Barrier to Gene Flow Between Eastern and Western Populations of *Cronartium ribicola* in North America

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Accepted for publication 10 May 2000.

ABSTRACT

Hamelin, R. C., Hunt, R. S., Geils, B. W., Jensen, G. D., Jacobi, V., and Lecours, N. 2000. Barrier to gene flow between eastern and western populations of *Cronartium ribicola* in North America. Phytopathology 90:1073-1078.

The population structure of *Cronartium ribicola* from eastern and western North America was studied to test the null hypothesis that populations are panmictic across the continent. Random amplified polymorphic DNA markers previously characterized in eastern populations were mostly fixed in western populations, yielding high levels of genetic differentiation between eastern and western populations (ϕ_{st} = 0.55; θ = 0.36; $P < 0.001$). An unweighted pair-group method, arithmetic mean dendrogram based on genetic distances separated the four eastern and four western populations into two distinct clusters along geographic lines. Similarly, a principal component analysis using marker frequency yielded one cluster of eastern populations and a second cluster of western populations. The population from New Mexico was clearly within the western cluster in both analyses, confirming the western origin of this recent introduction. This population was completely fixed (H_j = 0.000; n = 45) at all loci suggesting a severe recent population bottleneck. Genetic distances were low among populations of western North America (0.00 to 0.02) and among eastern populations (0.00 to 0.02), indicating a very similar genetic composition. In contrast, genetic distances between eastern and western populations were large, and all were significantly different from 0 (0.07 to 0.19; $P < 0.001$). Indirect estimates of migration were high among western populations, including the number of migrants among pairs of populations ($Nm > 1$) between New Mexico and British Columbia populations, but were smaller than one migrant per generation between eastern and western populations. These results suggest the presence of a barrier to gene flow between *C. ribicola* populations from eastern and western North America.

Additional keywords: analysis of molecular variance, white pine blister rust.

White pine blister rust, caused by the basidiomycete *Cronartium ribicola* J. C. Fisher, is a very severe disease of five-needle pines in North America. The history of introduction and spread of this exotic pathogen have been sufficiently documented (27). The first report of the disease in eastern North America occurred in 1906 (33) and was followed by additional introductions from several European sites (10,32). Extensive spread in the northeastern United States and adjacent Canada occurred through broad distribution of infected seedlings (27). Subsequently, the disease was introduced into western North America in 1910 (27) and originated from France on infected seedlings (5). Recent introductions have been reported in New Mexico (NM) (9) and in North Dakota (1). A better understanding of the source of these introductions and of the patterns of spread is important to efforts to control this disease. Resistant sugar pine (*Pinus lambertiana* Doug.) (16,18), western white pine (*P. monticola* D. Don) (11,13), and eastern white pine (*P. strobus* L.) (34) have been identified, but questions regarding stability of resistance have been raised (12,14,17,19, 28,31,38). Population subdivision and presence of races or biotypes, as well as potential for dissemination and spread, are therefore important concerns for white pine blister rust control programs. Recent developments through molecular approaches allow us to revisit some of the unanswered questions about the epidemiology of white pine blister rust and properly test key hypotheses.

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Publication no. P-2000-0727-02R

The population structure of white pine blister rust has been well documented in eastern and western North America. Genetic diversity was distributed at a very fine scale. Analysis of multiple dikaryotic aecidia per canker demonstrated that 70% of the genetic diversity occurred at the level of individual cankers, indicating that the spermatized cankers are a mosaic of diverse genotypes (6). The spermatia from unspermatized cankers consisted of genets within cankers, but almost all cankers possessed different random amplified polymorphic DNA (RAPD) profiles, and the pattern of marker distribution was consistent with random mating (8).

At the geographic scale, low levels of genetic differentiation among populations separated by several hundred kilometers in eastern North America (7) and among western populations (36) have been reported, and most of the genetic variability resided within populations. This has been interpreted as a consequence of long-distance migration leading to population homogenization.

However, no study yet has properly documented the genetic structure of populations at the continental level. Given that *C. ribicola* was introduced in distinct events in eastern and western North America, the question of whether or not populations are genetically different is relevant. A study using restriction fragment length polymorphism (RFLP), isozyme, and RAPD in populations of *C. ribicola* from western North America and a few samples from eastern North America, revealed as many genetic differences among western populations as between eastern and western populations (20).

The well-documented newly founded populations in New Mexico (9) and in North Dakota (1) can provide interesting insight into the biology and population genetics of this pathogen. Genetic characterization of these populations could help determine their

origin and document the effect of long-distance migration (i.e., long jump; 29) on the population structure of the rust. In one *C. ribicola* population from a plantation in Quebec, almost all aecidia possessed the same DNA profile, and heterozygosity was three times below the average of eastern populations suggesting that this rust population could have been recently founded, possibly from a nursery or from long-distance spore dispersal (7).

Comparison of the genetic structure of *C. ribicola* in western and eastern North America can address a fundamental question regarding the epidemiology of this disease. The presence of populations with similar genetic compositions in eastern and western North America could indicate the presence of a unique epidemiological unit on the continent. However, if the eastern and western populations differ in genetic composition, panmixis cannot be assumed and it can be extremely important, in terms of management of the disease, to assess whether other important characteristics such as virulence, aggressiveness, and host adaptation also differ between eastern and western rust populations. The objectives of our study were to compare the genetic structure of *C. ribicola* populations in eastern and western North America and to test the null hypothesis that genetic composition does not differ across the continent.

MATERIALS AND METHODS

Sampling. Single aecidia were collected from four natural stands and plantations of *P. monticola* in British Columbia (BC), Canada. Two collection sites were coastal and two were interior (Table 1). One coastal site contained four samples and was pooled with the other coastal site. Samples were collected from three mountain ranges (Silver Spring, Wills, and Board Canyon) in the Lincoln National Forest in the Sacramento ranger district, NM. At least 10 trees were sampled in each pine stand in the spring of 1997 and 1998. As reported previously, cankers were sampled prior to opening the aecidia, and aecidia were sampled separately to avoid the bulking of genotypes (6,7). Up to three single aecidia were collected individually on each canker (one canker per tree) by rupturing the aecidium with the tip of a sterile scalpel and collecting the aecidiospores in 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. To compare western and eastern populations, marker frequencies for four eastern populations were used in the study (2).

DNA extraction. DNA was extracted from aeciospores by a modification of a protocol described elsewhere (6,7,23). The lyophilized spores were ground for 2 to 4 min with ≈10 mg of diatomaceous earth (Sigma Chemical Co., St. Louis) and 50 µl of extraction buffer (700 mM NaCl, 50 mM Tris-HCl at pH 8.0, 10 mM EDTA, 1% b-2-mercaptoethanol, and 1% cetyl-trimethyl-ammonium-bromide) with disposable Kontes pestles (VWR-Canlab, Toronto). A volume of 350 µl of extraction buffer was added, and the samples were incubated at 65°C for 1 h.

The samples were extracted with 400 µl of chloroform/isoamyl alcohol (24:1), finger-vortexed, and centrifuged at 10,000 × *g* for 5 min. The upper phase was pipetted into 1.5-ml Eppendorf microcentrifuge tubes, and the DNA was precipitated by adding 75 µl of 7.5 M ammonium acetate and 500 µl of isopropanol and incubating at –20°C for at least 30 min. The DNA was pelleted by centrifuging for 5 min at 10,000 × *g* and washed with 70% ethanol, and the pellet was air dried and resuspended in 20 µl of Tris-EDTA (TE)-8 buffer (10 mM Tris-HCl at pH 8, and 1 mM EDTA). DNA concentration was estimated by comparing the band intensity on agarose gel with a known amount of λ-*Hin*dIII fragments (Gibco BRL, Bethesda, MD). DNA was diluted to ≈5 ng/µl depending on DNA concentration and stored in TE-8 buffer at –20°C.

RAPD amplification. Amplifications were carried out in volumes of 12.5 µl containing 10 mM Tris-HCl at pH 8.3, 50 mM KCl, 2 mM $MgCl_2$, 0.0001% gelatin, 100 µM of each dNTP (Pharmacia Biotechnology Inc., Uppsala, Sweden), 0.2 µM of each oligonucleotide, 2 µl of genomic DNA, and 0.5 unit of *Taq* (DNA polymerase, Boehringer GmbH, Mannheim, Germany) (37). Amplifications were carried out in a thermal cycler (Model PTC-60; MJ Research Inc., Watertown, MA) programmed for denaturation at 94°C for 3 min, followed by 1 cycle at 35°C for 4 min

TABLE 1. Estimated frequency of random amplified polymorphic DNA (RAPD) markers and expected heterozygosity (H_j) for populations of aecidia of *Cronartium ribicola*

Population[a]	n[b]	Estimated allele frequency (q)[c]							H_j
		OPA01-1700	OPA01-2000	OPC08-750	OPC08-900	OPE15-1600	OPK19-2000	OPK11-500	
Manning Park (coastal BC)	55 (21)	0.963	1.000	1.000	1.000	1.000	0.203	0.000	0.069
Smallwood (interior BC)	37 (14)	1.000	0.987	1.000	1.000	0.987	0.410	0.000	0.077
Shelter Bay (interior BC)	16 (6)	1.000	1.000	1.000	0.969	1.000	0.000	0.000	0.009
Sacramento (NM)	45 (15)	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000
Ste-Camille (Quebec)	30 (10)	0.417	1.000	0.966	0.610	0.733	0.375	0.490	0.347
Minden (Ontario)	30 (10)	0.522	1.000	0.983	0.522	0.582	0.455	0.553	0.369
Little Grand Lake (NFLD)	30 (10)	0.417	1.000	1.000	0.610	0.832	0.329	0.417	0.319
Moncton (New Brunswick)	30 (10)	0.564	0.969	0.985	0.641	0.655	0.440	0.313	0.344

[a] BC = British Columbia; NM = New Mexico; NFLD = Newfoundland. Quebec, Ontario, NFLD, and New Brunswick reproduced from Et-touil et al. (2); expected heterozygosity may differ from Et-touil et al. because two of the markers used in that study were not used here.
[b] Number of aecia and number of trees in parentheses.
[c] Allele frequency for dominant RAPD markers was estimated according to the method of Lynch and Milligan (27); frequencies are those of the null allele.

TABLE 2. Analysis of molecular variance (AMOVA) of random amplified polymorphic DNA (RAPD) haplotypes for populations of *Cronartium ribicola* from eastern and western North America

Hierarchical structure[a]	Source	df	Variance components	ϕ-Statistics	Proportion of variance components (%)	P value[b]
East (NFLD, QC, ON, NB) vs West (BC, NM)	Among regions	1	0.605	0.546	54.58	<0.001
	Among populations in regions	6	0.011	0.021	0.97	0.076
	In populations	262	0.493	0.556	44.45	<0.001
Interior BC vs Coastal BC	Among regions	1	0.000	0.000	0.00	0.78
	Among populations in regions	1	0.008	0.044	4.49	0.20
	In populations	105	0.174	0.032	95.51	<0.001

[a] NFLD = Newfoundland, QC = Quebec, ON = Ontario, NB = New Brunswick, BC = British Columbia, and NM = New Mexico.
[b] Probability of obtaining equal or larger value determined by 1,000 random permutations of the treatments.

for annealing, and 72°C for 2 min for extension, and 45 cycles at 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.

Primers (Operon Technologies Inc., Alameda, CA) OPA01, OPC08, OPE15, OPK11, and OPK19 were used because they were polymorphic in *C. ribicola* populations from eastern Canada (7).

Amplification products were separated by electrophoresis on 1.5% agarose gels with 1× Tris-acetate-EDTA (TAE) buffer (primers OPA01 and OPK19) or 1% agarose plus 0.5% synergel (Diversified Biotech, Newton Center, MA) in 0.5 Tris-phosphate-EDTA (TPE) buffer (primers OPC08, OPE15, and OPK11). Polymerase chain reaction products were visualized by UV fluorescence after ethidium bromide staining.

Data analysis. Markers were scored and entered in binary fashion into a matrix containing RAPD markers in columns (name of primer–size of marker in base pairs) and samples in rows. Because RAPD markers are dominant and aecidiospores are dikaryotic, data were interpreted as dominant markers in diploids. Usually, for diploid individuals, distinction between dominant homozygous and heterozygous is not possible, but allelic frequencies can be estimated by the square root of the frequency of homozygotes for the null allele (26). Allelic frequencies, expected heterozygosities (or gene diversity), *F* statistics (among populations and between eastern and western populations), and Nei's (30) unbiased genetic distances were calculated with corrections for bias in estimating the allele frequencies by tools for population genetic analysis (TFPGA, Version 1.3; M. Miller, Northern Arizona University, Flagstaff).

In addition, Euclidean distances among all pairs of RAPD multilocus phenotypes were subjected to an analysis of molecular variance (AMOVA) (3) using AMOVPREP (M. Miller) and ARLEQUIN (Version 1.1, S. Schneider, J. M. Kueffer, D. Roessli, L. Excoffier, University of Geneva, Switzerland). This analysis partitioned the total variance into hierarchical components (between eastern and western populations, among populations within regions, and within population). *F* statistic analogs were derived to estimate population differentiation. The statistical significance of each population parameter was tested by performing 1,000 random permutations of the haplotypes across populations or groups depending upon the parameter tested. For the AMOVA analysis, samples with missing markers were eliminated to avoid multilocus phenotype misidentification; therefore, a total of 270 aecial samples were analyzed. AMOVA on phenotypes estimates fairly accurate analyses of genetic differentiation based on genotypes (15). The number of migrants among pairs of populations (*Nm*) was estimated from pairwise coefficients of inbreeding (F_{st}) as $Nm = 1/4(1 - F_{st})/F_{st}$.

The unweighted pair-group method arithmetic mean (UPGMA) dendrogram based on pairwise Nei's unbiased genetic distances (30) was constructed using PHYLIP 3.56 implemented in TFPGA. Bootstrap confidence values on the nodes were calculated by 1,000 bootstrap resamplings over loci.

To further assess population clustering, a principal component analysis (Proc PRINCOMP, SAS Institute, Cary, NC) was conducted on a matrix of populations by null allele frequency estimated according to the method of Lynch and Milligan (26). This analysis reduced patterns in many loci based on correlated patterns among the loci. Scores for the eight populations from eastern and western North America were plotted along the first two principal components.

TABLE 3. *F* statistic calculated for seven putative random amplified polymorphic DNA (RAPD) loci based on estimated allele frequencies

Locus	θ_p Observed[a]
OPA1-1700	0.520
OPA1-2000	–0.005
OPC8-750	0.001
OPC8-900	0.423
OPE15-1600	0.275
OPK19-2000	0.095
OPK11-500	0.460
Average over loci	0.358
Lower 99% confidence interval[b]	0.133
Upper 99% confidence interval[b]	0.499

[a] Inbreeding coefficient. Genetic differentiation between eastern and western populations estimated according to the method of Lynch and Milligan (27).
[b] Calculated by 1,000 bootstrap replications (resampling with replacement) over loci.

RESULTS

A majority of the RAPD markers previously selected using eastern *C. ribicola* populations (6–8) were almost fixed in western populations. The null allele at locus OPA01-1700, with a frequency of ≈40 to 50% in eastern populations (Table 1) (2,7), was present at frequencies of 96 to 100% in western populations (Table 1). The null alleles at loci OPK11-500 and OPC08-900 were present at intermediate frequencies in eastern populations (31 to 55% for OPK11-500 and 52 to 64% for OPC08-900; Table 1) but were fixed in nearly all western populations (Table 1). The null alleles at three other loci (OPC08-750, OPA01-2000, and OPE15-1600) occurred at relatively high frequencies in eastern populations and were fixed in most western populations. A null allele at one locus (OPK19-2000) reported in this study was present in both eastern and western populations, but differences in frequency were observed, ranging from 33 to 45% in eastern populations and from 0 to 42% in western populations; two populations were fixed for the null allele (Table 1).

Expected heterozygosities were higher for eastern populations than western populations. A single population in Sacramento, NM, was fixed at all loci, and therefore exhibited an expected het-

TABLE 4. Unbiased genetic distance (lower diagonal) and estimated number of migrants (upper diagonal) among pairs of populations of *Cronartium ribicola* from eastern and western North America[a]

		West				East			
	Location	Manning Park	Smallwood	Shelter Bay	Sacramento	Ste-Camille	Minden	Little Grand Lake	Moncton
West	Manning Park	0.00	30.83	45.36	2.69	0.40	0.34	0.34	0.51
	Smallwood	0.01	0.00	5.16	1.25	0.48	0.42	0.40	0.62
	Shelter Bay	0.01	0.02	0.00	3.10	0.39	0.34	0.32	0.49
	Sacramento	0.01	0.02	0.00	0.00	0.18	0.16	0.15	0.22
East	Ste-Camille	0.13	0.14	0.15	0.15	0.00	12.82	45.65	24.85
	Minden	0.16	0.16	0.19	0.19	0.00	0.00	6.98	Inf[b]
	Little Grand Lake	0.11	0.11	0.12	0.12	–0.01	0.02	0.00	5.81
	Moncton	0.07	0.07	0.10	0.10	0.01	0.01	0.01	0.00

[a] Unbiased genetic distance determined according to the method of Nei (30); number of migrants estimated from pairwise coefficients of inbreeding. F_{st} = pairwise coefficients of inbreeding; value not significantly different from 0.
[b] Value of F_{st} not significantly different from 0.

erozygosity of 0. It is unlikely that sample size artifacts are the cause of this low level of heterozygosity ($n = 45$).

The AMOVA conducted on frequency of RAPD phenotypes and Euclidean distances among haplotypes revealed a high level of differentiation between eastern and western populations ($\phi_{st} = 0.55$; $P < 0.001$; Table 2). However, genetic differentiation among populations within regions was low and not significantly different from 0 ($\phi_{st} = 0.21$; $P = 0.076$; Table 2). Among the BC populations, those from the interior were not differentiated from coastal populations ($\phi_{st} = 0.00$; $P = 0.78$). Most of the genetic variability (99%) resided within populations (Table 2).

F statistics calculated with Lynch and Milligan's (26) estimates derived from the frequency of the putative homozygotes for null alleles yielded values ranging from –0.005 to 0.520 (average over loci = 0.358) for comparison among eastern and western populations (Table 3). Confidence intervals calculated by 1,000 bootstrap resamplings of the loci yielded values of θ from 0.199 (lower 99%) to 0.476 (upper 99%); these values were well above the null value expected under panmixis.

Genetic distances were low among populations of western North America (0.00 to 0.02) and among eastern populations (0.00 to 0.02), indicating a very similar genetic composition (Table 4). In contrast, genetic distances between eastern and western populations were relatively large, and all were significantly different from 0 (0.07 to 0.19; $P < 0.001$).

Migration among eastern and western populations was very low, below one migrant per generation (the minimum required to prevent genetic differentiation) for all pairwise comparisons (Table 4). In contrast, estimates of migration between pairs of western populations was above 1 for all comparisons and was very large between some populations (e.g., $Nm = 45$ between Shelter Bay in interior BC and Manning Park in coastal BC; Table 3). The lowest level of estimated migration among western populations was calculated between the NM population and populations from BC. High numbers of migrants were calculated among all pairwise comparisons in eastern North America.

A UPGMA dendrogram based on pairwise measures of genetic distances resulted in a tree with two clusters of populations de-

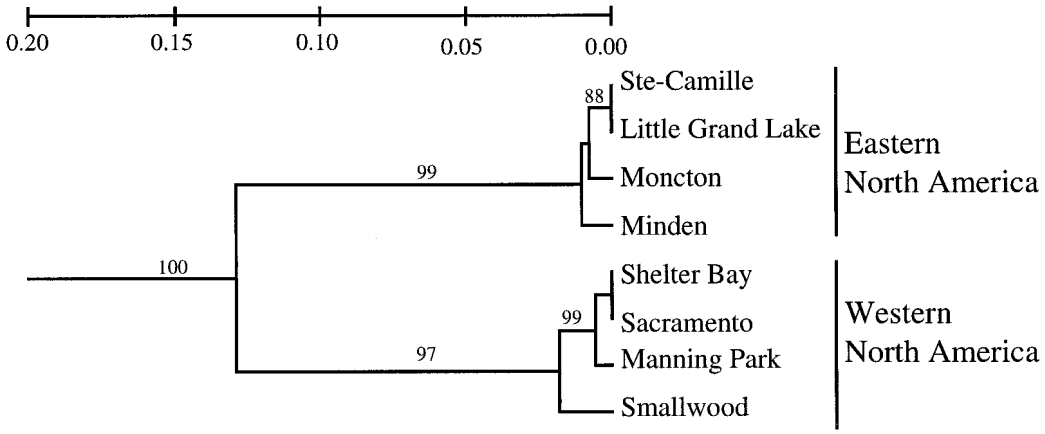

Fig. 1. Unweighted pair-group method with arithmetic averages (UPGMA) analysis of Nei's (30) unbiased genetic distance among eight populations of *Cronartium ribicola* from eastern and western North America. Numbers above branches represent percentage of 1,000 bootstrap replications over markers.

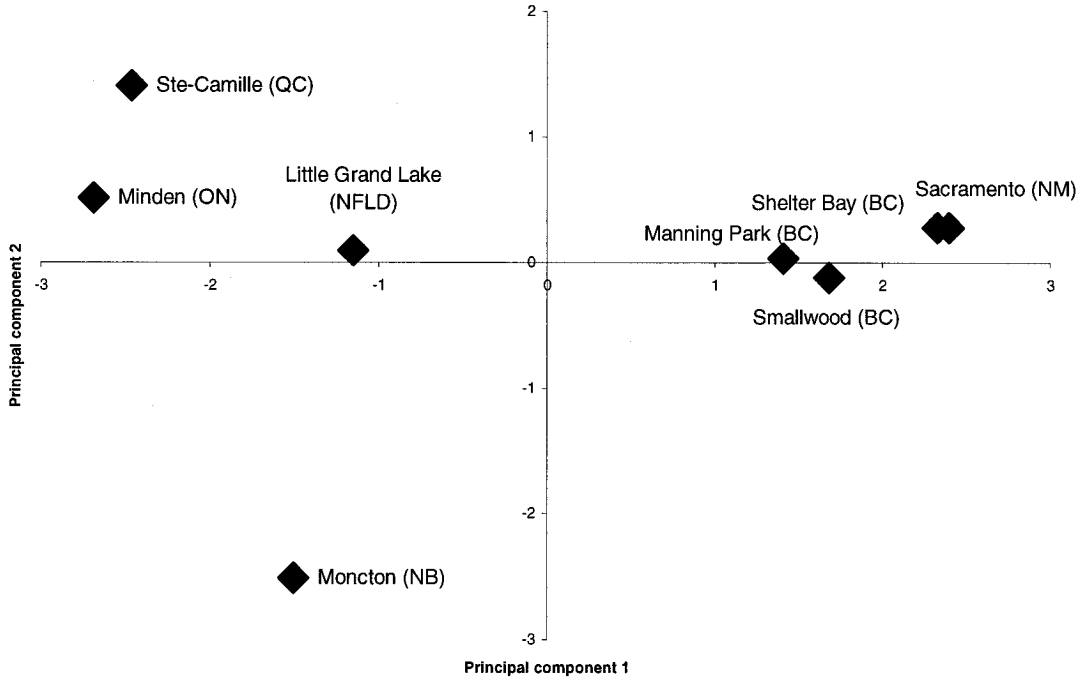

Fig. 2. Principal component analysis using allelic frequencies estimated by Lynch and Milligan's (27) method for eight populations of *Cronartium ribicola* from eastern and western North America.

lineated along geographic lines (Fig. 1). However, within clusters, there was no apparent correlation between geographic origin and genetic distance. For example, the population from Smallwood, interior BC was genetically closely related to the population from Manning Park, coastal BC but more distantly related to the population from Shelter Bay, interior BC. The population from NM was within the cluster of populations from BC.

A principal component analysis using estimated gene frequencies separated the populations along geographic lines. (Fig. 2). All eastern populations possessed negative values for the first principal component, whereas western populations possessed positive values. Populations from eastern North America were scattered more than those from western North America, as expected from the higher level of expected heterozygosity observed earlier (Table 1).

DISCUSSION

This is the first report of genetic differentiation associated with geographic origin among *C. ribicola* populations in North America. The level of differentiation observed between eastern and western populations of *C. ribicola* is very large, and we reject the null hypothesis that populations of this pathogen are panmictic in North America. Our results are in sharp contrast to other studies of white pine blister rust, where populations of distant geographic origin (sometimes >1,000 km) were genetically homogeneous (2,7, 20,36). In most of these studies however, populations were sampled within either the western or the eastern epidemiological units.

One possible explanation for our results is that host selection is the evolutionary force behind the observed genetic differentiation. A variety of five-needle pines occurs throughout the range of white pine blister rust. Accordingly, our rust samples were collected from *P. monticola* in BC, *P. strobiformis* Engelm. in NM, and *P. strobus* in eastern North America. Although it is possible that host selection selected rust phenotypes locally, it is unlikely for the following reason: The genetic differentiation reported here is at RAPD loci. These loci represent DNA amplified randomly from anonymous regions of the genome. Although it is not impossible that a marker would be tightly linked to a region of the genome involved in host adaptation, it is very unlikely that all five loci showing strong differentiation are linked to such regions. If *C. ribicola* were strictly a clonally reproducing fungus, this explanation would be more plausible, because absence of recombination would effectively result in complete linkage of the entire genome. However, populations were in Hardy-Weinberg equilibrium (4), and haplotype frequencies of the spermatial stage (the vegetative extension of the basidiospores) were consistent with those expected under the assumption of random mating (8). These observations suggest that recombination is extensive in this fungus and that the hypothesis of linkages between our markers and host adaptation traits is unlikely. A similar case can be made for environmental adaptation in the climatic zones encompassing the sampling range in our study.

A more likely explanation for the results reported in our study is the presence of a barrier to gene flow between eastern and western populations. The absence of hosts is probably the cause of this barrier. *C. ribicola* is a biotrophic heteroecious rust, requiring a pine (aecial) and a dicot (telial) host (*Ribes* spp. and *Scrophulariace* spp. in North America). Across the Great Plains, intensive agriculture and the absence of natural five-needle pines result in a zone of several hundred kilometers where aecial and telial hosts of *C. ribicola* are absent or rare. Although aeciospores and uredospores can be disseminated over long distances, the Great Plains severely restrict the dissemination of spores across the continent. In our opinion, the scarcity of infected *Ribes* and five-needle pines on the Great Plains is the most likely explanation for our results. To test this hypothesis, intensive sampling should be conducted in the populations adjacent to the Great Plains to determine whether gene flow is taking place. The threat posed by increased cultivation of *Ribes* in this zone to provide a bridge between eastern and western populations should be seriously considered.

Results similar to those reported here were found between eastern and western populations of cereal leaf rust in North America (21,24); however, the agricultural pathosystem is quite different. Wheat cultivars with different resistance genes are deployed east and west of the Rocky Mountains, and the rust population is asexual east of the Rockies and sexual west of the mountains. In addition, wheat is not grown in the mountains, therefore there is a host gap that may prevent spores from crossing the Rocky Mountains.

The greater variability observed in eastern *C. ribicola* samples is consistent with more rust importations in the east compared with the west. However, the markers used in the current study were developed after extensive screening of eastern populations of *C. ribicola* (2,6–8). Therefore, the markers selected show a polymorphic bias towards eastern populations. Although measures of differentiation should not be affected by that bias, the expected heterozygosities reported in Table 1 should be interpreted with caution. To determine whether genetic diversity is greater in eastern than in western populations, a random sampling of the genome should be conducted and all sampled markers scored. This is difficult because over 100 RAPD primers were screened to select the current set. Only 8% of 212 markers were polymorphic in western populations of *C. ribicola* (20) with an average heterozygosity of 0.025. Now that the population structure of the pathogen is more clearly understood, it would be possible to conduct a large sampling of the genome on a small number of individuals from the eastern and western populations.

The NM population was the only population fixed at all loci, including locus OPK19-2000 which was polymorphic in eastern and western North America (4,20). This population has a recent history of introduction. Although it was first reported in 1990 (9), the age of the oldest cankers suggests it was introduced around 1970 from either infected nursery stock or long-distance aerial dispersal (35). Our results (clustering of this population with those from BC, the high frequency of the same RAPD phenotypes in BC and NM populations, and the complete absence of expected heterozygosity) support the hypothesis of a single, recent introduction from a western North American source.

The eastern and western populations studied are not distinct biotypes, but relevant biological information can be associated with our results and the concept of epidemiological units clearly applies to these populations (22,25). For quarantine purposes, these genetically separated populations should be kept separate until the biological attributes associated with the observed genetic differences are better understood. From an epidemiological perspective, it is important to understand the barriers that prevent homogenization of these populations and to determine the limits of the eastern and western epidemiological units.

ACKNOWLEDGMENTS

We thank J. Bérubé and P. Cheers for reviewing the manuscript, and J. Pope and the U.S. Forest Service for collecting the New Mexico samples.

LITERATURE CITED

1. Draper, M. A., and Walla, J. A. 1993. First report of *Cronartium ribicola* in North Dakota. (Abstr.) Plant Dis. 77:9.
2. Et-touil, K., Bernier, L., Beaulieu, J., Bérubé, J. A., Hopkin, A., and Hamelin, R. C. 1999. Genetic structure of *Cronartium ribicola* populations in eastern Canada. Phytopathology 89:915-919.
3. Excoffier, L., Smouse, P. E., and Quattro, J. M. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data. Genetics 131:479-491.
4. Gitzendanner, M. A., White, E. E., Foord, B. M., Dupper, G. E., Hodgskiss, P. D., and Kinloch, B. B., Jr. 1996. Genetics of *Cronartium ribicola*. III. Mating system. Can. J. Bot. 74:1852-1859.

5. Güssow, H. T. 1923. Report of the dominion botanist for the year 1922. Canada Dept. Agric.
6. Hamelin, R. C. 1996. Genetic diversity between and within cankers of the white pine blister rust. Phytopathology 86:875-879.
7. Hamelin, R. C., Beaulieu, J., and Plourde, A. 1995. Genetic diversity in populations of *Cronartium ribicola* in plantations and natural stands of *Pinus strobus*. Theor. Appl. Genet. 91:1214-1221.
8. Hamelin, R. C., Dusabenyagasani, M., and Et-touil, K. 1998. Fine-level genetic structure of white pine blister rust populations. Phytopathology 88:1187-1191.
9. Hawksworth, F. G. 1990. White pine blister rust in New Mexico. (Abstr.) Plant Dis. 74:938.
10. Heimburger, C. 1972. Relative blister rust resistance of native and introduced white pines tested in eastern North America. Pages 257-269 in: Proc. Biol. Rust Resis. For. Trees, Moscow.
11. Hoff, R. J., and McDonald, G. I. 1971. Resistance to *Cronartium ribicola* in *Pinus monticola*: short shoot fungicidal reaction. Can. J. Bot. 49:1235-1239.
12. Hunt, R. S. 1994. Transferability of western white pine within and to British Columbia: Blister rust resistance. Can. J. Plant Pathol. 16:273-278.
13. Hunt, R. S. 1997. Relative value of slow-canker growth and bark reactions as resistance responses to white pine blister rust. Can. J. Plant Pathol. 19:352-357.
14. Hunt, R. S., and Meagher, M. D. 1989. Incidence of blister rust on "resistant" white pine (*Pinus monticola* and *P. strobus*) in coastal British Columbia plantations. Can. J. Plant Pathol. 11:419-423.
15. Isabel, N., Beaulieu, J., and Bousquet, J. 1995. Complete congruence between gene diversity estimates derived from genotypic data at enzyme and RAPD loci in black spruce. Proc. Natl. Acad. Sci. USA 92:6369-6373.
16. Kinloch, B. B., Jr. 1992. Distribution and frequency of a gene for resistance to white pine blister rust in natural populations of sugar pine. Can. J. Bot. 70:1319-1323.
17. Kinloch, B. B., Jr., and Byler, J. W. 1981. Relative effectiveness and stability of different resistance mechanisms to white pine blister rust in sugar pine. Phytopathology 71:386-391.
18. Kinloch, B. B., Jr., and Comstock, M. 1980. Cotyledon test for major gene resistance to white pine blister rust in sugar pine. Can. J. Bot. 58:1912-1914.
19. Kinloch, B. B., Jr., and Dupper, G. E. 1999. Evidence of cytoplasmic inheritance of virulence in *Cronartium ribicola* to major gene resistance in sugar pine. Phytopathology 89:192-196.
20. Kinloch, B. B., Jr., Westfall, R. D., White, E. E., Gitzendanner, M. A., Dupper, G. E., Foord, B. M., and Hodgskiss, P. D. 1998. Genetics of *Cronartium ribicola*. IV. Population structure in western North America. Can. J. Bot. 76:91-98.
21. Kolmer, J. A. 1991. Phenotypic diversity in two populations of *Puccinia recondita* f. sp. *tritici* in Canada during 1931 to 1987. Phytopathology 81:311-315.
22. Kolmer, J. A. 1992. Diversity of virulence phenotypes and effect of host sampling between and within populations of *Puccinia recondita* f. sp. *tritici* in Canada. Plant Dis. 76:618-621.
23. Lee, S. B., and Taylor, J. W. 1990. Isolation of DNA from fungal mycelia and single spores. Pages 282-287 in: PCR Protocols. M. A. Innis, D. H. Gelfand, J. J. Sninsky, T. J. White, eds. Academic Press, San Diego, CA.
24. Leonard, K. J., Roelfs, A. P., and Long, D. L. 1992. Diversity of virulence within and among populations of *Puccinia recondita* f. sp. *tritici* in different areas of the United States. Plant Dis. 76:500-504.
25. Long, D. L., Roelfs, A. P., and Leonard, K. J. 1993. Virulence and diversity of *Puccinia recondita* f. sp. *tritici* in the United States in 1991. Plant Dis. 77:786-791.
26. Lynch, M., and Milligan, B. G. 1994. Analysis of population genetic structure with RAPD markers. Mol. Ecol. 3:91-99.
27. Maloy, O. C. 1997. White pine blister rust control in North America: A case history. Annu. Rev. Phytopathol. 35:87-109.
28. McDonald, G. I., Hansen, E. M., Osterhaus, C. A., and Samman, S. 1984. Initial characterization of a new strain of *Cronartium ribicola* from the Cascade Mountains of Oregon. Plant Dis. 68:800-804.
29. Nagarajan, S., and Singh, D. V. 1990. Long-distance dispersion of rust pathogens. Annu. Rev. Phytopathol. 28:139-153.
30. Nei, M. 1978. Estimation of average heterozygosities and genetic distance from a small number of individuals. Genetics 89:583-590.
31. Powers, H. R. J. 1982. Pathogenic variability within the genus *Cronartium*. Pages 236-242 in: Proc. Resis. Dis. Pests For. Trees, Wageningen, the Netherlands.
32. Spaulding, P. 1929. White-pine blister rust: A comparison of European with North American conditions. U.S. Dept. Agric.
33. Stewart, F. C. 1906. An outbreak of the European currant rust (*Cronartium ribicola* Dietr.). N.Y. Agric. Exp. Stn. Tech. Bull. 61-74.
34. Stuckmeyer, B. E., and Riker, A. J. 1951. Wound-periderm formation in white-pine trees resistant to blister rust. Phytopathology 41:276-281.
35. Van Ardel, E. P., Conklin, E. P., Popp, J. B., and Geils, B. W. 1998. The distribution of white pine blister rust in the Sacramento Mountains of New Mexico. Pages 275-283 in: Proc. IUFRO Rusts For. Trees Working Party Conf., Finn. For. Res. Inst., Saariselkä, Finland.
36. White, E. E., Foord, B. M., and Kinloch, B. B., Jr. 1996. Genetics of *Cronartium ribicola*. II. Variation in the ribosomal gene cluster. Can. J. Bot. 74:461-468.
37. Williams, J. G. K., Kubelik, A. R., Livak, K. J., Rafalski, J. A., and Tingey, S. V. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. 18:6531-6535.
38. Yokota, S. 1983. Resistance of improved *Pinus monticola* and some other white pines to the blister rust fungus, *Cronartium ribicola*, of Hokkaido, Japan. Eur. J. For. Pathol. 13:389-402.