Genetic Diversity in Poplar Leaf Rust (*Melampsora medusae* f. sp. *deltoideae*) in the Zones of Host Sympatry and Allopatry

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ABSTRACT

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Poplar leaf rust caused by *Melampsora medusae* f. sp. *deltoideae* is a widespread disease in North America, where epidemics occur within zones of sympatry and allopatry of telial hosts (*Populus* spp.) and aecial hosts (*Larix* spp.). To test the hypothesis that epidemics originate in the zone of sympatry where the rust can complete its life cycle, populations in sympatry and allopatry were analyzed with single-strand conformational polymorphism for codominant detection of alleles directly from uredinia. More alleles were detected in rust populations in

the zone of host sympatry than in allopatry. Almost all alleles found in the zone of allopatry were a subset of the allelic diversity present in the zone of host sympatry. Distance analyses clustered populations according to geographic origin, but not sampling year or type of stand (plantation or natural stands). Large differences in allelic and genotypic frequency were observed between years in allopatry but not in sympatry, suggesting new colonizations in allopatric populations. Our results point to a dynamic and complex pattern of inoculum dissemination in poplar leaf rust. The hypothesis most consistent with our results is that populations in sympatry represent a source of inoculum for epidemics, with some annual recolonization in allopatry, possibly via intermediate population jumps.

Melampsora medusae f. sp. *deltoideae* Thuem is an indigenous fungus of North America causing poplar leaf rust, which is considered among the most serious diseases affecting poplars worldwide. It causes premature defoliation (23,28) which eventually weakens trees, thereby increasing their susceptibility to other pathogens (30). Poplar leaf rust has been reported to cause as much as 65% reduction in volume in poplar plantations in the north-central United States (31).

Annual epidemics of *M. medusae* f. sp. *deltoideae* occur throughout the range of distribution of *Populus deltoides* Bartr in eastern North America. The source of inoculum for these epidemics is uncertain. *M. medusae* f. sp. *deltoideae* is heteroecious and macrocyclic. To complete its life cycle, it must alternate between poplars of the section Aigeiros, where urediniospores, teliospores, and basidiospores are produced, and larch, where spermatia and aeciospores are produced (35). Although both hosts are required for the pathogen to complete its life cycle, epidemics occur in the zone of host sympatry (where both larch and poplar hosts coexist) and in the zone of allopatry (outside of the zone of natural occurrence of larch) (Fig. 1). Because only the urediniospores and the aeciospores are known to infect poplar, two potential sources of inoculum can be proposed: (i) aeciospores derived from larch or other unknown aecial hosts and (ii) overwintering urediniospores.

If the annual epidemics originated from aeciospores produced in the zone of host sympatry, followed by repeated asexual cycles, the populations would be expected to exhibit a gradient of diversity decreasing from northern to southern populations. In addition, large fluctuations in allelic and genotypic composition would be ex-

pected from year to year in populations distant from the source because sexual recombination would result in new genotypic combinations and their frequency would be augmented through repeated asexual cycles. In contrast, if local overwintering urediniospores were the source of primary inoculum, allele and genotype frequencies would be expected to be stable from year to year.

The source of inoculum also should influence population structure and equilibrium. In populations derived from locally produced aeciospores, Hardy-Weinberg equilibrium would be expected at neutral markers because they are the direct result of sexual recombination (9). However, populations derived from overwintering urediniospores or from aeciospores after repeated asexual cycles would be expected to exhibit deviation from Hardy-Weinberg equilibrium. In addition, a more differentiated population structure would be expected in populations with local asexual overwintering because of founder effects compared with those derived from locally produced aeciospores.

Investigations into the source of inoculum for *M. medusae* f. sp. *deltoideae* epidemics have been conducted previously. There was circumstantial evidence that *M. medusae* f. sp. *deltoideae* spread over long distances via windborne urediniospores (29,32), in a reverse "Puccinia path," from northern to southern locations (19,27). A correlation between spore source and pathotypes was reported using phenotypic virulence markers. *P. deltoides* clones were resistant to inoculum from northern locations along the Mississippi river valley but susceptible to isolates derived from southern locations from that same area (8,18,24). This suggested the presence of population structure with different races of *M. medusae* f. sp. *deltoideae* in different regions, which would imply different sources of inoculum with restricted migration.

However, questions regarding population biology are best answered using highly polymorphic neutral markers that can reveal demographic patterns and population history. Two highly polymorphic polymerase chain reaction (PCR)-based markers, codominant by single-strand conformational polymorphism (SSCP)

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analysis and specific to *M. medusae* f. sp. *deltoidae*, were developed previously (3). These codominant markers can be genotyped directly from single uredinia, allowing a large number of samples to be processed. This can lead to a better characterization of the genetic structure of rust populations and to the testing of hypotheses regarding the source of *M. medusae* f. sp. *deltoidae* primary inoculum.

Knowing the source of inoculum for poplar leaf rust epidemics is important, in particular in light of the extensive efforts deployed in poplar selection and breeding and the increasing interest in using poplar in intensively managed plantations. The source of inoculum could impact the epidemiological attributes of poplar leaf rust. Inoculum produced on larch is the result of sexual reproduction. The zone of poplar-larch sympatry could serve as a reservoir for greater allelic diversity and new genotypes. The extent of genetic exchange between regional populations and, more globally, between northern (zone of host sympatry) and southern (zone of allopatry of telial host) regions is an important epidemiological feature that can be measured using molecular approaches.

The objective of this study was to analyze populations of *M. medusae* f. sp. *deltoidae* using highly polymorphic codominant molecular markers to determine the potential origin of annual poplar leaf rust epidemics. To test the hypothesis that the zone of host sympatry represents the center of genetic diversity from which rust epidemics originate, we compared allelic and genotypic composition and diversity, population structure, and migration in *M. medusae* f. sp. *deltoidae* populations throughout the range of distribution of *P. deltoides* in eastern North America.

MATERIALS AND METHODS

Sample collection. Infected *P. deltoides* (except in one North Dakota population where a *P. deltoides* × *P. balsamifera* hybrid was sampled) leaves showing uredinia were collected in September and October 1997 and 1998. Sampling was carried out

at 14 stands, 7 in the zone of host sympatry and 7 in the zone of host allopatry (Fig. 1). Only 11 stands were sampled in 1998. The seven stands in sympatry were located in Canada and included one site in Ontario (Ottawa) and six sites in Quebec (Montreal, Sutton, Trois-Rivieres, Villeroy, Cap-Rouge, and Lévis). In the zone of sympatry, all populations were sampled from natural stands except the Cap-Rouge and Villeroy populations, which were collected in plantations. Populations in allopatry were from five U.S. states: North Dakota, Iowa, Illinois, Missouri, and Mississippi. Six stands were identified by the name of the city from which they originate: Ames (Iowa), Cairo (Illinois), Anniston (Missouri), Stoneville (Mississippi), and Fargo (North Dakota, two sites); one was identified by the county of origin: New Madrid (Missouri). Populations were sampled from natural stands except for Anniston, Stoneville, and one population at Fargo.

Three leaves with uredinia were removed from 10 trees at least 10 m apart in each stand, stored in a standard paper mailing envelope, placed in a Ziploc plastic bag, and shipped to the laboratory. Leaves were used immediately for harvesting single uredinia or stored in the plastic bag at 4°C until they were processed.

Uredinia were excised individually from leaves with a sterile scalpel after removing urediniospores (to avoid cross contamination) and used for DNA extraction (3).

DNA extraction. DNA was extracted by grinding uredinia with diatomaceous earth (Sigma-Aldrich, St. Louis) in 400 µl of Qiagen extraction buffer (100 mM Tris-HCl, pH 9.5, 2% cetyltrimethylammonium bromide, 1.4 M NaCl, 1% polyethylene glycol 8000, 20 mM EDTA, pH 8.0, and 1% β-mercaptoethanol). Suspensions were mixed, incubated at 65°C for 1 h, extracted once with 400 µl of phenol:chloroform:isoamyl alcohol (25:24:1), and centrifuged at 10,000 × g for 5 min. The aqueous phase was transferred into a new microtube, precipitated with one volume of cold isopropanol, and centrifuged at 10,000 × g for 5 min. The resulting pellets were washed with 70% ethanol, air dried overnight, and resuspended in 20 µl of TE buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA, pH 8.0). DNA extracts in TE buffer were stored at -20°C.

Amplification of markers. Because of the small amount of fungal DNA in each extract, 1 µl of extract in TE buffer was added directly to a 12-µl reaction mix composed of 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, 200 µM each dNTP, 1 µM each primer, and 0.5 U of Taq DNA polymerase. The two markers were amplified with primers c1c3a2f (5'-GGGGGTCTT-TAGGACAAA-3') and c1c3a2r (5'-TTCGAGCCAGCATGAAAC-AC-3') (locus A), and c1c3a3f (5'-TTCGAGCCAGAAAGTTGTT-TC-3') and c1c3a3r (5'-TTCGAGCCAGCATCACTT-3') (locus B) (3). PCR products of 500 bp (GenBank accession numbers AY489127 to AY489134) and 600 bp (GenBank accession numbers AY490805 to AY490813) are targeted by these primers. Amplifications were carried out in an MJ Research PTC-60 thermal cycler (MJ Research Inc., Watertown, MA) with the following conditions: denaturation for 3 min at 95°C; 35 cycles of 92°C for 30 s, 55°C for 30 s, and 72°C for 1 min; and then a final extension at 72°C for 10 min. PCR products were visualized by electrophoresis in 1.5% (wt/vol) agarose in TAE buffer (0.04 M Tris-acetate, 1 mM EDTA, pH 8) and stained with ethidium bromide.

SSCP analysis of markers. Amplification products were analyzed by SSCP (15) following an adapted protocol (2). PCR product (5 µl) was added to 3 µl of formamide loading buffer (0.05% bromophenol blue, 0.05% xylene cyanol in formamide). The resulting mixture was denatured at 100°C for 3 min and quickly chilled on ice before a volume of 6 µl was loaded on gel. Locus A was separated on 0.6× Tris-borate EDTA (TBE), 0.8× Hydrolink MDE (FMC BioProducts, Rockland, ME) nondenaturing polyacrylamide gel (size: 260 by 125 by 0.5 mm) at 2 W for 18 h at 10°C using a Multiphor II system with a MultiTemp II Thermostatic Circulator and an ECPS 3000/150 power supply (Pharmacia, Uppsala, Sweden). Locus B was separated on 0.6×

Fig. 1. Distribution of *Melampsora medusae* f. sp. *deltoidae* populations sampled in eastern North America. Distribution areas of the two hosts, larch and poplar, are shown, including the zones of sympatry (where the two hosts coexist) and telial host allopatry (where no known alternate host occurs). Populations identified with a dot are natural stands and populations identified with an asterisk are plantations.

TBE, 0.8× Hydrolink MDE at 8 W for 20 h at 4°C. SSCPs were visualized by silver staining using a Bio-Rad Silver Stain Kit (Bio-Rad Laboratories, Hercules, CA). Isolates showing patterns of one or two bands were designated homozygotes, whereas isolates revealing patterns of three or four bands were considered heterozygotes. Alleles not identified previously were extracted from the acrylamide gel and the DNA was sequenced as reported before (3).

Data analyses. General population genetics statistics included the observed number of alleles (N_a); observed heterozygosity (H_o), which is the proportion of observed heterozygotes; and expected heterozygosity (H_e), which is calculated as 1.0 minus the sum of the squared gene frequencies. They were calculated with Genalex 5.1 (R. Peakall and P. E. Smouse, The Australian National University, Canberra, Australia). Nei's unbiased genetic distances (14) were calculated among populations and a dendrogram was generated with the neighbor-joining (NJ) method using PHYLIP software package (version 3.572c; Department of Genetics, University of Washington, Seattle). Inbreeding coefficients F_{is} (the proportion of the variance in the subpopulation contained in an individual) and genetic differentiation calculated with F_{st} (the proportion of the total genetic variance contained in a subpopulation relative to the total genetic variance) were calculated in Arlequin (Genetics and Biometry Laboratory, Department of Anthropology and Ecology, University of Geneva, Geneva, Switzerland). A pairwise F_{st} matrix among all population pairs was used as input in a principal component analysis with Genalex 5.1 (R. Peakall and P. E. Smouse, The Australian National University, Canberra, Australia). Exact tests of genic and genotypic differentiation among sampling years and exact tests of Hardy-Weinberg equilibrium were conducted with Genepop (Laboratoire de Génétique et Environnement, Montpellier, France). A Markov chain procedure was used with 1,000 dememorization steps and 1,000 iterations per batch. A Bonferroni correction was applied to take into account the multiple comparisons.

The proportion of individuals that are suspected migrants was determined using BayesAss (33). BayesAss is a program that estimates recent migration rates between populations using Monte Carlo Markov Chains. Each individual can be assigned as an immigrant from a specific population, a nonimmigrant, or the offspring of an immigrant and a nonimmigrant. The number of iterations was 3,000,000, of which 999,999 were burnin, and the sampling frequency was 2,000. The delta value for allele frequency, inbreeding, and migration was set at 0.15, the default value. This analysis was performed separately for the two sampling years.

Genetic and geographic distance matrices were tested for correlation with ISOLDIS in Genepop. Spearman correlations between the geographic and genetic distance matrices were calculated for all populations separately in the two sampling years, and for populations within the zones of host sympatry and allopatry. Significance of the correlation was tested with 1,000 random permutations of the matrix.

RESULTS

All uredinal samples yielded an amplification product of the expected size for both markers. PCR products were resolved on SSCP gels to reveal the codominant genotypes at both loci. Most samples yielded one to four bands, consistent in this dikaryon with the presence of a homo- or heterokaryon. The rare cases where more than four bands were observed in an individual were interpreted as the result of contamination, spurious amplification, or gene duplication, and were excluded from the analysis. In all, 7 and 26 alleles were identified at loci A and B, respectively. At locus A, all seven alleles detected were present in the zone of host sympatry but only five alleles were found outside the zone of host sympatry. The two private alleles (alleles that are specific for one populations) in the zone of sympatry at locus A were found in

both sampling years. At locus B, 24 alleles were present in the rust populations from the zone of sympatry but only 15 alleles were detected outside of the zone of host sympatry (Fig. 2). Allelic composition at the more polymorphic locus B was further examined by breaking down the populations into three zones: sympatry, northern allopatry (Fargo-N, Fargo-P, and Ames), and southern allopatry (Anniston, New Madrid, Cairo, and Stoneville). Eleven private alleles were found in the zone of host sympatry (Fig. 2). Only two private alleles were found outside of the zone of host sympatry. All private alleles were rare and occurred in a single year in a single population, except for three private alleles in sympatry that were found in both sampling years. Five alleles were observed in the zones of sympatry and northern allopatry and eight alleles were shared among populations from all three zones (Fig. 2).

A greater diversity was observed in the populations from the zone of sympatry compared with those from the zones of telial host allopatry. Observed and expected heterozygosities generally were higher in populations of host sympatry than in populations in the zones of northern and southern allopatry (Table 1). There was a north-south gradient in diversity: populations from sympatry had the highest diversity, followed by populations in northern allopatry, then populations in southern allopatry (Table 1). The only exception was in 1998 where, in spite of higher allelic diversity in sympatry than in allopatry, observed heterozygosities were similar, on average, in populations from sympatry and allopatry.

There were marked differences in the inbreeding coefficients among the host zones. There was an excess of homozygotes at locus A in sympatry but not at locus B for both sampling years (Table 2). By contrast, there was an excess of heterozygotes at both loci in northern and southern allopatric zones for both years, except at locus A in 1997 in northern allopatry (Table 2). There was a north-south gradient in F_{st} , with smaller F_{st} , on average, in sympatry than in allopatry. F_{st} among regions was 0.065 and 0.079 in 1997 and 1998, respectively.

An NJ tree using a matrix of Nei's unbiased genetic distances revealed that populations generally clustered according to geographic origin (Fig. 3), but not sampling year or type of stand (plantation or natural stands). The populations from southern allopatry (Cairo, Anniston, New Madrid, and Stoneville) formed a distinct cluster that included the plantation from Fargo in 1997. Most populations from sympatry (Cap-Rouge, Levis, Villeroy, Montreal, Sutton, Trois-Rivieres, and Ottawa) formed a cluster at

Fig. 2. Allele distribution at locus B in three zones of host distribution of *Melampsora medusae* f. sp. *deltoideae* in eastern North America: sympatry, northern allopatry, and southern allopatry. Numbers represent 26 alleles observed in 1997 and 1998 at locus B.

the other extremity of the NJ tree. Populations from northern allopatry (Ames and Fargo) and some populations from sympatry (Ottawa and Trois-Rivières in 1997) were intermediate between populations from southern allopatry and other populations from sympatry (Fig. 3).

A principal component analysis using a matrix of pairwise F_{st} among all populations for the two sampling years revealed a similar trend (Fig. 4). Populations from the zone of sympatry formed a compact group mostly in the upper-right quadrant for both sampling years, whereas no population from allopatry was in that quadrant (Fig. 4). Populations from allopatry were dispersed among the three remaining quadrants. Populations from northern allopatry were in the bottom right quadrant, with the exception of Fargo-P in 1997. Populations from allopatry for which sampling had been conducted for 2 years either were in different quadrants for the different years, such as Anniston and Fargo-P, or at opposite ends of a quadrant, such as Stoneville, Fargo-N, and Ames (Fig. 4).

Genic and genotypic frequencies were compared in *M. medusae* f. sp. *deltoideae* populations in different sampling years. Pairwise F_{st} among populations for 1997 and 1998 ranged from 0.001 to 0.026, with an average of 0.013 in sympatry, and from 0.024 to 0.115, with an average of 0.070 in allopatry (Table 3). Comparison of allelic and genotypic frequencies between sampling years revealed that no comparisons in the zone of host sympatry were

significant (Table 3). In contrast, three comparisons from the zone of host allopatry were significant, indicating changes in both allelic and genotypic composition between the two sampling years.

Tests of Hardy-Weinberg (H.W.) equilibrium were conducted to test whether or not patterns were consistent with the presence of sexual reproduction. Clonality or inbreeding would be expected to cause deviations from H.W. equilibrium. Only six tests revealed a statistically significant deviation from expectations under the H.W. model (Table 4). Of those, there were three in populations in sympatry (loci A and B in Cap-Rouge in 1997 and locus A in Ottawa in 1998), and three in populations in allopatry (locus B in the two Fargo populations in 1997 and locus B in Stoneville in 1998).

The proportion of estimated recent migrants (in the current and the previous generation) was estimated by assigning individuals as immigrants, nonimmigrants, or the offspring of immigrants or nonimmigrants. Striking differences were found in the patterns observed in 1997 and 1998. In 1997, populations from sympatry were estimated to have received migrants mostly from other populations in sympatry, or from populations from northern allopatry (Table 5). An exception was the population from Sutton, where 12% of the individuals were assigned to populations in southern allopatry. Populations from allopatry in 1997 were estimated to have received migrants mostly from other populations in allopatry. By contrast, in 1998, a large proportion of individu-

TABLE 1. Measures of genetic diversity at two polymerase chain reaction single-strand conformational polymorphism loci in *Melampsora medusae* f. sp. *deltoideae* populations from the zones of host sympatry and telial host allopatry in eastern North America^a

Year, zone, province or state	City or county	Stand ^a	Locus A			Locus B		
			n_a^b	H_O	H_E	n_a^b	H_O	H_E
1997								
Sympatry								
Québec	Lévis	N	5	0.33	0.41	11	0.90	0.81
Québec	Cap Rouge	P	5	0.37	0.54	11	0.87	0.84
Québec	Villeroy	P	5	0.37	0.42	11	0.72	0.81
Québec	Trois-Rivières	N	5	0.60	0.62	11	0.71	0.76
Québec	Sutton	N	6	0.50	0.57	7	0.90	0.79
Québec	Montreal	N	5	0.30	0.48	9	0.69	0.81
Ontario	Ottawa	N	6	0.33	0.56	11	0.90	0.85
Mean	...	...	5	0.40	0.51	10.14	0.81	0.81
Northern allopatry								
North Dakota	Fargo-N	N	5	0.63	0.66	4	0.46	0.64
North Dakota	Fargo-P	P	2	0.03	0.03	3	0.97	0.56
Iowa	Ames	N	5	0.27	0.30	9	0.70	0.69
Mean	...	...	4	0.31	0.33	5.33	0.71	0.63
Southern allopatry								
Illinois	Cairo	N	4	0.33	0.35	5	0.86	0.57
Missouri	Anniston	P	4	0.47	0.38	4	0.69	0.59
Missouri	New Madrid	N	3	0.27	0.24	4	0.38	0.56
Mississippi	Stoneville	P	3	0.07	0.07	4	0.78	0.66
Mean	...	...	4	0.29	0.26	4.25	0.68	0.60
1998								
Sympatry								
Québec	Lévis	N	4	0.29	0.47	12	0.92	0.83
Québec	Villeroy	P	5	0.41	0.53	8	0.79	0.77
Québec	Trois-Rivières	N	6	0.50	0.56	12	0.79	0.83
Québec	Sutton	N	5	0.34	0.56	11	0.81	0.79
Québec	Montreal	N	5	0.27	0.41	11	0.78	0.83
Ontario	Ottawa	N	5	0.30	0.54	11	0.80	0.75
Mean	...	...	5	0.35	0.51	10.83	0.81	0.80
Northern allopatry								
North Dakota	Fargo-N	N	5	0.67	0.54	6	0.83	0.67
North Dakota	Fargo-P	P	5	0.50	0.52	9	0.86	0.75
Iowa	Ames	N	3	0.70	0.57	8	0.69	0.67
Mean	...	...	4	0.62	0.54	7.67	0.79	0.70
Southern allopatry								
Missouri	Anniston	P	2	0.04	0.04	7	0.87	0.74
Mississippi	Stoneville	P	3	0.57	0.42	4	0.83	0.74
Mean	...	...	3	0.31	0.23	5.50	0.85	0.74

^a Type of stand: N = natural and P = plantation.

^b For each locus, n_a = observed number of alleles, H_O (observed heterozygosity) = observed proportion of heterozygotes, and H_E (expected heterozygosity) is calculated as 1.0 minus the sum of the squared gene frequencies.

als (on average, 22%) from populations in sympatry and northern allopatry were assigned to populations in sympatry, suggesting high rates of migration from the zone of host sympatry. Contrary to 1997, very low proportions of individuals were assigned to populations from northern allopatry in 1998 (<6%). Low proportions of individuals also were assigned to the zone of southern allopatry, with the exception of the southernmost population (Stoneville), for which 29% of the individuals were assigned to other populations in allopatry in 1998.

To test the hypothesis of isolation by distance, pairwise geographic distances were plotted against $F_{st}/(1 - F_{st})$. There was a positive correlation between genetic and geographic distances (Fig. 5). The Spearman correlation coefficient between the matrices of geographic distances and $F_{st}/(1 - F_{st})$ was highly significant for both sampling years (Spearman rank correlation was 0.60 and 0.62 in 1997 and 1998, respectively; $P < 0.001$). However, there was no significant correlation ($P > 0.10$) between genetic and geographic distance matrices when calculated separately for the populations from allopatry and sympatry for either sampling years.

DISCUSSION

Epidemics of poplar leaf rust occur annually throughout the range of distribution of poplars in eastern North America. However, the aecial and telial hosts are sympatric only in the northern portion of the range. In this heteroecious rust, alternation between these hosts is necessary for completion of the life cycle and sexual reproduction. Presence of the alternate host, then, is expected to influence population genetic parameters.

Three models can account for the *M. medusae* f. sp. *deltoidae* epidemics in eastern North America. In the first one, aeciospores derived from aecial hosts in the zone of host sympatry would infect poplars and develop into epidemics through repeated asexual uredinal cycles throughout the range of poplars. In a second model, urediniospores would overwinter in southern locations, either on evergreen poplars or in leaf litter (5). In a third model, aeciospores would be produced locally, outside of the natural zone of host sympatry, on either ornamental conifers or other, unreported aecial hosts.

Our results point to a dynamic and complex pattern of inoculum dissemination in polar leaf rust and are consistent with a combination of the first and third models. The high allelic diversity and the presence of several private alleles in the zone of host sympatry, and the fact that almost all alleles in allopatry form a subset of the alleles in sympatry, are in agreement with the zone

of sympatry acting as a reservoir of diversity and a source of inoculum. However, although expected heterozygosity generally was higher in sympatry than in allopatry, observed heterozygosity often was higher in allopatry. In addition, observed heterozygosities almost always were higher than expected heterozygosities in allopatry. This observation could be explained by the presence of heterozygotes in the primary inoculum, which was increased by the repeated asexual cycles. Whether or not this can be generalized for other loci or sampling years cannot be determined with the current data set. More loci and sampling years would be required to determine if there is a selective advantage to heterozygosity in the absence of the alternate host.

The low values of population differentiation measured by F_{st} and the extensive cross assignments suggest that extensive migration takes place in this pathosystem. However, the different patterns observed in the two sampling years points to the possibility that different inoculum sources are important in different years. The hypothesis most consistent with our analysis is that of annual recolonization in allopatry via intermediate population jumps, with possibly some local aecial sources. The populations from the zone of northern allopatry in the Midwest were clearly outside of the natural distribution range of larch; however, they could be near enough to allow some migration to take place and act as a bridge between sympatric and allopatric populations located further south. Populations from the Midwest generally had genetic compositions that were intermediate between populations from the zones of host allopatry and sympatry. Rust spores, in particular aeciospores and urediniospores, are particularly resistant and well suited to survival and long-distance dispersal. Circumstantial evidence of long-range migration events has been reported for *M. medusae* f. sp. *deltoidae* between Australia and New Zealand, a distance roughly equivalent to those between populations from the zone of sympatry and those in northern allopatry in our study (31,32).

The highly significant and positive correlation between genetic and geographic distances could be in agreement with an isola-

TABLE 2. Inbreeding coefficient statistics for North American *Melampsora medusae* f. sp. *deltoidae* populations at two polymerase chain reaction single-strand conformational polymorphism loci for two sampling years^a

Zone, locus	1997		1998	
	F_{is}	F_{st}	F_{is}	F_{st}
Sympatry				
Locus A	0.212	0.029	0.312	0.012
Locus B	-0.020	0.045	-0.019	0.025
Mean	0.070	0.039	0.111	0.020
Northern allopatry				
Locus A	0.044	0.138	-0.152	0.025
Locus B	-0.142	0.025	-0.134	0.025
Mean	-0.078	0.067	-0.142	0.025
Southern allopatry				
Locus A	-0.106	0.043	-0.311	0.125
Locus B	-0.165	0.129	-0.145	0.017
Mean	-0.147	0.104	-0.187	0.047

^a F_{is} (inbreeding coefficient) is the proportion of the variance in the subpopulation contained in an individual. An F_{is} of 0 indicates no inbreeding. F_{st} is the proportion of the total genetic variance contained in a subpopulation relative to the total genetic variance. Values can range from 0 to 1, with 0 indicating no differentiation.

Fig. 3. Neighbor-joining tree derived from a pairwise Nei's distance matrix among populations of *Melampsora medusae* f. sp. *deltoidae* sampled in eastern North America in 1997 and 1998 and genotyped at two polymerase chain reaction single-strand conformational polymorphism loci.

tion–distance model. However, when the data were analyzed separately in the zones of allopatry and sympatry, the correlations were not significant. This suggests that factors other than distance could be important in explaining this correlation. A likely explanation is that there is a confounding effect between genetic distance and presence of the alternate host and that the latter is the important factor.

Although several conifers are listed as potential hosts of *M. medusae* f. sp. *deltoideae*, previous searches for aecial hosts for *M. medusae* f. sp. *deltoideae* outside of the range of natural distribution of larches have failed to uncover a host among other conifers (25). Also, no conifers other than larch were infected by *M. medusae* f. sp. *deltoideae* in an inoculation experiment with several southern conifers (25,28). However, *M. medusae* f. sp. *deltoideae* has been observed on ornamental larch in Kentucky (25) and it is possible that locally produced aeciospores serve as primary inoculum. It also remains possible that nonconiferous aecial hosts are present. Some members of the *Melampsora* genus alternate between poplars and *Allium* spp.

Overwintering urediniospores have been considered as another potential source of primary inoculum. Winter survival of poplar rust urediniospores has been reported in the Texas plains, but the actual role of this inoculum in the epidemics has been questioned because spores did not appear to survive the summer drought (6). Attempts to recover viable urediniospores from the previous year's leaves in the spring to reinfect poplar failed (R. C. Hamelin, unpublished). On the other hand, uredinia of *M. larici-populina* Kleb., a close relative of *M. medusae* f. sp. *deltoideae*, have been reported to overwinter in Japan and France and to be the source of primary inoculum (1,5). Even in *Puccinia recondita*, where massive annual migrations are known to be at the origin of the annual epidemics along the Puccinia path in North America, local overwintering of uredinia has been suspected from the observed differences between phenotypes of rust collections along the Puccinia path and those east of the Mississippi (12,13,22) and in the Canadian prairies (11).

Our results and analyses do not support the model of local asexual overwintering in southern populations. Only three tests were not in agreement with the H.W. model in populations in al-

TABLE 3. Genetic differentiation between sampling years for North American *Melampsora medusae* f. sp. *deltoideae* populations^a

Site	<i>F</i> _{st}	Genotypic	Genic
Lévis	0.026	NS	NS
Villeroi	0.003	NS	NS
Trois-Rivières	0.019	NS	NS
Sutton	0.005	NS	NS
Montreal	0.001	NS	NS
Ottawa	0.025	NS	NS
Fargo-N	0.024	NS	NS
Fargo-P	0.115	**	**
Ames	0.038	NS	NS
Anniston	0.079	*	*
Stoneville	0.093	**	**

^a Exact tests of genotypic and genic differentiation between 1997 and 1998; NS = not significant, * = significant at *P* = 0.01 to 0.05, and ** = significant at *P* < 0.01; all tests adjusted by Bonferroni procedures for multiple testing.

TABLE 4. Test of Hardy-Weinberg equilibrium in North American *Melampsora medusae* f. sp. *deltoideae* populations^a

Site	1997		1998	
	Locus A	Locus B	Locus A	Locus B
Lévis	NS	NS	NS	NS
Cap-Rouge	*	*	...	...
Villeroi	NS	NS	NS	NS
Trois-Rivières	NS	NS	NS	NS
Sutton	NS	NS	NS	NS
Montreal	NS	NS	NS	NS
Ottawa	NS	NS	**	NS
Fargo-N	NS	**	NS	NS
Fargo-P	NS	**	NS	NS
Ames	NS	NS	NS	NS
Cairo	NS	NS	...	...
Anniston	NS	NS	NS	NS
New Madrid	NS	NS	...	...
Stoneville	NS	NS	NS	*

^a For both loci, NS = not significant, * = significant at *P* < 0.05, and ** = significant at *P* < 0.01; all tests adjusted by Bonferroni correction for multiple tests.

Fig. 4. Principal component analysis using a pairwise *F*_{st} matrix among populations of *Melampsora medusae* f. sp. *deltoideae* sampled in eastern North America and genotyped at two polymerase chain reaction single-strand conformational polymorphism loci.

lopatry, the same number as in populations in sympatry. Disequilibrium would be expected in the absence of sexual reproduction. Also, the large difference in allelic frequency between years in allopatry is consistent with these populations being founded from distinct populations each year. In addition, the large and significant difference in genotypic composition between years is not consistent with local asexual colonization.

The presence of an alternate host and of sexual reproduction was found to have an effect on population structure of other rusts. Swedish populations of *M. larici-epitea* on *Salix* spp. (willow), where the aecial host occurs, had a population structure consistent with sexual reproduction with higher diversity and random association among loci (21); on the other hand, populations in the United Kingdom, where alternate hosts were absent, had lower di-

versity and nonrandom association among loci (16,21). Phenotypic diversity for virulence and isozyme loci were higher for populations of wheat leaf rust *P. recondita* (7,10,34), wheat stem rust *P. graminis* Pers.:Pers. (4,7,19,20), and oat crown rust *P. coronata* Corda (7,26) where the aecial host was present than where it was absent or rare. However, in the cereal rust studies, there was a confounding effect of the presence of the aecial host and of telial host diversity. Cereal rust populations in the American Midwest were exposed to a highly homogeneous host, both genetically and spatially, in addition to being in a zone of host allopatry.

Two potential weaknesses of this study should be discussed. The populations from allopatry were geographically more dispersed and this could affect our estimation of genetic diversity. However, when pairwise genetic distances among populations

TABLE 5. Estimation of migrant origin in North American *Melampsora medusae* f. sp. *deltoideae* populations^a

Zone, site	1997			1998		
	Sympatry	North allopatry	South allopatry	Sympatry	North allopatry	South allopatry
Sympatry						
Lévis	0.168	0.036	0.024	0.221	0.037	0.024
Cap-Rouge	0.038	0.014	0.018	...	...	...
Villeroie	0.070	0.033	0.025	0.266	0.026	0.010
Trois-Rivières	0.100	0.138	0.045	0.265	0.033	0.015
Sutton	0.091	0.092	0.122	0.079	0.023	0.012
Montreal	0.135	0.052	0.040	0.211	0.027	0.057
Ottawa	0.108	0.072	0.067	0.263	0.034	0.011
Northern allopatry						
Fargo-N	0.031	0.035	0.018	0.221	0.008	0.023
Fargo-P	0.010	0.003	0.007	0.247	0.052	0.021
Ames	0.060	0.164	0.042	0.186	0.056	0.070
Southern allopatry						
Cairo	0.042	0.165	0.102	...	...	...
Anniston	0.022	0.095	0.038	0.020	0.009	0.002
New Madrid	0.018	0.014	0.034	...	...	...
Stoneville	0.011	0.006	0.007	0.025	0.012	0.285

^a Proportion of putative migrants assigned to populations from host zones.

Fig. 5. Plot of pairwise genetic ($F_{st}/[1 - F_{st}]$) and geographic distances among populations of *Melampsora medusae* f. sp. *deltoideae* genotyped at two polymerase chain reaction single-strand conformational polymorphism loci for two sampling years; ♦ and ▲ represent the 1997 and 1998 sampling years, respectively.

with similar geographic distances were compared, allopatric populations exhibited large genetic distances. For example, nearby population pairs from North Dakota and Missouri had F_{st} values of 0.148 and 0.105, respectively, in 1997. By comparison, the Levis and Villeroy and the Levis and Cap-Rouge populations had pairwise F_{st} values of 0.003 and 0.027, respectively. In addition, less diversity, not more, should be expected in the populations from sympatry because less geographic range was covered. Yet, the populations from sympatry had higher diversity than those in allopatry in spite of covering a smaller geographic area.

The small number of markers sampled is another point that needs to be addressed. We decided to conduct this study with a very large number of samples using PCR-SSCP analyses to assess codominant genotypes. However, the corollary to this decision was that the number of markers had to be limited to allow DNA amplification, scoring, and allele sequencing of those samples. We believe that the high level of polymorphism observed compensated for this low number of loci. Indeed, 37 polymorphic sites were found at locus B (3) and 7 polymorphisms were observed at locus A. Preliminary results with a smaller sample size and more markers confirmed the results reported here (M. Bourassa, L. Bernier, and R. C. Hamelin, *unpublished results*).

The results of our study suggest that the source of inoculum of the epidemics might be distant from the sites where resistance screening is performed and where material is deployed, in particular outside the range of natural larch. In addition, the source and genetic composition might vary from year to year outside of the zone of host sympatry. If the model of annual recolonization with intermediate jumps connecting allopatric populations to the more diverse gene pool in sympatry is verified, adaptation of the pathogen to resistant hosts could be influenced by this population structure. The race composition in populations of *M. larici-populina* was influenced mainly by the structure of the host populations, with lower race complexity in natural stands (17). Presence of an aecial host also appeared to be an important parameter in the population structure and complexity. This could be important for North American poplar breeders to predict *M. medusae* f. sp. *deltoideae* population composition and adopt appropriate breeding strategies of host deployment.

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