	*Populus nigra*	50°05′N	15°10′E	I. Salkova
France	FRA-1	La Quincy, Picardie	Hybrid poplars	49°25′N	03°26′E	P. Frey
	FRA-2	Champenoux, Lorraine	Hybrid poplars	48°45′N	06°20′E	B. Barrès
	FRA-3	Charrey, Bourgogne	Hybrid poplars	47°05′N	05°09′E	J. Pinon
	FRA-4	Prelles, Alpes	*Populus nigra*	44°51′N	06°34′E	P. Frey
Germany	DEU	Ziltendorfer Niederung, Brandenburg	*Populus nigra*	52°12′N	14°40′E	I. Zaspel
Italy	ITA	Zerbolo, Pavia	*Populus nigra*	45°12′N	09°03′E	L. Vietto
The Netherlands	NLD	Gendt, Gelderland	*Populus nigra*	51°51′N	05°58′E	G. Kranenborg
Poland	POL	Warta River, Poznan	*Populus nigra*	52°12′N	16°53′E	T. Tylkowski
United Kingdom	GBR	Castlearchdale, Northern Ireland	Hybrid poplars	54°28′N	07°43′W	P. Frey
Canada	CAN	Lotbinière, Québec	Hybrid poplars	46°30′N	71°55′W	P. Frey
Iceland	ISL	Skalholt, Arnessysla	*Populus trichocarpa*	64°07′N	20°31′W	H. Sverrisson

standards of 400 and 600 bp (Beckman Coulter), labeled with D1 dye, were used to genotype *Set A* and *Set B*, respectively, in a mixture containing 30 μl of Sample Loading Solution (SLS, Beckman Coulter), 0.5 μl of internal size standard, and 1 μl of each of the marker sets. When chromatograms were of poor quality, or when a locus failed to amplify, PCRs of the entire set were performed again. If the analysis failed again, the individual was considered as missing data. It should be noted that μMLP22 is a mitochondrial microsatellite locus (Barrès et al., 2006). Each allele at this locus was therefore considered as a different mitotype. The loci μMLP12 and μMLP13 are physically linked but are not redundant (Barrès et al., 2006). We tried to keep all the information carried by these two loci by (i) reconstructing the two haplotypes within an individual using the PHASE program (Stephens et al., 2001) and (ii) summing both allele sizes, which amounts to performing a PCR on a single microsatellite locus with a compound motif. All further analyses were made using this compound locus, named μMLP38, instead of the linked locus pair μMLP12 and μMLP13.

2.4. Data analyses

From pathotype frequency in each population, we calculated an evenness index (E) and a richness index (Shannon relative index, H_{SR}) to illustrate both relative abundance of pathotypes and mean pathotype diversity, respectively. The evenness index (E) is derived from the Simpson's index corrected for sample size after Fager (1972). We first calculated the complement of Simpson's index (D) for each population as $D = 1 - \sum_i [k_i(k_i - 1)/N_p(N_p - 1)]$, where k_i is the number of isolates of pathotype i and N_p is the number of isolates analyzed for virulence in the population. Then we calculated E, which is not influenced by sample size, as $E = (D - D_{min})/(D_{max} - D_{min})$ where $D_{min} = [(n_p - 1)(2N_p - n_p)]/[N_p(N_p - 1)]$ and $D_{max} = [(n_p - 1)N_p]/[n_p(N_p - 1)]$, where n_p is the number of pathotypes found in the population. The richness index H_{SR} is derived from the Shannon's index, corrected for sample size and calculated as follows: $H_{SR} = -\sum_i [(k_i/N_p)\ln(k_i/N_p)]/\ln(N_p)$. We also computed an index of pathotype complexity (C_i), defined as the mean number of virulences carried by a single isolate (Andrivon and de Vallavieille-Pope, 1995).

We subsequently used the eight virulence markers to estimate a pathotypic distance matrix between populations. Pathotypic distance was computed using DARWIN 5.0 software (Perrier et al., 2003). We chose the Manhattan distance, which is commonly used for continuous variables and is less sensitive to large differences than is the Euclidian distance. A matrix of pairwise geographical distances was built using CIRCÉ software (http://www.ign.fr). The correlation between the pathotypic distance matrix and the geographic/genetic distance matrices was assessed from Mantel tests with the ZT program (developed by E. Bonnet and Y. Van de Peer; url: http://www.psb.ugent.be/~erbon/mantel/), assuming 10,000 permutations.

Identical multilocus genotypes were identified using GIMLET version 1.3.3 (Valière, 2002) on a dataset pooling all individuals from the different populations. Potential insufficient power of our molecular markers could lead to scoring individuals as having the same multilocus genotype without, however, being clones. In order to identify multilocus genotypes that are statistically over-represented (assuming panmixia), and that could thus be considered as belonging to the same clonal lineage, the method described by Halkett et al. (2005) was used. The probability of observing n times a multilocus genotype in a population was computed using MLGSIM software (Stenberg et al., 2003). Using a Monte Carlo simulation method, the program determines the significance threshold for the probability values, indicating the multiple copies of the same multilocus genotype that did not occur by chance (true clones). Calculation was done for each population, taking into account sample size and allele frequencies. The significance level was set to 0.01. Hence, a clone-corrected dataset was built, keeping a single individual per identical multilocus genotype. These individuals were considered as clones for each population. We called n_g the number of multilocus genotypes in a given population after this clone correction. Relative genotypic diversity (n_g/N_g) was computed for each population in order to estimate the impact of asexual reproduction, where N_g is the number of sampled isolates from a given population that were genotyped. The clone-corrected dataset was used for all further analyses.

Genotypic linkage disequilibrium and deviation from Hardy–Weinberg equilibrium were computed using GENEPOP 3.4 (Raymond and Rousset, 1995) and FSTAT (Goudet, 1995), respectively. Significant levels were subsequently adjusted using the sequential Bonferroni correction method (Rice, 1989). Allelic richness (A_r), gene diversity (H_E), and inbreeding coefficients (F_{IS}) were estimated for each population using FSTAT 2.9.3.2 (Goudet, 1995).

A test to detect recent founder effects was conducted using BOTTLENECK version 1.2 (Piry et al., 1999) on the Canadian and the Icelandic populations. This test is based on the assumption that populations that have experienced a recent reduction of their effective size exhibit a higher reduction of their allele number than of their gene diversity. The program computes the Wilcoxon's test for gene diversity excess after estimating the expected gene diversity at mutation-drift equilibrium, using three mutation models: infinite allele model, stepwise mutation model and two-phase model. Permutation tests were carried out using FSTAT in order to test whether allelic richness and gene diversity were significantly different between *M. larici-populina* populations collected from their native distribution area (i.e. European populations) vs. recently founded populations (i.e. Canadian and Icelandic populations), and also between European populations sampled from hybrid poplars (FRA-1, FRA-2, FRA-3, GBR) vs. European populations sampled from *P. nigra* (BIH, CZE, DEU, FRA-4, ITA, NLD, POL). In order to determine the putative source population of Canadian and Icelandic individuals, assignment tests were performed with a frequencies-based method (Paetkau et al., 1995) using GENECLASS2 software (Piry et al., 2004). The probability that an individual belongs to a reference population was computed using the resampling method developed by Paetkau et al. (2004).

Significance of genotypic differentiation was assessed from exact tests conducted using GENEPOP 3.4. Pairwise F_{ST} values were estimated using the method of Weir and Cockerham (1984). Shared allele distances (DAS, Bowcock et al., 1994) were computed between the 13 populations of *M. larici-populina* with the 9 nuclear microsatellite markers using the POPULATION program (O. Langella, http://bioinformatics.org/project/?group_id=84). Principal component analyses were performed from pairwise matrix distances (both genotypic and pathotypic distances) using NUEES (O. Langella, http://bioinformatics.org/project/?group_id=84).

Spatial analyses were performed using two methods. First, we tested the hypothesis of isolation by distance by plotting pairwise $F_{ST}/(1 - F_{ST})$ ratios against log-transformed geographic distances according to Rousset's method (1997). Geographic distances were log-transformed because *M. larici-populina* populations evolve in a two-dimensional space. Significance of the correlation between $F_{ST}/(1 - F_{ST})$ ratios and log-transformed geographic distance matrices was assessed through a Mantel test using the ISOLD program, implemented in GENEPOP (Raymond and Rousset, 1995). Second, we performed an autocorrelation analysis (Smouse and

Peakall, 1999). We used GENALEX 6 (Peakall and Smouse, 2005) to generate a spatial autocorrelogram which figures the decrease in average genetic correlation (r) between pairs of individuals taken within a given distance interval as a function of log-transformed distances (Hardy and Vekemans, 1999). Eight distance classes were computed, starting from the logarithm of distance equaling five (approximately 150 km) and with a 0.5 log interval. To test for statistical significance, we performed 1000 random permutations of individuals among distance classes, thus estimating the 95% confidence interval of r about the null hypothesis of no spatial genetic structure (Smouse and Peakall, 1999).

3. Results

3.1. Pathotypic diversity

A total of 791 *M. larici-populina* isolates were analyzed for virulence (Table 2), among which 57 distinct pathotypes were found. The evenness index (E) showed a large disparity between populations: the POL population was characterized by the predominance of a single pathotype, whereas the BIH, CZE, FRA-1, FRA-2, FRA-3, ITA and CAN populations displayed balanced pathotype frequencies. Pathotype richness (H_{SR}) was moderately high, with the highest value found for the NLD population. Above all, pathotype complexity (C_i) discriminated between two population groups: FRA-1, FRA-2, FRA-3, and GBR were characterized by a mean number of around four virulences per isolate, and the remaining populations displayed a mean number of one to two virulences per isolate (Table 2).

3.2. Genetic diversity and equilibrium tests

A total of 313 *M. larici-populina* isolates were genotyped in this study (Table 3). Among these, 224 distinct multilocus genotypes were identified. Very few identical multilocus genotypes were detected in different populations, except two pairs (FRA-1/FRA-2 and FRA-3/POL). The mean expected heterozygosity over all loci and all sites was moderately high ($H_E = 0.352 \pm 0.239$). All loci were found to be polymorphic and exhibited numbers of alleles ranging from two to 16, for μMLP37 and μMLP38 loci, respectively. No pair of loci was significantly linked after Bonferroni correction for multiple comparisons.

Most identical multilocus genotypes were considered as non-clonal according to the analysis performed using MLGSIM, except in the BIH population, where 21 isolates were found to result from asexual reproduction (Table 3). As a result, the relative genotypic diversity was high in all the populations (0.78–1.00), except in the BIH population (0.32). The subsequent analyses were performed using the clone-corrected dataset.

All the European populations exhibited a high number of polymorphic loci (8 or 9 out of 9), except the GBR population (5 out of 9). Allelic richness was moderately high ($A_r = 2.62 \pm 0.33$, mean ± S.D.), ranging from 1.78 to 3.05, in the GBR and ITA populations, respectively. Gene diversity also was relatively high ($H_E = 0.345 \pm 0.059$, mean ± S.D.), the lowest and highest values also being found in GBR ($H_E = 0.236$) and ITA ($H_E = 0.431$) populations, respectively. None of the European populations was found to deviate significantly from Hardy–Weinberg proportions. Mean allelic richness and mean gene diversity were not significantly different between populations collected from *P. nigra* and on hybrid poplars ($P = 0.14$ and 0.17, respectively).

Whereas European populations appeared to be consistent with Hardy–Weinberg proportions, the CAN population did not ($F_{IS} = 0.771$, $P < 0.001$). Nonetheless, the ISL population did not significantly deviate from Hardy–Weinberg proportions ($F_{IS} = -0.007$, $P = 0.52$). Only three and five loci were found to be polymorphic in the Canadian and Icelandic populations, respectively. The mean allelic richness ($A_r = 1.43 \pm 0.19$, mean ± S.D.) and mean gene diversity ($H_E = 0.131 \pm 0.091$, mean ± S.D.) were significantly lower in these two overseas populations compared to the European populations ($P < 0.05$). Besides, all the alleles found in the CAN and ISL populations were found in the European populations. Two to four mitotypes were found in the European populations, whereas only mitotype D was identified in the CAN and ISL populations (Table 3). Therefore, the genetic diversity of the Canadian and Icelandic populations represents only a subset of the extant diversity in the European populations. Regardless of the mutation model assumed, the tests performed with BOTTLENECK were not significant for both CAN and ISL populations.

3.3. Population differentiation

Pathotypic distances between populations were well explained by the first axis of the principal components analysis (PCA) (Fig. 2A), which accounted for more than 68% of the total inertia. The second axis accounted for only 15% of the pathotypic distance inertia. The first axis sorted the populations into complex and simple pathotypes. Indeed, the increase in pathotype complexity from the right to the left of the diagram matched a great variation in vir7 frequency between FRA-1, FRA-2, FRA-3, and GBR

Table 2
Pathotypic characteristics of the *M. larici-populina* populations

Population ID	N_p[a]	Vir1	Vir2	Vir3	Vir4	Vir5	Vir6	Vir7	Vir8	n_p[b]	E[c]	H_{SR}[d]	C_i[e]
BIH	108	–	–	0.50	0.62	–	0.06	–	–	7	0.78	0.30	1.18
CZE	12	–	–	0.67	0.50	–	–	–	–	4	0.92	0.54	1.17
FRA-1	106	0.35	0.10	0.92	0.94	0.44	–	0.74	–	19	0.84	0.48	3.49
FRA-2	103	0.73	0.32	0.75	0.93	0.88	0.22	0.90	0.23	36	0.90	0.68	4.97
FRA-3	104	0.76	0.19	0.82	0.97	0.91	0.12	0.88	0.17	26	0.74	0.53	4.83
FRA-4	103	0.01	0.15	0.01	0.90	0.02	0.05	0.02	–	7	0.44	0.21	1.16
DEU	12	–	0.08	0.83	1.00	0.08	–	–	–	4	0.29	0.40	2.00
ITA	12	–	0.75	–	0.33	–	–	–	–	3	0.96	0.43	1.08
NLD	12	0.25	0.08	0.83	0.67	0.25	–	0.25	–	8	0.57	0.78	2.33
POL	12	–	0.08	0.92	0.92	–	–	–	–	3	0.00	0.23	1.92
GBR	11	0.82	–	1.00	1.00	0.64	–	1.00	–	3	0.61	0.38	4.45
CAN	97	–	0.69	0.99	0.22	–	–	0.03	–	6	0.75	0.27	1.93
ISL	99	0.01	–	0.34	0.94	0.01	0.02	0.01	–	6	0.60	0.21	1.33

[a] Number of isolates analyzed for virulence.
[b] Number of pathotypes found.
[c] Pathotype evenness index (Simpson's index corrected for sample size).
[d] Pathotype richness index (Shannon's index corrected for sample size).
[e] Pathotype complexity (mean number of virulences per individual).

Table 3
Genetic characteristics of the *M. larici-populina* populations

Population ID	N_g[a]	n_g[b]	n_g/N_g[c]	Polymorphic loci	A_r (±S.D.)[d]	H_E (±S.D.)[e]	F_{IS}[f]	Mitotype
BIH	31	10	0.32	8/9	2.71 (±1.41)	0.401 (±0.238)	−0.137	B, C, D, E
CZE	10	9	0.90	8/9	2.67 (±1.00)	0.321 (±0.234)	0.115	C, D
FRA-1	27	27	1.00	8/9	2.74 (±1.30)	0.298 (±0.240)	−0.020	B, C, D
FRA-2	45	45	1.00	9/9	2.70 (±1.00)	0.305 (±0.221)	0.068	B, C, D
FRA-3	45	45	1.00	9/9	2.47 (±0.92)	0.299 (±0.222)	0.067	C, D
FRA-4	46	43	0.93	8/9	2.42 (±0.97)	0.384 (±0.226)	0.098	A, B, C, D
DEU	12	10	0.78	9/9	2.82 (±1.31)	0.401 (±0.239)	0.059	C, D
ITA	12	10	0.78	9/9	3.05 (±1.34)	0.431 (±0.258)	0.073	C, D, E
NLD	12	11	0.92	8/9	2.65 (±1.03)	0.349 (±0.238)	0.104	C, D
POL	10	10	1.00	8/9	2.83 (±1.40)	0.373 (±0.237)	0.225	B, C, D
GBR	11	9	0.82	5/9	1.78 (±0.83)	0.236 (±0.270)	−0.203	C, D
CAN	30	29	0.97	3/9	1.30 (±0.48)	0.067 (±0.121)	0.771[g]	D
ISL	22	22	1.00	5/9	1.57 (±0.61)	0.196 (±0.244)	−0.007	D

[a] Number of isolates genotyped.
[b] Number of genotypes after clone-correction.
[c] Relative genotypic diversity.
[d] Allelic richness.
[e] Expected heterozygosity.
[f] Inbreeding coefficient. A_r, H_E and F_{IS} were calculated on the clone-corrected dataset.
[g] Significant departure of F_{IS} from 0.

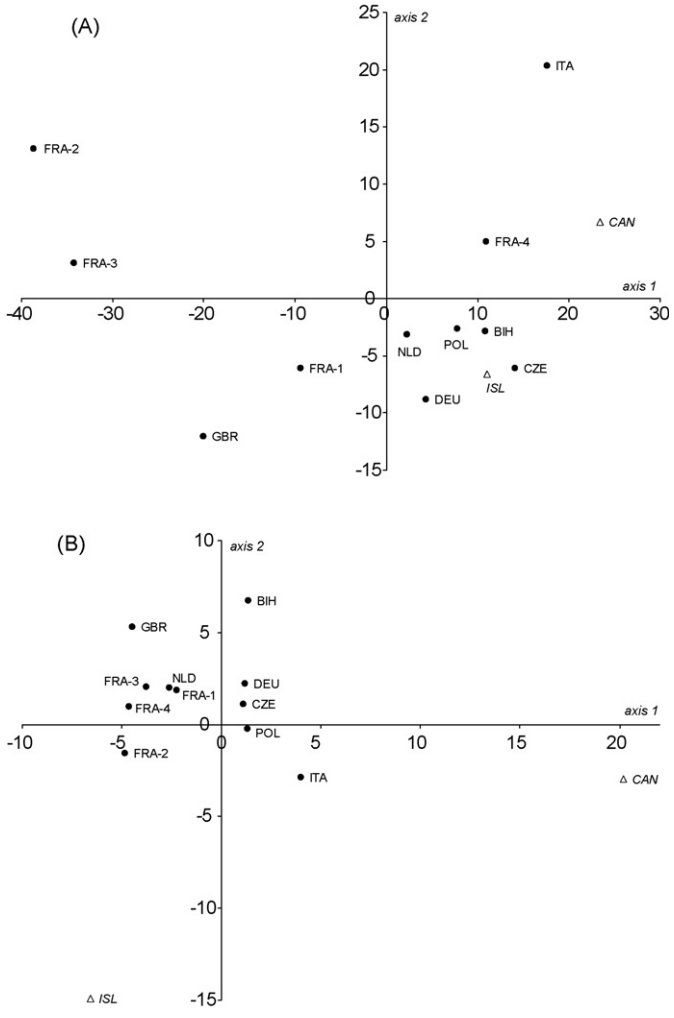

Fig. 2. Principal component analysis based on (A) the pathotypic distance matrix and (B) the genetic distance matrix between all population pairs. Pathotypic distances between populations were estimated using the Manhattan method reduced by the expected range. Shared allele distances (DAS) were used for genetic distances. Black circles represent European populations; open triangles represent overseas populations.

populations (in which most isolates carried this virulence) and the remaining populations (Table 2). It is worth noting that populations carrying the virulence 7 were collected from hybrid poplars, which either carried the corresponding resistance gene or were surrounded by such resistant cultivars. This was not the case for the hybrid poplars from Canada.

The PCA performed on genetic distances showed a very different pattern (Fig. 2B). Based on the genetic distance analysis, all the European populations were clumped together, regardless of the original hosts. The discrepancy between the genetic and pathotypic PCA analyses is further highlighted by the lack of correlation between genetic and pathotypic distance matrices ($r = -0.051$, $P = 0.47$). In the PCA analysis performed on genetic distances, the first and second axis accounted for approximately 54% and 32% of total inertia, and separated the Canadian and Icelandic populations from the European cluster. This is further supported by pairwise population differentiation analyses: pairwise F_{ST} values between the European and the two recently founded populations were all very high and significant ($P < 0.001$), ranging from 0.198 to 0.653 (Table 4). The genetic differentiation between the CAN and ISL populations was also very high ($F_{ST} = 0.653$, $P < 0.001$). Albeit much weaker, a significant genetic differentiation was detected among the European populations (global $F_{ST} = 0.057$, $P < 0.001$). Pairwise F_{ST} ranged from 0 to 0.162, nearly half being significant at the 0.001 level (Table 4). The highest differentiation values within Europe were found between GBR and continental populations. According to the assignment tests performed with GENECLASS2 software, none of the Canadian or Icelandic individuals could be clearly assigned to one of the European populations.

3.4. Spatial genetic structure

We found no evidence for a spatial structure of pathotype diversity, as the correlation between pathotypic and geographic distances was weak and non-significant (either taking all populations into account, $r = 0.006$, $P = 0.39$, or excluding the ISL and CAN populations, $r = 0.062$, $P = 0.36$).

In contrast, we detected a significantly positive correlation between the $F_{ST}/(1 - F_{ST})$ ratios and the log-transformed geographic distances when performed on all populations (dashed regression line, $P < 0.001$, Fig. 3). This overall IBD pattern seemed, however, disrupted by an inflection point occurring when the

Table 4
Pairwise F_{ST} estimated with Weir and Cockerham's ϕ (1984) on the clone-corrected dataset between *M. larici-populina* populations (above diagonal)[a], and pairwise geographic distances between locations in kilometers (below diagonal)[b]

	BIH	CZE	FRA-1	FRA-2	FRA-3	FRA-4	DEU	ITA	NLD	POL	GBR	CAN	ISL
BIH		0.056 **[c]	0.065 ***	0.098 ***	0.097 ***	0.056 ***	0.029 ns[d]	0.061 ***	0.040 **	0.045 *	0.152 ***	0.474 ***	0.317 ***
CZE	742		0.032 *	0.037 ***	0.064 ***	0.038 *	0.026 ns	0.072 *	0.027 ns	-0.008 ns	0.141 ***	0.537 ***	0.297 ***
FRA-1	1256	850		0.029 ***	0.023 *	0.020 ***	0.047 **	0.097 ***	0.015 ns	0.047 *	0.059 **	0.488 ***	0.264 ***
FRA-2	1033	660	225		0.072 ***	0.041 ***	0.081 ***	0.103 ***	0.033 **	0.044 ***	0.118 ***	0.471 ***	0.198 ***
FRA-3	1042	812	289	205		0.065 ***	0.073 ***	0.150 ***	0.052 **	0.075 ***	0.114 ***	0.470 ***	0.274 ***
FRA-4	886	873	561	434	272		0.060 ***	0.051 ***	0.005 ns	0.046 **	0.075 ***	0.415 ***	0.231 ***
DEU	981	240	851	706	893	1014		0.053 ***	0.013 ns	0.001 ns	0.126 ***	0.469 ***	0.273 ***
ITA	698	715	633	446	367	199	885		0.062 **	0.057 **	0.162 ***	0.382 ***	0.250 ***
NLD	1258	677	324	346	533	780	600	774		-0.007 ns	0.106 ***	0.551 ***	0.295 ***
POL	961	267	999	844	1022	1120	153	973	753		0.147 ***	0.485 ***	0.260 ***
GBR	2187	1631	950	1161	1222	1483	1509	1581	960	1654		0.653 ***	0.367 ***
CAN	7198	6621	5946	6166	6191	6422	6459	6558	5967	6587	5014		0.653 ***
ISL	3306	2622	2180	2356	2468	2739	2421	2801	2060	2527	1307	4147	

[a] Unbiased estimate of the *P*-value of log-likelihood-based exact test of genotypic distribution using a Markov chain method (dememorization = 5000, batches = 50, iterations = 2000) in GENEPOP version 3.4.
[b] Pairwise geographical distances were computed using CIRCÉ software.
[c] Significance levels are indicated by stars (*, ** and *** for $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively).
[d] Not significant.

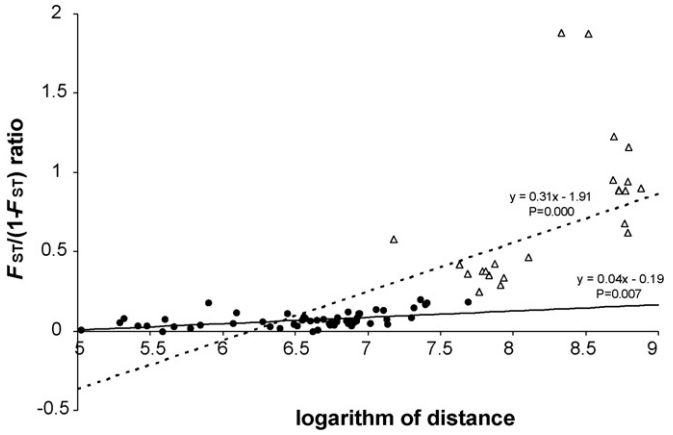

Fig. 3. Regression plot between $F_{ST}/(1 - F_{ST})$ ratios and log-transformed geographic distances. *P* values were obtained by a Mantel test (10,000 permutations). Black circles represent pairs of European populations; open triangles represent pairs with at least one overseas (Canada or Iceland) population.

logarithm of distance equals 7.5 (around 2000 km). This inflection point corresponds to the overseas populations (CAN and ISL) being included in the spatial regression analysis. Partial regression performed on only European populations yielded a weaker correlation, albeit still significant, between genetic and log-transformed geographic distances (full regression line, $P < 0.01$, Fig. 3). This drop in statistical confidence may be due to the large decrease in the value of the slope of the regression line (from 0.31 to 0.04). The departure of overseas populations from the IBD pattern was best figured by means of an autocorrelogram (Fig. 4). The autocorrelation index *r* yielded positive and significant values for 5.5–6.5 log distance classes, with an *x*-intercept at roughly 7.6 (approximately 2000 km). These five distance classes, which corresponded to within-Europe distance intervals, determined the extent of non-random genetic structure, i.e., an IBD pattern, as

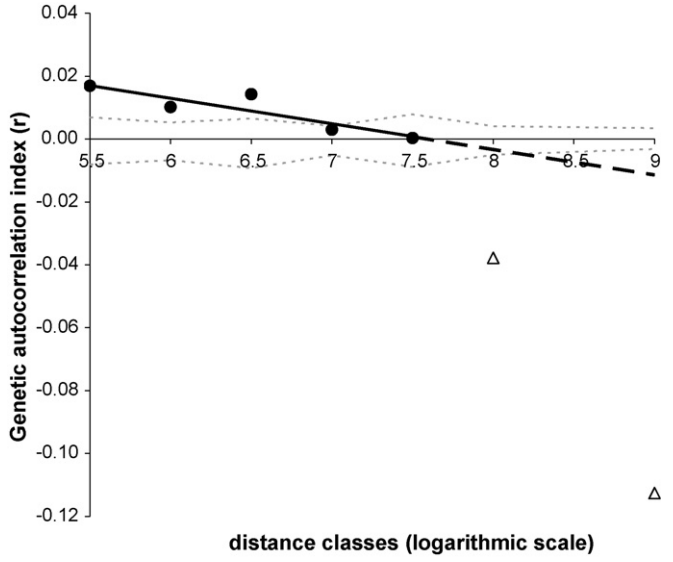

Fig. 4. Correlogram showing the genetic autocorrelation index *r* as a function of log-transformed geographic distance classes. A 95% confidence interval around the null hypothesis of the random distribution of *Melampsora larici-populina* individuals (dotted line) was determined by 1000 permutations of the dataset. Black circles represent within-Europe distance classes. Open triangles represent distance classes including at least one overseas country (Canada or Iceland). The regression line between positive values of *r* (within-Europe distance classes) and log-transformed geographic distances was plotted (dashed line after it crosses the *x*-axis).

the corresponding values of r (black circles) well aligned along a regression line (Hardy and Vekemans, 1999). The two other distance classes (open triangles), which included all overseas distance intervals, showed significant negative values of r (which reflects that CAN and ISL are highly differentiated from European populations and from each other). These two points did not fit the expected decrease in r values when extrapolating the IBD pattern (dashed line). Whereas gene flows across all the European populations exhibited an IBD pattern, the overseas populations displayed evidence for stochastic LDD events.

4. Discussion

4.1. Evidence for both IBD pattern and LDD events in *M. larici-populina* populations

The main outcome of this study was that both an IBD pattern and stochastic LDD events occurred among *M. larici-populina* populations. Interestingly, Mantel tests performed according to Rousset's method (1997) were all significant, whether taking overseas populations into account, or not. From these tests, it was therefore not possible to determine whether or not the IBD pattern extended overseas. Evidence for a break in the dispersal regime occurring between European and overseas populations was better assessed through the autocorrelogram analysis. According to this method, a non-random spatial genetic structure was observed when only the European populations were included. The points corresponding to intra-Europe distance intervals aligned well and showed a regular decrease of the relationship coefficient (r) as geographic distances increased (Fig. 4). This is considered to be a signature of an IBD (Hardy and Vekemans, 1999). The two last points of the autocorrelogram – those that include overseas populations in the analysis – did not fit with this regression line, indicating that these populations separated from the European populations through stochastic differentiation processes.

Interestingly, the IBD pattern and LDD events did not overlap. The inflection point figured in the graphical representation of the Mantel test enabled the geographic range for gradual dispersal to be clearly delineated. In the autocorrelogram, this switch point matched the intersection between the regression line and the x-axis. However, it is important to notice that this coincidence has no biological meaning; first, because the interception point is largely dependent on the sampling design, and second, because the extent of the IBD pattern should not be regarded as an estimation of the distance dispersal of an organism (Hardy and Vekemans, 1999; Vekemans and Hardy, 2004). In *M. larici-populina*, it is likely that gradual dispersal occurs across the whole native range of the pathogen, and that the IBD pattern might have been larger, provided we had sampled locations eastward within Europe. Conversely, the distance at which we observed the shift from gradual to stochastic dispersal might reflect the sufficient distance interval – characterized by a full length of unsuitable habitat – for a stochastic genetic differentiation to appear; that is, a sea distance of 2000 km was sufficient to create stochastic dispersal of *M. larici-populina*.

In principle, the mean dispersal distance has to be estimated through the slope of the regression line between the genetic and geographic distances (Rousset, 1997; Hardy and Vekemans, 1999). Here, it is obvious that including LDD would have biased the estimate of the dispersal distance by artificially inflating the value of the slope of the regression line. Significant IBD does not necessary mean a spatially homogeneous gene flow (e.g. Garnier et al., 2004). This raises the problem of how to distinguish LDD events from an IBD pattern when these two processes occur within the same geographic range. To our knowledge, there is no specific methodology to deal with this situation. Recent advances in Bayesian computing in population genetics enable genetic discontinuities to be easily revealed (Pritchard et al., 2000; Falush et al., 2003). However, these methods are more likely to detect physical barriers to gene flow when clustering populations are at Hardy–Weinberg equilibrium (Manel et al., 2003), than to distinguish between IBD and LDD, the latter being often far from genetic equilibrium (see below). The most promising method (described in Garnier et al., 2004) is to perform successive resampling of populations, test for IBD (e.g. with Rousset's method, 1997), and then exclude the populations that greatly increased the slope of the regression line. This method was successfully applied to the ground beetle *Carabus soleri* to confirm that a geographic barrier (the Alps) prevented secondary contacts during postglacial recolonization from southern France and Italy (Garnier et al., 2004). However, a standardized procedure is still missing, which would aim to provide a statistically based exclusion rule.

4.2. Evidence for isolation by distance within Europe

An IBD pattern was found among all *M. larici-populina* populations sampled across the European continent (including the GBR population from Northern Ireland). This correlation between geographic and genetic distances results from the regular and gradual dispersal of the pathogen over the spatial scale covering the sampled regions. The IBD pattern proved that there is no ubiquitous mixing in *M. larici-populina* across Europe, but instead that gene flow decreased over geographic distances. Nonetheless, the genetic differentiation between European populations of *M. larici-populina* was moderately low, which resulted in a weak value of the slope of the regression line. Therefore, even if dispersal is limited over Europe, an important gene flow occurred at this spatial scale.

The detection of an IBD pattern greatly depends on the spatial scale studied (Rousset, 1997; Castric and Bernatchez, 2003), but the appropriate sampling scale to reveal an IBD pattern for plant pathogenic fungi is not obvious at first sight. This may account for the limited number of studies that detected an IBD on plant pathogens. Considering the high dispersal ability of airborne pathogens (Brown and Hovmøller, 2002), it is not surprising that most IBD patterns were found only at the continental scale (e.g. *Cronartium ribicola* in Eastern Canada, Et-touil et al., 1999; *Rhynchosporium secalis* in Australia, McDonald et al., 1999; or *Plasmopara viticola* in Central Europe, Gobbin et al., 2006), or even at the worldwide scale for *Rhynchosporium secalis* (Zaffarano et al., 2006). Sampling at smaller spatial scales often resulted in the failure to detect IBD, except for the particular example of *Microbotryum violaceum,* for which no IBD was found between populations (Delmotte et al., 1999; Giraud, 2004) but an IBD pattern was detected within populations (Giraud, 2004). For some airborne plant pathogenic fungi, IBD can be difficult to detect even at the largest spatial scale, because of a high gene flow (Brown and Hovmøller, 2002; Zeller et al., 2004). In a few cases, no genetic structure was observed, which implies that all the populations behaved as a single panmictic unit (Et-touil et al., 1999). Nonetheless, it is also quite likely that at these large spatial scales (world or continent), some barriers to gene flow produce a large and stochastic genetic differentiation that would impede the detection of IBD. Many physical barriers – mountains, seas or any vast area without suitable hosts – can disrupt the natural dispersal of the pathogen, leading to reproductive isolation. In these cases, significant population differentiation is expected (Manel et al., 2005). The effects of drift, together with selection, will generate this genetic differentiation; it may also be strengthened by founder effects, as observed in *Mycosphaerella fijiensis* during the

worldwide spread of the black leaf streak disease of banana. Founder effects lead to highly stochastic differentiation processes and a reduction of population genetic diversity (Rivas et al., 2004). Conversely, the effect of physical barriers to gene flow can be much weaker, as observed in *Plasmopara viticola,* the causal agent of grapevine downy mildew, for which the strength of the IBD pattern across Europe was only slightly reduced when taking into account the Greek populations (Gobbin et al., 2006). In the present study, the English Channel, the North Sea and the Irish Sea could have acted as physical barriers limiting spore dispersal of *M. larici-populina* from the continent to Northern Ireland. Although the GBR population showed a lower level of genetic diversity and a large genetic differentiation with the remaining European populations, this population nonetheless fell under the IBD pattern.

Another explanation for a lack of correlation between genetic and geographic distances among pathogen populations is the existence of selection driven by the host. Such selection could indeed lead to local adaptation, which would interfere with an underlying IBD pattern by selecting for the individuals most adapted to their host plants (e.g. the bean anthracnose fungus *Colletotrichum lindemuthianum*, Capelle and Neema, 2005). This would particularly be the case if sexual reproduction occurs on the same host plant where the selection acts, leading to reproductive isolation (Giraud et al., 2006). Hence, the population genetic structure of the pathogen would be more conditioned by the distribution of the different host plants than by geographic distances between pathogen populations. Local adaptation processes are usually measured using selected markers such as virulences. A well-known example of such studies is given by the flax rust fungus, *Melampsora lini*, an autoecious rust fungus which completes its whole life cycle on *Linum marginale* (Thrall and Burdon, 2002). Patterns of metapopulation dynamics driven by local adaptation were also shown in the *Plantago lanceolata–Podosphaera plantaginis* interaction (Laine, 2005; Laine and Hanski, 2006). In the present study, we showed that the host type also conditioned the virulence profile of the *M. larici-populina* isolates. This was highlighted by the higher pathotype complexity of the isolates collected from the cultivated poplars, and the large pathotype differentiation between populations from cultivated vs. wild poplars, as has already been shown in a previous study (Gérard et al., 2006). Indeed, the extensive use of cultivars carrying specific resistance genes toward *M. larici-populina* in poplar cultivation has strongly affected the pathogen populations (Pinon and Frey, 2005). For example, the massive plantation of the cultivar 'Beaupré' carrying the resistance factor 7 had a great impact on the population dynamics and virulence profile of the poplar rust (Gérard et al., 2006). Nonetheless, we found no correlation between selected (virulence) and neutral (microsatellite) markers. In addition, microsatellite markers revealed little (albeit significant) population structure with respect to the sampled host plant ($F_{ST} = 0.025$, $P < 0.001$). We can therefore argue that there is little reproductive isolation between the populations sampled from cultivated and wild poplars. It is noteworthy that *M. larici-populina* has to alternate on larch, the aecial host, to reproduce sexually. In accordance with previous results (Gérard et al., 2006), the genetic characteristics of *M. larici-populina* populations strongly suggest that sexual reproduction occurs widely and frequently: (i) all the European *M. larici-populina* populations were at Hardy–Weinberg equilibrium; (ii) none of the loci pairs were linked in any of the studied populations; (iii) there were very few clones, except in Bosnia and Herzegovina. This is consistent with the wide distribution of the European larch (*Larix decidua*). Although native to the mountains of Central Europe (Alps, Carpathians, Sudetes, Tatras) and lowlands in Northern Poland, it is now well distributed in lowlands throughout Europe because it has been widely planted for timber and ornamental purposes. Because it promotes genetic intermixing, the need to alternate on larch is likely to explain the lack of reproductive isolation of *M. larici-populina* populations.

4.3. Evidence for founder effects overseas

Introduction of *M. larici-populina* in Eastern Canada and Iceland are recent events (Innes et al., 2004; H. Sverrisson, personal communication). Such long distance dispersal events may have originated with a limited number of individuals. Here we report several lines of evidence that Canadian and Icelandic populations exhibited signatures of strong founder effects. First, we observed a reduction of genetic diversity compared to populations from the native range. In the Canadian and Icelandic populations, most of the loci were monomorphic, and allelic richness and gene diversity were significantly lower than in the European populations. Second, Cornuet and Luikart (1996) showed that recent founder effects should result in a gene diversity excess at selectively neutral loci. However, we did not detect such a genetic disequilibrium in the Canadian and Icelandic populations. This may be explained by the low number of polymorphic loci in these populations, resulting in a lack of power of the tests (Luikart et al., 1998). In addition, the Canadian population showed an important heterozygote deficiency. This large deviation from Hardy–Weinberg equilibrium may result from the sexual reproduction among a very restricted number of individuals (even clone mates), therefore resulting in a high rate of selfing (Giraud, 2004; Raboin et al., 2006). Third, high genetic differentiation was observed between the two newly founded populations and the European populations. Indeed, founder effects often result in high genetic differentiation between populations because the reduced effective number of individuals favors a rapid divergence of gene frequencies through drift (Boileau et al., 1992; Carlier et al., 1996; Rivas et al., 2004). Moreover, great differentiation was observed between Canadian and Icelandic populations, suggesting the independence of the two introductions, and illustrating again the stochastic character of these events. Nevertheless, the origin of the Canadian and Icelandic populations could not be determined from the assignment tests, possibly because of too large population differentiation between European and overseas populations.

4.4. Putative mechanisms of long distance dispersal

For plant pathogenic fungi, long distance dispersal events can either result from passive transport by wind or from human activity (Brown and Hovmøller, 2002). Although there is no direct evidence, the migration from Europe to Iceland is likely to have resulted from an airborne LDD event, as shown for other taxa. Several insect species, especially Lepidopteran, were shown to be transported by wind from Europe to Iceland (Downes, 1988). In addition, large amounts of birch pollen originating from continental Europe were detected in Iceland in May 2006, several weeks before the flowering of local birch trees (M. Hallsdóttir, personal communication). Similar airborne LDD events have already been reported for *M. larici-populina* between Australia and New Zealand via trans-Tasman air-currents (Wilkinson and Spiers, 1976), and also for other rust fungi such as coffee leaf rust (*Hemileia vastatrix*, Bowden et al., 1971), sugarcane rust (*Puccinia melanocephala*, Purdy et al., 1985), and soybean rust (*Phakopsora pachyrhizi*, Pan et al., 2006). The fact that poplars remained free from any rust fungus for decades in Iceland reflects the very low probability of viable spores reaching susceptible poplar leaves. Nevertheless, the development of poplar cultivation in Iceland during the 1990's increased the net trapping effect, and severe poplar rust epidemics in Western Europe during the period

1996–2000 certainly strengthened the inoculum pressure (Lonsdale and Tabbush, 2002; Pinon and Frey, 2005).

The wind dispersal hypothesis seems less realistic for explaining the introduction of *M. larici-populina* in Canada, considering (i) the higher distance and (ii) the direction of prevailing winds in the Northern hemisphere. Moreover, most of the *Populus* species in North America are susceptible to *M. larici-populina*. Therefore, if wind-dispersed migration could have occurred in the past, *M. larici-populina* should have been discovered far sooner than its first report in North America (Newcombe and Chastagner, 1993). Wilkinson and Spiers (1976) suggested the possible spread of *M. larici-populina* by infected plant material to explain the introduction of the pathogen in Australia. However, no evidence of survival of *M. larici-populina* as urediniospores attached to poplar buds, or as mycelium in buds, has been reported so far. Hence, the introduction of *M. larici-populina* in Canada seems more likely to be due to human transport (e.g. by urediniospores carried on clothes).

It appears that *M. larici-populina* has become durably established in Canada and Iceland. Indeed, larches (*Larix* spp.) are present in both countries and aecia of *M. larici-populina* are observed each spring on larch needles in the vicinity of poplars in Canada (Grondin et al., 2005) and Iceland (H. Sverrisson, personal communication). The lack of significant linkage disequilibrium between microsatellite loci is in agreement with the occurrence of sexual reproduction in the Canadian and Icelandic populations. These findings underline the importance of pest alert networks and quarantine regulations in order to avoid the introduction of exotic pathogens to healthy areas, as these pathogens can become durably established under favorable conditions. Hybridization with indigenous species is also a great danger of such introductions (Brasier, 2001). Hybridization between *M. larici-populina* and *M. medusae* f. sp. *deltoidae*, a North American poplar rust fungus, has already been reported in New Zealand (Spiers and Hopcroft, 1994) and South Africa (Frey et al., 2005). The hybrid taxon was shown to exhibit the cumulative host range of both parental species, and could thus be a potential threat to poplar cultivation.

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