Fine-Level Genetic Structure of White Pine Blister Rust Populations

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ABSTRACT

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The fine-level genetic structure of the white pine blister rust agent, *Cronartium ribicola*, was investigated by sampling multiple monokaryotic spermatia directly on cankers in four eastern Canadian white pine (*Pinus strobus*) plantations and assessing genetic variability, using random amplified polymorphic DNA (RAPD) markers. Ninety-eight percent of the cankers surveyed contained a single DNA haplotype, suggesting spermatia within cankers are the result of clonal reproduction. A single canker contained two haplotypes that were divided between the upper and lower parts of the canker, suggesting it represented two confluent cankers. In contrast, genotypic diversity was high among cankers. Thirty-seven haplotypes were found among forty-three cankers sampled, and an analysis of molecular variance indicated that 93% ($P < 0.001$) of

the total genetic diversity was attributable to sampling of different cankers, strongly suggesting that multiple infections do not take place in the white pine blister rust pathosystem, i.e., a canker is the result of infection by a single genotype. This result is in contrast with the high level of genetic diversity previously reported among dikaryotic aecidia within cankers and is consistent with the hypothesis that variability in the aecidial stage is the result of outcrossing between resident spermatia and alien spermatia. The genetic structure of the spermatial stage, which is the vegetative extension of infection by basidiospores and, therefore, the indirect result of meiosis, was consistent with random mating; the observed genotypic diversity was not significantly different ($P > 0.05$) from the genotypic diversity expected under the assumption of panmixis. The results indicate that monokaryotic cankers can be genotyped by sampling a single unopened spermatium per canker.

Additional keyword: AMOVA.

White pine blister rust, caused by the basidiomycete *Cronartium ribicola* J.C. Fisch., is a very severe disease of white pine. The pathogen was introduced into North America during the early twentieth century (30) and has spread to most of the distribution range of white pine. Reforestation and regeneration of white pine are greatly hampered by blister rust in economically important species, such as *Pinus strobus*, *P. monticola*, and *P. lambertiana*. Mortality rates of up to 95% have been reported in white pine plantations in high hazard zones. In addition, some pine species adapted to specialized ecological niches, such as whitebark pine (*P. alba*), are so vulnerable they are endangered by blister rust (13,14).

An important component of managing blister rust is knowledge about the biology of the pathogen. Early efforts focused on attempts to disrupt the life cycle of the fungus by eradicating the telial host, *Ribes* spp. (2). Recently, resistance breeding, aided by molecular mapping and selection, has shown promise for helping to manage this disease (4,21). However, many questions remain about the population biology of *C. ribicola* (e.g., the role and contribution of different spore types to the life cycle). Spermatia are apparently strictly sexual uninfertile spores that unite two nuclei (28). Aeciospores and urediniospores are presumed to be involved in middle- to long-distance spread. Basidiospores are suspected to be disseminated fairly locally, possibly up to 8 km, depending on topography and wind pattern (33,34). However, such knowledge often is based on circumstantial evidence. Experimental demonstration of these hypotheses is lacking.

Use of molecular methods can provide new means of elucidating the population biology of *C. ribicola* and testing hypotheses concerning infection and dissemination patterns. Four approaches to sampling and analyses have been used to study the population

genetics of pine rusts: (i) direct sampling of single-gall, bulk aeciospore collections combined with isozymes and restriction fragment length polymorphism (RFLP) (24,32,35-37); (ii) direct sampling of single aecidia combined with polymerase chain reaction (PCR)-based random amplified polymorphic DNA (RAPD) (9,10); (iii) basidiospore-derived axenic cultures combined with isozymes, RFLP, and RAPD (5,6,8,15); and (iv) direct sampling of single spermatia with RAPD (5,6,9,11).

With the first approach, codominant markers requiring large amounts of material, such as isozymes and RFLP, can be used, but in heterothallic rusts, the samples can comprise a mosaic of genotypes (9,18), and data interpretation can be difficult (37). In the second approach, PCR-based techniques, mostly dominant RAPD markers, must be used because of the small amount of material available. Because the aecial stage is dikaryotic, analyses are conducted using statistical corrections to account for dominance (10, 20) or, alternatively, using analyses of molecular variance (AMOVA) on phenotypes (7,9). Large numbers of samples can be processed, but exact marker frequencies cannot be obtained. In the third approach, a large amount of material can be grown, and a variety of markers, including codominant isozymes and RFLPs, as well as RAPDs, can be used. Segregation analyses can be conducted because single telia produce a population of sibling progeny (5,6,8). However, the technique can be tedious, and large sample sizes can be difficult to obtain. In addition, selection does not necessarily reflect natural conditions, either on the pine host or growth medium.

The fourth approach is potentially interesting, because spermatia are the extension of basidiospores, which are the direct result of meiosis. By sampling the fungus directly on the host, it may be possible to study populations of *C. ribicola* resulting from sexual reproduction and test whether the population structure is consistent with the assumptions of random mating. Also, by directly comparing the monokaryotic spermatial DNA profile (pre-spermatization) with that of the dikaryotic aecidia (postspermatization), the actual rate of outcrossing of individual cankers can be calculated.

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Knowledge of the fine-level genetic structure of the spermatogonial stage also is important to better understand the biology of *C. ribicola* and devise the best sampling strategy for population studies. Comparing the genetic structure obtained by analyzing different spore stages, including aeciospores, urediniospores, spermatogonia, and basidiospore-derived cultures, it may be possible to better understand their respective roles in the epidemiology of *C. ribicola* and to assess the effect of host selection pressure on the population structure.

However, genetic variability has been reported among spermatogonial drops within cankers in *C. ribicola* and *C. quercuum* f. sp. *fusiforme* (5,9), which is surprising, because it suggests that either there were multiple infections, i.e., a single canker was the result of infection by several genotypes, or heterokaryons were established after dikaryotization. It also is possible that the spermatogonial drops were already spermatized at the time of sampling and the observed intracanker variability among monokaryotic spermatogonia was, in fact, the result of outcrossing. This could not be

completely ruled out, because some of the spermatogonia were already opened at the time of sampling (5,9).

The objective of this study was to elucidate the fine-level genetic structure of *C. ribicola* populations by sampling unopened monokaryotic spermatogonia in a hierarchical fashion (within cankers, among cankers, and among sites) directly on white pine blister rust cankers to determine whether (i) genetic variability occurs among spermatogonia within cankers; (ii) the population structure of the spermatogonial spore stage is similar to that of the aecidial stage; and (iii) the population structure of spermatogonia is consistent with random mating.

MATERIALS AND METHODS

Sampling. Samples were harvested from cankers in two white pine plantations in south-central Quebec Province (Plessisville [PL], $n = 19$; Saint-Cyprien [CY], $n = 14$), where *C. ribicola* populations have been monitored since 1993, and two plantations on the Gaspé Peninsula in eastern Quebec (CO, $n = 5$; SB, $n = 5$). A total of 43 cankers was sampled. A single canker was sampled on each tree, except for two trees on which two cankers were sampled. Although some markers differed in frequency between the PL and CY rust populations, expected heterozygosities were relatively small, as were overall genetic differentiations (9,10). Tagged trees with active cankers were observed from the last week of June until the end of July 1996 for appearance of spermatogonial blisters. Spermatia were harvested by carefully piercing closed spermatogonia with the tip of a Pasteur pipette and collecting the nectar drop in a 1.5-ml Eppendorf microtube containing a 1.5 M sorbitol solution. Samples were kept on ice until processing. Between 3 and 14 single spermatogonia, depending on availability of unopened spermatogonia, were sampled on each canker, for a total of 324 samples (Table 1). The position of spermatogonia on large cankers was noted to identify patterns of distribution of haplotypes within cankers. Spermatogonia samples collected in 1994 from cankers that were still active in 1995 were assayed for comparison between years of the same canker.

DNA extraction. DNA was extracted from spermatogonia using a modification of a protocol described previously (10). Lyophilized spores were ground for 2 to 4 min with ≈ 10 mg of diatomaceous earth (Sigma Chemical Co., St. Louis) and 100 μ l of extraction buffer (700 mM NaCl, 50 mM Tris-HCl [pH 8.0], 10 mM EDTA, 1% β -2-mercaptoethanol, 1% cetyl-trimethyl-ammonium-bromide) with disposable Kontes pestles (VWR-Canlab, Toronto). Extraction buffer (300 μ l) was added, and samples were incubated at 65°C for 1 h.

Samples were extracted with 600 μ l of chloroform/isoamyl alcohol (24:1, vol/vol), vortexed, and centrifuged at $10,000 \times g$ for 5 min. The upper phase was pipetted into 1.5-ml Eppendorf microcentrifuge tubes, and DNA was precipitated by adding 75 μ l of 7.5 M ammonium acetate and 600 μ l of isopropanol and placing the tubes on ice for at least 30 min. DNA was pelleted by centrifugation at $10,000 \times g$ for 5 min and washed with 70% ethanol, after which the pellet was air-dried and resuspended in 20 μ l of TE-8 buffer (10 mM Tris-HCl [pH 8], 1 mM EDTA). DNA concentration was estimated by comparing band intensity on an agarose gel with a set quantity of λ -HindIII fragments (Gibco-BRL, Gaithersburg, MD). DNA was diluted from 1:2 to 1:25, depending on DNA concentration, and stored in TE-8 buffer at -20°C. Our results indicate that reproducible RAPD patterns were obtained with concentrations of 1 to 100 ng.

DNA amplifications. DNA amplifications were performed in 12.5- μ l volumes containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2 mM MgCl₂, 0.0001% gelatin, 100 μ M each of dNTP (Pharmacia Biotechnology, Uppsala, Sweden), 0.2 μ M oligonucleotides, 2 μ l (≈ 10 ng) of genomic DNA, and 0.5 units of *Taq* DNA polymerase (Boehringer Mannheim, Laval, QC, Canada) (38). Amplifications were performed in a thermal cycler (model PTC-60, MJ

TABLE 1. Random amplified polymorphic DNA haplotypes of *Cronartium ribicola* spermatogonia from four white pine plantations in Quebec Province

Canker ^a	n ^b	Haplotype ^c	Designation
CY101	8	100101110	1
CY102	10	000111111	2
CY21	3	010101010	3
CY22	14	100101000	4
CY23	11	000111011	5
CY28-1	4	110111100	6
CY28-2	10	100101000	4
CY35	10	010111011	7
CY38	10	100101101	8
CY39	6	010101101	9
CY40	10	010111100	10
CY42	10	000111000	11
CY44	10	101111001	12
... ^d	...	001111001	13
CY65	12	100011111	14
PL1	3	001101010	15
PL11	9	100110010	16
PL16	14	000101100	17
PL18	11	100101000	4
PL23	7	110110100	18
PL24	10	000111001	19
PL25	11	110101110	20
PL26	12	001111000	21
PL29	10	000011000	22
PL33-1	3	100111111	23
PL33-2	5	000111000	11
PL34	9	011000110	24
PL35	9	100111010	25
PL37	9	010111000	26
PL39	5	110101001	27
PL45	7	110111001	28
PL5	3	100101111	29
PL41	9	001101010	15
CO2	5	001101110	30
CO6	5	101100100	31
CO11	5	001111101	32
CO18	5	001111000	21
CO20	5	101101110	33
SB1	5	011001001	34
SB3	5	011111100	35
SB8	5	101101111	36
SB10	5	100101101	8
SB19	5	100111001	37

^a Letters represent the site (CY = Saint-Cyprien, PL = Plessisville, CO and SB = two plantations on the Gaspé Peninsula). Numbers represent canker identification.

^b Number of spermatogonia sampled per canker.

^c Markers are (Operon primer-size [in base pairs] of marker): OPA01-1700, OPC08-900, OPE15-1600, OPE15-2500, OPK19-2000, OPK19-1700, OPK19-1500, OPK11-550, and OPK11-900; for conciseness, monomorphic markers are not indicated in the haplotype binary designation.

^d Two haplotypes were found within this canker.

Research, Watertown, MA) programmed for denaturation at 94°C for 3 min, followed by 1 cycle at 35°C for 4 min and 72°C for 2 min, and 45 cycles of 94°C for 1 min, 35°C for 1 min, and 72°C for 2 min. Reactions were ended with a 10-min extension at 72°C.

Primers (Operon Technologies, Alameda, CA) OPA01 and OPC08 were selected based on previous results (9,10). Additionally, primers OPE15, OPK11, and OPK19 were assayed based on a screening of 60 primers that indicated reproducible polymorphisms in these primers (K. Et-touil and R. C. Hamelin, *unpublished data*). Amplification products were separated by electrophoresis on 1.5% agarose gels using 1× Tris-acetate-EDTA buffer (primer OPA01) or 1% agarose plus 0.5% Synergel (Diversified Biotech, Newton Center, MA) in 0.5× Tris-phosphate-EDTA buffer. PCR products were visualized by UV fluorescence after ethidium bromide staining.

Marker frequencies for OPA01 and OPC08 were reported for *C. ribicola* populations in Quebec (10), including plantations CY and PL sampled in the current study. Mendelian segregation was demonstrated for marker OPK19-1900 in western populations of *C. ribicola* (8). Amplifications of DNA templates and primer combinations were repeated regularly to ensure the reproducibility of amplification profiles. All major diagnostic DNA fragments used in our analysis were reproducible between runs and present in spermogonia, aecidiospores, and urediniospores of *C. ribicola* (9,10) (R. C. Hamelin, *unpublished data*).

Data analysis. A matrix of samples × markers containing a binary scoring for each marker (1 for presence, 0 for absence) was recorded. An AMOVA (7) was performed using a matrix of Euclidean distances and Nei and Li's (26) genetic distance between all pairs of haplotypes. The two metric distances yielded similar results, so only the former was used subsequently.

AMOVA uses a classical analysis of variance (ANOVA) approach to compute molecular variance components at different hierarchical levels. The analysis is performed on a matrix of squared molecular distances between pairs of observations, in our case RAPD haplotypes. A conventional sum of squares was calculated as the sum of squared deviations (SSD) between individual observations. The total SSD could be partitioned in a traditional ANOVA framework:

$$\text{SSD}(\text{total}) = \text{SSD}(\text{among regions}) + \text{SSD}(\text{among populations within regions}) + \text{SSD}(\text{within population})$$

and the corresponding mean squared deviations (MSD) were obtained by dividing the SSD by the appropriate degrees of freedom. Variance components were obtained by equating the MSD with its expectations as σ_a^2 (among regions), σ_b^2 (among populations within groups), and σ_c^2 (within populations). *F* statistic analogs (Φ statistics) were calculated as

$$\Phi_{ST} = \sigma_a^2 / (\sigma_a^2 + \sigma_b^2), \Phi_{CT} = \sigma_b^2 / \sigma_c^2, \text{ and } \Phi_{SC} = \sigma_b^2 / (\sigma_a^2 + \sigma_b^2)$$

where Φ_{ST} is the correlation between random haplotypes within populations and random haplotypes drawn from all populations, Φ_{CT} is the correlation between random haplotypes within regions (east: CO and SB; west: CY and PL) and random haplotypes from all populations, and Φ_{SC} is the correlation between random haplotypes within populations and random haplotypes within region (7). Nonparametric tests of Φ_{ST} , Φ_{CT} , and Φ_{SC} were conducted with 100 random permutations of the data set. The hierarchical level "within cankers" was not included in AMOVA because of the apparent clonality of haplotypes.

The hypothesis of panmixis was tested using analysis of genotypic diversity. The observed genotypic diversity, $\hat{G}$ (31), was calculated using $\hat{G} = 1/\sum p_i^2$, where p_i is the frequency of the i th haplotype in the population. The maximum value for this variable is the sample size. Observed genotypic diversity was compared with the expected genotypic diversity generated assuming random mating and performing 100 simulations using the observed marker frequency. A *t* statistic was calculated as $t = [G - \hat{G} \text{var}(G)]$, where G is the mean and $\text{var}(G)$ is the variance determined from

the simulations (23). The simulations were performed using a macro written in Microsoft Excel spreadsheet software.

RESULTS

Of the 43 cankers sampled, 42 (97.6%) were monomorphic with regard to the RAPD profiles of the spermogonia (Table 1; Fig. 1). Even large cankers that spanned more than 0.5 m and from which 20 spermogonia samples were collected contained unique DNA profiles. Spermogonial DNA profiles also were distinct among cankers sampled on the same tree. The sole canker (CY44; Table 1) in which two haplotypes were found was very large and may have been two confluent cankers. Haplotype 12 was present only on the upper part of canker CY44, and haplotype 13 was present only on the lower part. This canker could not be resampled because the tree died after the 1995 collections.

From 43 cankers sampled, 37 haplotypes of *C. ribicola* were found. Thirty-two haplotypes (86.5%) were unique, while five (13.5%) occurred more than once. There was no clear association between the occurrence of RAPD markers or haplotypes and the geographic origin of the sample (Table 1). One haplotype (4; Table 1) was represented three times: twice at CY and once at PL, two plantations separated by ≈100 km (Table 1; a map is presented in Hamelin et al. [10]). Most of the remaining haplotypes did not occur more than once within a site (Table 1).

Because samples within cankers were apparently clonally derived, cankers, not spermogonia, were considered the sampling unit for subsequent analyses of genetic diversity, avoiding the potential problem of duplicate sampling (23). AMOVA indicated that most of the genetic variability (93%) was found among cankers within sites and that spermogonial DNA profiles were not significantly more likely to be different among populations ($\Phi_{ST} = 0.074$; $P = 0.096$; Table 2). The proportion of genetic variance between regions (east versus west, separated by ≈700 km) was significant but not very large (8%), and the genetic differentiation index was low but significantly larger than random expectation ($\Phi_{CT} = 0.085$, $P < 0.001$), whereas the level of genetic differentiation among populations within regions was low and not significant ($\Phi_{SC} = -0.011$; $P = 0.610$; Table 2). The negative values reported for the variance component among populations within region can be interpreted by analogy to correlation coefficients: haplotypes drawn from different populations within regions were genetically slightly (but not statistically) more related than random haplotypes drawn from the same population.

Observed genotypic diversity, when considering cankers as the sampling unit, was high in all populations sampled (Table 3). The maximum value for this measure of genotypic diversity is the total number of samples. Values observed were close to the sample size

Fig. 1. Random amplified polymorphic DNA of *Cronartium ribicola* spermogonia, using oligonucleotide primers A, OPK11 and B, OPA01. Lanes 1 and 14, 100-bp ladder; lanes 2 through 7, individual spermogonia samples from canker CY35; and lanes 8 through 13, individual spermogonia samples from canker PL25.

in all populations. The genotypic frequencies expected under the assumption of random mating also were similar to the observed genotypic frequency and not significantly different, as determined by a *t* test ($P > 0.05$; Table 3). The genetic profiles of cankers sampled in 1994 that were still active in 1995 were compared between years. In all cases, genetic profiles were identical in both years (data not shown).

DISCUSSION

One of the objectives of this study was to determine whether unspermatized cankers of white pine blister rust comprise more than one monokaryotic haplotype of *C. ribicola*. This question is important because it influences the sampling strategy for conducting population studies on *C. ribicola*. The presence of multiple haplotypes within a canker would imply that multiple infections take place in this pathosystem. The results reported here clearly indicate that spermogonia within a canker are clonal and that an unspermatized canker consists of a single genotype. Furthermore, the high level of genetic diversity within each site, combined with the almost complete absence of genetic diversity within cankers, strongly suggests that multiple infections do not occur in this pathosystem.

This is particularly interesting because infection patterns usually are characterized by multiple spots per needle. Cultures derived from these spots have different genetic profiles (8). The results reported here suggest that a selection process takes place between the time of infection and establishment of a single monokaryotic haplotype that forms a canker. One possible explanation for these results is that once a genotype is present in the infection court, it has the ability to exclude others. The mechanism responsible for this exclusion is unknown.

The monomorphism among spermogonia within cankers reported here is in contrast to previous studies in which some genetic variability was reported among spermogonia within cankers of white pine blister and fusiform rusts (5,9). The discrepancy can be explained by the possibility that spermatization had already occurred at the time of sampling in previous studies. In the study reported here, only unopened spermogonia were sampled, thereby avoiding the potential for contamination or outcrossing. The presence of the DNA markers assayed in the spermogonia, aecidiospores, and urediniospores at similar frequencies strongly suggests that they are not sampling artifacts (9,10) (R. C. Hamelin, unpublished data). In addition, Mendelian segregation was reported for at least one of the markers used in our study (8).

The fine structure of the genetic diversity of the spermogonial phase was in sharp contrast to that of the dikaryotic aecidial phase. Seventy percent of the total genetic diversity was present among aecidia within cankers (9), in contrast to the nearly zero percent reported here, but the proportion of genetic differentiation among

cankers within and among sites was similar in this and other studies of white pine blister rust that used aecidia (9,10). The impact of these studies on sampling strategy is important, because the probability of obtaining samples with different DNA markers was high when sampling spermogonia or aecidia from different cankers but increased only slightly when sampling new sites or regions (9). The difference in the fine-level genetic structure of aecidia and spermogonia of blister rust can be explained by the fact that the unspermatized canker represents a monokaryon that vegetatively propagates within the host, whereas aecidia are the result of outcrossing between "resident" monokaryotic spermogonia and "alien" spermatia (8,9,12,28).

Low levels of genetic differentiation among geographically separated pine rust populations have been found, using isozymes, RFLP, and RAPD (8,11,35–37). The data have been interpreted primarily as being the result of interpopulation homogenization by long-distance migration. Evidence of long-distance dispersal for rusts is mostly circumstantial, but some cases are well documented for cereal, bean, and poplar leaf rusts (25,27). Aeciospore deposition gradients in fusiform and western gall rusts were fairly steep but were highly dependent on wind and other meteorological factors (1,29). Disease gradients were rather flat for these rusts, possibly due to the long tail in the dispersal gradient or secondary spread. Whether basidiospores, urediniospores, or aeciospores are responsible for gene flow among *C. ribicola* populations remains to be demonstrated.

Because spermogonia are the indirect result of meiosis on the telial host, it was hypothesized that the *C. ribicola* population structure should be consistent with the assumption of random mating. The values reported here strongly support this hypothesis and are consistent with another study that indicated western populations of *C. ribicola* were in Hardy-Weinberg equilibrium (8), in contrast to studies on some ascomycetes in which observed genotypic diversity values were often smaller than values expected under the assumption of random mating (3,16,17,19,22). The difference can be attributed to the presence of mixed mating systems in ascomycetes, with the asexual and sexual stages often occurring concurrently. In contrast, sexually derived basidiospores can only infect the pine host; therefore, any infection occurring on the pine host is the result of meiosis.

From a practical standpoint, the presence of a single monokaryon within cankers of white pine blister means that a canker can be genotyped by collecting a single unopened spermogonial drop per canker. This would allow large population studies to be conducted and, possibly, exhaustive genotyping of entire *C. ribicola*

TABLE 2. Analysis of molecular variance of spermogonial samples of *Cronartium ribicola* from four white pine plantation populations in Quebec Province

Source	df	Variance	Total variance (%)	Φ Statistic	P^a
Among regions ^b	1	0.167	8.45	$\Phi_{CT} = 0.085$	<0.001
Among populations within region	2	-0.020 ^c	-1.00 ^c	$\Phi_{SC} = -0.011$	0.610
Among cankers within population	39	1.825	92.55	$\Phi_{ST} = 0.075$	0.097 ^d

^a Probability of a larger value obtained by chance.
^b East: CO and SB (two plantations on the Gaspé Peninsula); west: CY (Saint-Cyprien) and PL (Plessisville).
^c Negative molecular variance components are possible when values are not significantly different from zero, because the associated fixation index analogs are correlation coefficients. A negative value indicates that haplotypes drawn from different populations are more related than haplotypes drawn from the same population.
^d The null hypothesis that there is no population structure is not rejected.

TABLE 3. Genotypic diversity and random mating in *Cronartium ribicola* populations from Quebec Province

Site ^a	No. of haplotypes	No. of cankers	$\hat{G}^b$	G^c	p^d	t^e
PL	17	19	16.200	16.721	0.84	0.273 NS
CY	14	15 ^f	13.235	13.802	0.83	0.332 NS
CO	5	5	5.00	...	...	...
SB	5	5	5.00	...	...	...
Total ^h	37	43	32.438	34.434	0.82	0.251 NS

^a Letters represent the site (PL = Plessisville, CY = Saint-Cyprien, CO and SB = two plantations on the Gaspé Peninsula).
^b Observed genotypic diversity.
^c Mean value of genotypic diversity generated by 100 simulations, using observed frequencies under the assumption of random mating.
^d Proportion of simulated distribution with values equal to or larger than the observed genotypic frequency.
^e *t* Statistic calculated as $t = [G - \hat{G}var(G)]$ (23). NS indicates nont significant at 5% level.
^f For the purpose of this analysis, canker CY44 was assumed to be two confluent cankers.
^g Simulations were not performed because the data set was too small.
^h Because haplotypes can occur over more than one site, the total number of haplotypes is not equal to the sum of the haplotypes found in individual sites.

populations in white pine plantations, as well as comparison of the genetic profiles of different spore stages using aecidial sampling (9,10) in addition to basidiospore-derived cultures (8,15). Comparison of genetic profiles will be particularly useful for molecular epidemiology studies of white pine blister rust, for example in investigating the level of outcrossing of individual cankers and studying the spatial relationship of matings.

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