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Genetic diversity in populations of *Cronartium ribicola* in plantations and natural stands of *Pinus strobus*

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Abstract Genetic diversity was studied in 22 populations of the white pine blister rust fungus *Cronartium ribicola* from natural stands and plantations of eastern white pine, *Pinus strobus*. Pseudo-allelic frequencies were estimated at each of 7 putative RAPD loci by scoring for presence or absence of amplified fragments in dikaryotic aecidiospores. Analysis of genetic distance between all pairs of populations did not reveal any trend with regard to geographic origin or type of white pine stand. In addition, when hierarchical population structure was analysed, total genetic diversity (H_s=0.214) was mostly attributable to diversity within populations ($\bar{H}_s$=0.199; AMOVA ϕ_{st}=0.121, $P< 0.01$). Genetic diversity of populations relative to region of origin (east, centre, and west) or type of stand (natural stands vs plantations) was not significantly different from zero ($P>0.10$) Nevertheless, a significant proportion of genetic differentiation was found between populations within region or stand type ($\hat{F}_{st}$=0.114; ϕ_{sc}=0.132, $P<0.001$). This result indicates that some population structure exists but that it appears to be independent of region of origin or type of stand. At least for 2 populations from white pine plantations, it appears possible that a recent introduction of a limited number of propagules was responsible for low levels of genetic diversity. We interpret these results as meaning that either long-distance dispersal is taking place between populations more than 1000 km apart or that these populations share a common recent ancestor. In addition, we suggest that *C. ribicola* may still be expanding its distribution by colonizing new plantations.

Key words Population genetics · White pine blister rust · RAPD

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Introduction

Cronartium ribicola J. C. Fischer ex Rabenh. (class basidiomycetes) is the causal agent of white pine blister rust (WPBR), a very damaging disease of five-needle pines in North America, Asia and Europe. This fungus was first reported in North America at the beginning of the century in New York State where it is believed to have been introduced from Europe on white pine seedlings (Spaulding 1916). The disease has since expanded its geographic range from the Atlantic Coast to the Pacific Coast (Mielke 1943; Boyce 1961), causing losses of revenues of millions of dollars, while reducing the genetic basis and endangering some populations of five-needle pines (Hoff and Hagle 1990; Keane and Arno 1993; Keane et al. 1994).

White pine blister rust has a complex life cycle that consists of five spore stages on two different host species (Ziller 1974). Each spore stage has distinct epidemiological characteristics, some of which have been studied under natural and artificial conditions. Sexual recombination takes place on the telial host (*Ribes* spp.) where karyogamy and meiosis within the dikaryotic teliospores result in the production of monokaryotic basidiospores. The latter are drought- and UV-sensitive and are most likely disseminated locally. Basidiospores can only infect the aecial host (white pines) where uninfective spermatia (sex cells) are produced on cankers. Dikaryotization occurs when receptive hypha are fertilized by spermatia carried presumably by insects. The fungus then produces dikaryotic aecidiospores that can only infect the telial host. Asexual propagation takes place on the telial host where several cycles of urediospores can be produced during a growing season. Both the aecidiospores and urediospores are drought- and UV-resistant and are believed to be involved in long-distance dispersal (Aylor 1990; Nagarajan and Singh 1990).

Dependent on the dissemination potential of each spore stage, different population structures could be expected for this pathogen. For example, if basidiospores and aecidiospores contribute mainly to the primary inoculum in a new infection area, greater genetic diversity is to be expected

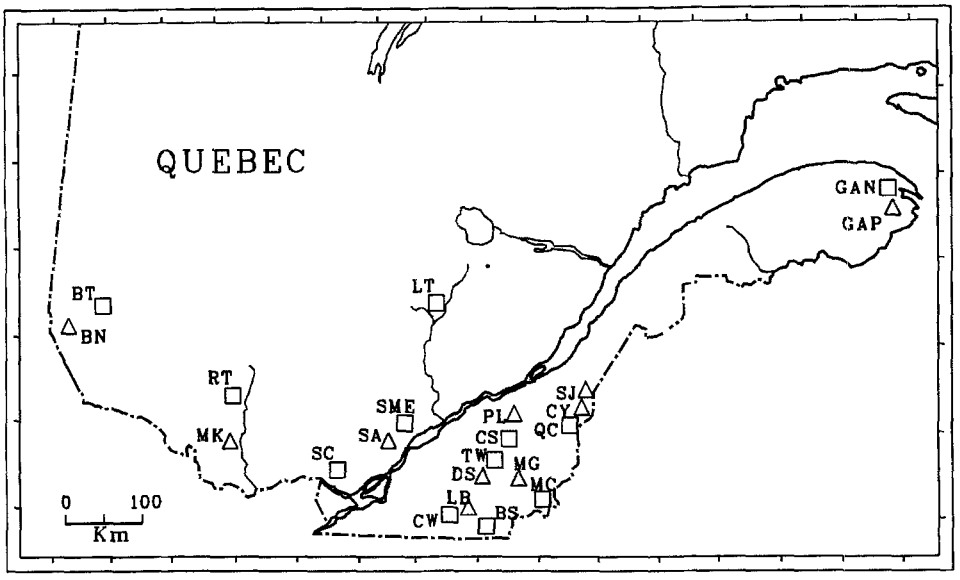

Fig. 1 Geographical location of white pine plantations (*triangles*) and natural stands (*squares*) sampled

than if asexual urediospores serve as primary inoculum. Also, if any spore type has the potential for long-distance dispersal, gene flow between populations would reduce the likelihood of local population differentiation.

Information concerning the population genetics and epidemiology of *C. ribicola* is important for management of the disease, in particular for the testing and deployment of resistant material. Reforestation with genetically resistant white pines is the most promising control method against this disease. Several resistance mechanisms have been found in natural populations of *P. monticola* (Hoff and McDonald 1971; Kinloch and Comstock 1980; Kinloch and Byler 1981; Kinloch 1992), and breeding programmes aimed at developing WPBR resistance are in place for *P. strobus* (Kriebel 1983).

However, the capacity of adaptation of the pathogen cannot be underestimated. For example, a major resistance gene in *P. monticola* was overcome by a virulent race in a rust population in California (Kinloch and Byler 1981; Kinloch and Comstock 1981). In spite of the presumed long-distance dispersal ability of rust pathogens, this new virulent race does not seem to be spreading outside the local area where it was initially discovered (Kinloch and Comstock 1981; Kinloch and Dupper 1987; Kinloch and Dulitz 1990).

Nevertheless, *C. ribicola* is apparently still being introduced to new areas of North America (Draper and Walla 1993). In view of the adaptation capacity of this pathogen and its long-distance dispersal potential, it is important to develop a better understanding of its population biology in order to attain sound genetic control against this disease. The objectives of this study were to (1) compare genetic diversity and genetic differentiation in populations of white pine blister rust from different regions in northeastern North America, and (2) compare genetic diversity and genetic differentiation in plantations and natural stands of white pine.

Materials and methods

Sampling

A total of 22 sites comprising ten plantations and 12 natural stands were sampled from throughout the natural range of *Pinus strobus* in Quebec (Fig. 1; Table 2). Whenever possible, sites were paired by region to avoid confounded effects of type of stand with region of origin (i.e. at least 1 natural stand and 1 plantation were sampled per region). Each site was visited in early May 1993 and a minimum of ten trees showing signs of cankers were tagged, labelled and mapped. In natural stands, all trees under approximately 20 years of age were examined (older trees had escaped infection or branch cankers were too high to be sampled). In plantations, trees were sampled following a systematic pattern (e.g. the closest infected tree was selected every 20 m) to provide the best possible representation of the population.

Cankers were revisited starting on May 15, 1993 after blisters started appearing but before they ruptured, in order to avoid airborne contamination or cross-contamination between aecidia. Between one and three single aecidia were collected individually on each canker by rupturing the aecidium with the tip of a sterile scalpel and harvesting the aecidiospores into a 1.5-ml Eppendorf microtube. All samples were placed in a desiccator containing a silica-based desiccant, lyophilized and stored at –80°C until DNA extraction.

DNA extraction

DNA was extracted from the aecidiospores by a modification of a protocol described elsewhere (Lee and Taylor 1990). The lyophilized spores were ground for 2–4 min with approximately 10 mg of diatomaceous earth (Sigma) and 100 µl extraction buffer (700 m*M* NaCl, 50 m*M* Tris-HCl (pH 8.0), 10 m*M* EDTA, 1% β-2-mercaptoethanol, 1% cetyl-trimethyl-ammonium-bromide) using disposable Kontes pestles (Canlab). Three hundred microliters of extraction buffer was added, and the samples were incubated at 65°C for 1 h.

The samples were then extracted with 600 µl chloroform:isoamyl alcohol (24:1), finger vortexed and centrifuged at 10000×*g* for 5 min. The upper phase was pipetted into 1.5-ml Eppendorf microtubes, and the DNA was precipitated with 75 µl of 7.5 *M* ammonium acetate and 600 µl isopropanol and placed on ice for at least 30 min. The DNA was pelleted by centrifugation for 5 min at 10000×*g* and then washed with 70% ethanol after which the pellet was air-

Table 1 List of oligonucleotides used to generate RAPD profiles in *Cronartium ribicola* with the approximate size of fragments that were scored and the name of the putative loci

Oligo-nucleo-tides[a]	Sequence	Fragment size (bp)[b]	Putative locus
OPA01	CAGGCCCTTC	1700	*OPA01-1700*
OPA01	CAGGCCCTTC	2000	*OPA01-2000*
OPA09	GGGTAACGCC	1350	*OPA09-1350*
OPA09	GGGTAACGCC	1150	*OPA09-1150*
OPA09	GGGTAACGCC	950	*OPA09-950*
OPC08	TGGACCGGTG	750	*OPC08-750*
OPC08	TGGACCGGTG	900	*OPC08-900*

[a] All oligonucleotides were purchased from Operon Technology (see Materials and methods)
[b] Only RAPD markers that could be reliably scored and that met the $x < 3/n$ rule are listed here

dried and resuspended in 20 µl of TE-8 buffer (10 m*M* TRIS-HCl pH 8, 1 m*M* EDTA). DNA concentration was estimated by comparing the band intensity on an agarose gel to a known amount of λ-*Hind*-III fragments (Gibco-BRL). DNA was aliquoted 1:2 to 1:25, depending on DNA concentration, and stored in TE-8 buffer at –20°C until needed. Our results indicate that reproducible random amplified polymorphic DNA (RAPD) patterns were obtained with concentrations of 10 ng plus or minus a tenfold dilution.

DNA amplifications

Amplifications were performed in volumes of 25 µl containing 10 m*M* TRIS-HCl, pH 8.3, 50 m*M* KCl, 2 m*M* $MgCl_2$, 0.0001% gelatin, 100 µ*M* of each dNTP (Pharmacia), 0.2 µ*M* of oligonucleotides, 2 µl (approximately 10 ng) of genomic DNA and 0.5 unit *Taq* DNA polymerase (Boehringer Mannheim) (Williams et al. 1990). Amplifications were carried out in a thermal cycler (MJ-Research model PTC-60) programmed for a denaturation step at 94°C for 3 min, followed by 1 cycle at 35°C for 4 min, 72°C for 2 min, then 45 cycles of 94°C for 1 min, 35°C for 1 min and 72°C for 2 min. The reactions ended with a 10-min extension at 72°C. Amplification products were separated by electrophoresis on 1.5% agarose gels using 1×TAE buffer (primer OPA01) or 1% agarose plus 0.5% synergel (Diversified Biotech) in 0.5×TPE buffer (primers OPA09 and OPC08). Polymerase chain reaction (PCR) products were visualized by UV fluorescence after ethidium bromide staining.

Sixty oligonucleotides (Kits A, B, C; Operon Technologies) were screened with seven *C. ribicola* samples selected from different geographic areas in order to identify primers yielding reproducible and interpretable banding patterns. Primers OPA01, OPA09, and OPC08 produced a total of 20 interpretable RAPD fragments and were retained for the population study (Table 1). Samples for which banding patterns were not clear were reamplified after a new dilution from the original DNA stocks had been prepared.

Analysis

All RAPD markers were scored as separate putative loci with two alleles. One allele was amplifiable by PCR (the marker allele) while the other was not, either because of point mutations or insertions/deletions. Since the aecidiospores are presumably dikaryotic, markers were interpreted in a similar fashion as dominant markers in diploids (Williams et al. 1990). The absence of co-migration of heterologous DNA fragments was also assumed. This phenomenon has been found to be infrequent in plants (Grattapaglia and Sederoff 1994).

Allelic frequencies were estimated from the proportion of the sampled individuals not exhibiting the marker x. If the assumption is made that the populations were in Hardy-Weinberg equilibrium, the frequency of the null allele was obtained as $q=x$. Then an asymptotically unbiased estimate was calculated by carrying out the correction for small sample size (Lynch and Milligan 1994). To obtain less biased estimates of population genetic parameters, putative loci that did not meet the $x < 3/n$ rule (including all monomorphic loci) were excluded from the analysis (Lynch and Milligan 1994).

To explore for the presence of any population structure, particularly related to epidemiologically relevant characters such as geographical origin or stand type, Nei's (1978) unbiased genetic distance was measured between all pairs of populations. The relationship between populations was then investigated by constructing a phenogram using the unweighted pair-group method with arithmetic averages (UPGMA) method of the PHILIP software package (version 3.5).

Knowing that estimates of genetic diversity based on RAPD loci using classical analyses of population genetics are biased, we used unbiased allelic frequencies to estimate $\hat{H}_t$ and $\hat{F}_{st}$ (Lynch and Milligan 1994). In addition, unbiased allelic frequencies were used in a hierarchical analysis of genetic diversity in order to subdivide the total genetic diversity (H_t) in its components, i.e. among and within geographic regions (east, centre and west) and stand type (natural stand and plantation) to give an indication of the proportion of genetic diversity attributable to the different levels of structure. Data were analysed with the BIOSYS software package (version 2.0).

An analysis of molecular variance (AMOVA) was performed on the RAPD haplotypes and the Euclidean distances between all pairs of haplotypes (Excoffier et al. 1992; Huff et al. 1993). The AMOVA was used to partition the total variance into within population, among populations within regions, between regions and between stand type. The variance components were tested statistically by non-parametric randomization tests using 1000 repetitions. Both the AMOVA and $\hat{F}_{st}$ calculated with the Lynch and Milligan method gave the most accurate estimate of observed heterozygosities when both genotypic and phenotypic RAPD data were compared for conifers (Isabel et al. 1995).

Results

A total of 18 RAPD markers were scored for the 515 aecidiospore samples studied. Of these, 11 putative loci were nearly fixed for the marker allele and were pruned out of the numerical analysis (Lynch and Milligan 1994). Pseudo-allelic (allelic for the rest of this paper) frequencies were calculated for the remaining 7 loci (Table 2). Estimated frequency of the null allele averaged over all populations ranged from 0.443 for locus *OPA01-1700* to 0.959 for locus *OPA09-1150* (Table 2). Some populations were polymorphic at all loci (e.g. Bolton-sud, Durham-sud, Plessisville, Saint-Canut), while another one was polymorphic at 1 locus only (Sainte-Marguerite) (Table 2). An example of the RAPD profiles obtained is shown for primers OPA01 (Fig. 2A) and OPAC08 (Fig. 2B).

Genetic distances between pairs of populations were variable. However, there was no obvious trend with regard to geographic origin or stand type (Fig. 3). For example, a low genetic distance (0.001) was found between the Béarn (a natural stand from western Quebec) and Saint-Cyprien (a plantation from central Quebec) populations. However, a higher genetic distance (0.055) was measured between Bolton-sud and Chesterville (2 natural stands from central Quebec). In all cases, genetic distances were relatively low,

Table 2 Frequency of RAPD markers and average expected heterozygosities at 7 putative RAPD loci for 22 populations of *Cronartium ribicola* from natural stands and plantations of *Pinus strobus*

Frequency of null allele[a]

Populations	Stand type[b]	*n*	Percentage polymorphic loci	*OPA01-1700*[c]	*OPA01-2000*	*OPA09-1350*	*OPA09-1150*
Béarn (BN)	P	13	57	0.314	1.000	1.000	0.962
Bolton-sud (BS)	N	25	100	0.232	0.843	0.754	0.903
Belleterre (BT)	N	19	57	0.423	1.000	1.000	1.000
Chesterville (CS)	N	18	42	0.630	1.000	1.000	1.000
Cowansville (CW)	N	18	86	0.712	0.944	0.868	1.000
Saint-Cyprien (CY)	P	49	71	0.384	1.000	0.989	0.956
Durham-sud (DS)	P	39	100	0.511	0.863	0.610	0.974
Gaspésie-A (GAP)	P	19	71	0.585	1.000	0.932	0.932
Gaspésie-B (GAN)	N	35	86	0.474	0.968	0.922	0.969
Lac Brome (LB)	P	22	86	0.488	0.901	0.673	0.944
La Tuque (LT)	N	15	71	0.292	0.967	0.896	1.000
Lac aux Araignées (MC)	N	18	57	0.423	0.972	1.000	0.932
Sainte-Marguerite (MG)	P	24	14	0.306	1.000	1.000	1.000
Maniwaki (MK)	P	10	86	0.471	0.841	0.781	0.950
Plessisville (PL)	P	37	100	0.477	0.928	0.721	0.934
Quatre-Chemins (QC)	N	31	86	0.514	0.934	0.785	0.944
Ruisseau Tortue (RT)	N	17	57	0.649	1.000	0.967	0.932
Saint-Alexis (SA)	P	31	71	0.270	0.964	0.850	1.000
Saint-Canut (SC)	N	25	100	0.227	0.939	0.873	0.980
Saint-Juste (SJ)	P	30	86	0.463	0.983	0.981	0.963
Sainte-Mélanie (SME)	N	11	57	0.539	1.000	0.917	0.825
Tingwick (TW)	N	9	71	0.375	0.944	0.629	1.000
Average	–	–	–	0.443	0.954	0.870	0.959

Frequency of null allele[a]

Populations	Stand type[b]	*OPA09-950*[c]	*OPA08-750*	*OPC08-900*	H_s[d]	$\hat{H}_j$[e]
Béarn	P	0.962	1.000	0.789	0.135	0.138
Bolton-sud	N	0.832	0.668	0.777	0.328	0.330
Belleterre	N	0.673	0.947	0.765	0.204	0.206
Chesterville	N	0.914	1.000	0.789	0.141	0.142
Cowansville	N	0.868	0.785	0.903	0.219	0.219
Saint-Cyprien	P	0.989	1.000	0.744	0.142	0.142
Durham-sud	P	0.672	0.709	0.986	0.310	0.268
Gaspésie-A	P	0.820	0.974	1.000	0.160	0.161
Gaspésie-B	N	0.905	0.922	1.000	0.157	0.157
Lac Brome	P	0.630	0.772	1.000	0.299	0.301
La Tuque	N	0.932	0.859	1.000	0.153	0.155
Lac aux Araignées	N	0.896	1.000	1.000	0.126	0.127
Sainte-Marguerite	P	1.000	1.000	1.000	0.062	0.063
Maniwaki	P	0.716	0.885	1.000	0.273	0.276
Plessisville	P	0.699	0.719	0.986	0.292	0.292
Quatre-Chemins	N	0.819	0.742	1.000	0.255	0.256
Ruisseau Tortue	N	0.779	1.000	1.000	0.146	0.147
Saint-Alexis	P	0.637	0.881	1.000	0.202	0.198
Saint-Canut	N	0.605	0.712	0.980	0.241	0.244
Saint-Juste	P	0.963	0.966	1.000	0.113	0.114
Sainte-Mélanie	N	1.000	0.907	1.000	0.168	0.170
Tingwick	N	0.754	0.822	1.000	0.258	0.263
Average	–	0.821	0.876	0.942	0.199	0.199

[a] Estimated by Lynch and Milligan's method (1994)
[b] P Plantation, N natural stand
[c] Putative RAPD locus as described in Table 1
[d] Expected heterozygosity averaged over seven loci
[e] Expected heterozygosity adjusted by Lynch and Milligan's method (1994) and averaged over 7 loci

Table 3 Analysis of molecular variance (AMOVA) for 22 populations of *Cronartium ribicola* from *Pinus strobus* plantations and natural stands using haplotypes of 7 polymorphic markers

Hierarchical structure[a]	Source	*df*	Variance components	ϕ-statistics	Proportion of variance component (%)	*P*-value[b]
By region	Among regions	3	–0.007	–0.013	–1.28	>0.10
	Among populations within regions	18	0.073	0.132	13.35	<0.001
	Within populations	490	0.478	0.121	87.93	<0.001
By stand type	Among types of stand	1	–0.005	–0.009	–0.87	>0.10
	Among populations within type of stand	20	0.072	0.131	13.20	<0.001
	Within populations	490	0.478	0.123	87.93	<0.001

[a] The present version of the AMOVA software only allowed two hierarchical levels to be analysed simultaneously
[b] Probability of obtaining equal or larger value determined by 1000 randomization of the treatments

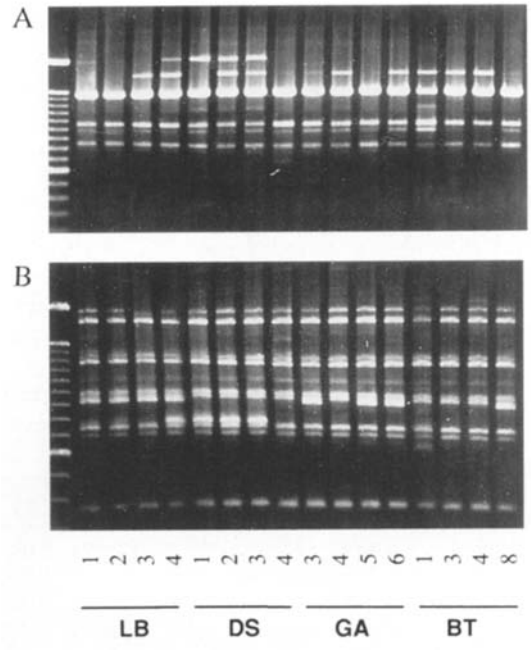

Fig. 2A, B Random amplified polymorphic DNA profiles for aecidiospore samples of *Cronartium ribicola* from 4 separate trees on 4 sites. DNA was amplified with primers OPA01 (**panel A**) and OPC08 (**panel B**)

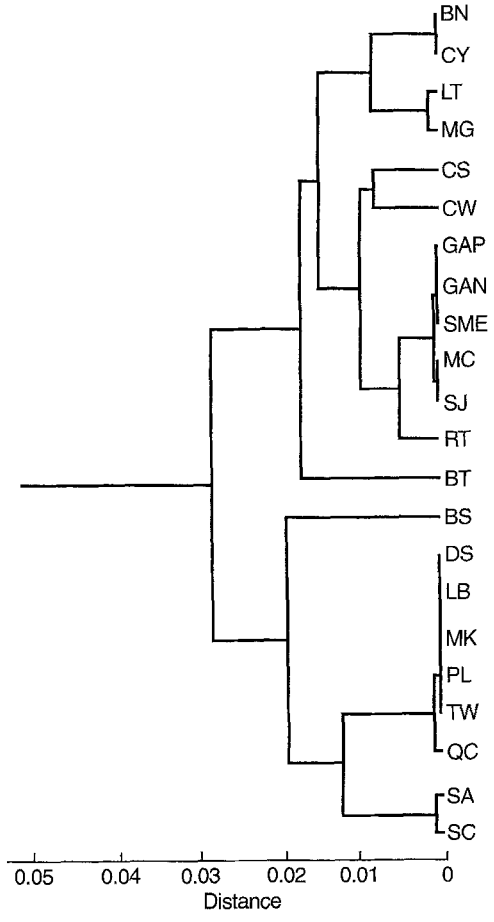

Fig. 3 Unweighted pair-group method with arithmetic averages (UPGMA) analysis of Nei's (1978) unbiased genetic distance between *Cronartium ribicola* populations from ten plantations and 12 natural stands

and no single population appeared to be genetically very differentiated from the others.

Expected heterozygosities averaged over all loci were also variable among all 22 populations (Table 2). An expected heterozygosity of 6.3% was calculated for Sainte-Marguerite, a plantation from central Quebec, but an expected heterozygosity of 29% was found for Plessisville, another plantation from central Quebec. Both high and low levels of expected heterozygosity were found in natural stands, for example, 33% for Bolton-sud in central Quebec, and 15.5% for La Tuque in the northern range of WPBR in Quebec (Table 2).

Hierarchical analysis of genetic diversity indicated that total genetic diversity (H_t=0.214) was mostly attributable to diversity within populations ($\bar{H}_s$=0.199; AMOVA ϕ_{st}=0.121, P< 0.01). The genetic diversity of populations relative to region of origin (east, centre and west) or type

of stand (natural stands vs plantations) was not significantly different from zero ($P>0.10$; Table 3). Nevertheless, the populations of *C. ribicola* were structured independently of their region of origin or stand type since a significant proportion of genetic diversity was found among populations within a region or stand type ($\hat{F}_{st}=0.114$; $F_{st}=0.075$; $\phi_{sc}=0.132$, $P< 0.001$) (Table 3).

F_{st} values estimated without the corrections proposed by Lynch and Milligan (1994) for heterozygosities and fixation indices but using the unbiased allelic frequencies were approximately 34% lower ($F_{st}=0.075$) than those obtained with the adjustments ($\hat{F}_{st}=0.114$). In addition, in all cases the adjusted F_{st} values were closer to the *F*-statistic analogues (ϕ-statistics) calculated by AMOVA than were the unadjusted values ($\hat{F}_{st}=0.114$ versus $\phi_{sc}=0.132$). However, there were only small differences between expected heterozygosity estimates (H_s) calculated with unadjusted equations but using unbiased allelic frequencies and those calculated with unbiased allelic frequencies using adjusted equations ($\hat{H}_j$; Table 2).

Discussion

The analysis of genetic distance between populations provides a first indication of the absence of any clear trends in the distribution of genetic variability with regard to geographic origin or type of white pine stand. The low genetic distances between populations separated by about 1000 km suggests either that extensive gene flow is homogenizing the populations, or that these populations share a common recent ancestor.

Gene flow through the long-distance transport of urediospores and possibly aecidiospores is likely for this fungus. Long-distance dissemination of rust urediospores is well-documented for several rust pathosystems (Aylor 1990; Nagarajan and Singh 1990). For example, isozyme patterns of *Puccinia graminis* f. sp. *tritici* were found to be identical between Angolan (Africa) collections and newly found races of the fungus in Australia (Burdon et al. 1982). Virulence surveys of *P. recondita* f. sp. *tritici* have indicated that the central United States is a single epidemiological unit from the Gulf Coast States to the Great Plains (Long et al. 1993). Since the alternate overwintering host has been functionally eradicated over most of the wheat growing areas of central and eastern North America, it is believed that urediospores are transported by wind over distances of 1000 km annually from Mexico along what is known as the "Puccinia Path" (Stakman and Harrar 1957; Roelfs 1986, 1989).

The appearance of poplar leaf rust in New Zealand at two different locations 450 km apart following the recent spread of the disease in western Australia and the analysis of weather systems during this epidemic also provide compelling circumstantial evidence that spores of this tree rust were carried across the ocean for over 3000 km (Van Kraayenoord et al. 1974; Wilkinson and Spiers 1976; Pedgley 1986).

The mechanisms for the long-distance transport of urediospores have been hypothesized. Aerial dispersal appears to be important in long-distance spread (Bowden et al. 1971; Pedgley 1982), but other dispersal mechanisms have also been suggested, such as migratory birds (Warner and French 1970) or infected plant material (Wellings et al. 1987).

Dispersal of basidiospores, on the other hand, appears to be more localized. For example, infection by *C. ribicola* was greatly reduced in white pine plots where the *Ribes* host was eradicated over a distance of 300 m compared with the infection rate in control plots (Martin 1944). However, local topographical features can apparently affect the dispersal of basidiospores (Peterson and Jewell 1968). For example, complex air movements along valleys, near large bodies of water or in mountainous areas resulted in pine infections up to 600 m away from the nearest *Ribes* bush (Van Arsdel 1961), and a 300-m *Ribes*-free zone was not effective in preventing WPBR infections in northern Idaho (Toko et al. 1967).

Fungi for which measures of genetic diversity have been calculated often show low levels of differentiation, in particular for ascomycetes or basidiomycetes producing spores with potential for long-distance dispersal. For example, isolates of *Uromyces appendiculatus* (bean rust pathogen) from Germany, Mexico and Puerto Rico had isozyme patterns that were very similar to isolates from the continental United States (Lu and Groth 1987), and no relationship between geographic origin and phenotypic distance was found (Linde et al. 1990).

Long-distance dispersal of *Mycosphaerella graminicola*, the causal agent of Septoria leaf blotch of wheat, was suggested to explain the low levels of genetic differentiation between populations separated by 750 km ($F_{st}=0.039$). The estimated gene flow was 12 individuals per generation, which is sufficient to prevent population differentiation. The authors suggested that the airborne dispersal of sexual ascospores is probably accountable for such low levels of population differentiation (Boeger et al. 1993). A similarly low level of genetic differentiation [$G_{st}=0.061$; this measure of genetic differentiation was shown to be equivalent to F_{st}; (Nei 1973)] was measured between populations of *Cronartium quercum* f. sp. *fusiforme*, the causal agent of fusiform rust, from different regions in the southern United States (Hamelin et al. 1994).

A moderate level of genetic differentiation ($G_{st}=0.16$) was found between populations of *Rhynchosporium secalis* (the causal agent of scald disease of barley) in Europe, Australia and the United States. But when genetic differentiation was measured between locations within regions, the results were very similar to those of other fungal pathogens with long-distance dispersal ability (e.g. $G_{st}=0.06$ between eastern and western U. S. and $G_{st}=0.035$ between northern and southern Norway) (Goodwin et al. 1993).

Another possible reason for the presence of low levels of genetic differentiation between populations is the sharing of a common ancestor. For example, the worldwide distribution of the Irish potato famine fungus (*Phytophtora infestans*) seems to have stemmed from a narrow genetic

base, resulting in low genetic differentiation between continents (Fry et al. 1992; Goodwin et al. 1992, 1994). However, since long-distance dispersal of this fungus is mostly believed to be associated with the transport of infected material by humans, this estimate of genetic differentiation is not informative about its natural dispersal.

The hypothesis of recent colonization of white pine stands by a limited number of propagules is supported by the low level of genetic diversity observed in some rust populations. For example, only two haplotypes were found out of 20 individuals, and 6/7 loci were fixed in Sainte-Marguerite (a plantation). This resulted in an expected heterozygosity of only 6.3%, approximately 3 times as low as the average expected heterozygosity and almost 5 times as low as the population with the highest heterozygosity (Bolton-sud, $\hat{H}_j$=33%).

Another population (SJ, also a plantation) has the second lowest expected heterozygosity ($\hat{H}_j$=11.4%). This plantation was located in the Alleghany Mountains where natural stands of *P. strobus* were practically non-existant, and no rust was found in the few naturally occurring pines observed. Nevertheless, over 50% of the white pines were infected in the plantation. For both of these plantations, the closest white pines identified on the Forestry Canada Forest Insect and Disease Survey maps were more than 5 km away.

We propose two scenarios to explain our results; first, it is possible that symptomless infected nursery material was planted on these sites and an epidemic developed between the plantations and the local *Ribes* populations. The biology of this fungus is consistent with this scenario since infections can remain latent for up to 3 years (Ziller 1974). Alternatively, it is also possible that a limited number of wind-blown urediospores started the local epidemic. Since these are asexually propagated, this would also result in a population bottleneck. A similar situation has been reported for the bean rust fungus. One population had a low level of genetic diversity compared with the rest of the populations surveyed and was believed to have undergone several cycles of asexual reproduction leading to genetic drift (McCain et al. 1992).

The lack of significant genetic differentiation between natural stands and plantations can be viewed as an indication that gene flow is occurring between populations of *C. ribicola* in plantations and natural stands. This result is not unexpected given the dissemination potential of rust fungi. There was also no apparent genetic differentiation between *Peridermium harknessii* populations from plantations, windbreaks or natural stands in North Dakota (Tuskan et al. 1990).

The potential practical implication of our findings is that the rust populations studied share very similar genetic profiles. Therefore, our results do not provide evidence that white pine breeding programmes should be developed for the different regions studied. However, it remains to be seen whether virulence genes are also homogeneously distributed in these populations.

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