Genetics of *Cronartium ribicola*. IV. Population structure in western North America

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Abstract: Population genetic parameters were estimated for six populations of *Cronartium ribicola* in western North America from British Columbia to the southern Sierra Nevada, and two outgroups from eastern North America, using isozyme, random amplified polymorphic DNA (RAPD) and restriction fragment length polymorphism (RFLP) markers on cultured haploid clones. Diversity was low, with only 8% polymorphism in the 212 markers identified. Each polymorphic locus had only two alleles, except for an RFLP marker in the ribosomal DNA complex with multiple alleles, that resulted from variable numbers of tandem repeats. Expected heterozygosity within populations, estimated from diploid teliospores, was only 0.025. The three types of markers were highly consistent with each other for these parameters. Yet, populations were highly differentiated; the proportion of the total variation attributable to differences among populations was 0.205. Multivariate statistical analysis as well as different clustering algorithms based on contrasting evolutionary assumptions (drift, mutation) all showed similar relationships and differences among populations. Genetic distances were not associated with geographic distances; western populations within a few kilometres of each other were often more distant from each other genetically than they were from eastern populations across the continent. The lack of pattern over the landscape of this metapopulation is consistent with aspects of the life cycle and epidemiological behavior of the pathogen, in which genetic drift appears to play a major role.

Key words: white pine blister rust, isozymes, RAPDs, RFLPs, multivariate analysis, genetic drift.

Résumé : À l'aide de l'analyse isozymique, de l'amplification aléatoire de l'ADN (RAPD) et de marqueurs issus du polymorphisme de la longueur des fragments de restriction (RFLP) obtenus à partir de clones haploïdes en culture, les auteurs ont évalué les paramètres génétiques des populations dans six populations de *Cronartium ribicola* dans l'ouest de l'Amérique du nord, allant de la Colombie canadienne au sud de la Sierra Nevada, ainsi que dans deux groupes externes de l'est de l'Amérique du nord. La diversité est faible, avec seulement 8% de polymorphisme dans les 212 marqueurs identifiés. Chaque lieu polymorphique ne comporte que deux allèles, excepté pour un marqueur RFLP du complexe rADN avec de multiples allèles, résultant d'un nombres variables de répétitions en tandem. L'hétérozygocité attendue dans la population, évaluée à partir de téliospores diploïdes, n'est que de 0,025. Les trois types de marqueur sont fortement congruents les uns avec les autres pour ces paramètres. Cependant, les populations sont hautement différenciées les unes par rapport aux autres; la proportion de la variation totale attribuable aux différences entre les populations est 0,205. L'analyse statistique multivariée ainsi que différents algorithmes de regroupement basés sur des suppositions évolutives contrastées (dérive, mutation) montrent tous des similarités et des différences entre les populations. Les distances génétiques ne sont pas associées aux distances géographiques; les populations de l'ouest, séparées de quelques kilomètres, sont souvent plus distantes les unes des autres génétiquement qu'elle ne le sont des populations de l'est situées à l'autre extrémité du continent. L'absence de patron dans le paysage de cette métapopulation est congruente avec les aspects du cycle vial et le comportement épidémiologique du pathogène, chez lequel la dérive génétique semble jouer un rôle majeure.

Mots clés : rouille du pin blanc, isozymes, RAPDs, RFLPs, analyse multivariée, dérive génétique.

[Traduit par la rédaction]

Introduction

Variation in virulence is the trait of most practical importance in pathogen populations but is often difficult to determine, because it depends on test inoculations of resistant host genotypes, which may not be known. Insight into the potential magnitude of such variation may be gained indirectly, however, by understanding the genetic structure of the pathogen population; that is, the amount, distribution, and maintenance of genetic variation overall, as determined by selectively neutral markers. Population genetic parameters of practical concern to disease management are rates of outcrossing and

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gene flow, because these affect the pathogen's ability to recombine and diffuse new genotypes of wider virulence throughout the population. Such knowledge may be especially important in assessing risks to host populations of introduced pathogens, where founder effects may be important, and where resistance loci and alleles in host populations may be few in number and rare in frequency.

White pine blister rust is a case in point. The pathogen was introduced separately into eastern and western North America around the turn of the century, in both cases on infected eastern white pines (*Pinus strobus* L.) exported from European nurseries for use as planting stock. The western introduction is believed to be from a single shipment of several hundred infected seedlings to a nursery near Vancouver, B.C. (Mielke 1943). From this point, the disease spread southward through the Coast Ranges, Cascades, and Sierra Nevada, and east and south through the Rocky Mountains, often in long-range jumps (Smith 1996). More recently, an isolated outbreak was reported on southwestern white pine (*Pinus strobiformis* Engelm.) in the Sacramento Mountains of New Mexico. The source of this infection is unknown but is at least 1000 km south and 1400 km east of the nearest known infection centers in Wyoming and California, respectively (Hawksworth 1990). All North American white pines are highly susceptible to blister rust, and many have sustained great damage in at least some part of their range. Yet, several resistance mechanisms that exist at low frequencies have been reported in two of the commercial species, sugar pine (*Pinus lambertiana* Dougl.) and western white pine (*Pinus monticola* Dougl.; Kinloch 1982).

Until recently, lack of marker loci has impeded study of the genetic structure of *C. ribicola*. Hamelin et al. (1995) used random amplified polymorphic DNA (RAPD) markers to survey variation in Quebec, and an earlier study in this series used restriction fragment length polymorphism (RFLP) markers at the ribosomal DNA (rDNA) gene complex to describe variation of this rust in British Columbia populations (White et al.1996). These intensive studies of 22 and 24 populations, respectively, showed variation both within and among populations, without any recognizable geographic or other pattern. Because DNA was extracted from aeciospores, a dikaryotic stage of the rust, conclusions were based on phenotypic data of the isolates analyzed. The ability to culture the haploid stage of the fungus (Kinloch and Dupper 1996) subsequently enabled genetic interpretation of the rDNA gene complex and many other molecular markers (Gitzendanner et al.1996). The latter study showed that *C. ribicola* was highly outcrossing and that populations were in genetic equilibrium.

We undertook the present study to obtain an initial, broad profile of genetic variation of blister rust populations in western North America. How much variation did the rust arrive with? Has it since stratified into detectable subpopulations? If so, how are they structured, and what are the dynamics of change? The interval between the rust's northern extention in British Columbia to its present southern limit (excluding New Mexico) in the southern Sierra Nevada of California spans over 17° of latitude, in which changes in climate and alternate host species could act as agents of natural selection. The distribution of the rust is not continuous within this range, however, especially in southern portions, but occurs as a patchwork of infection centers of varying size and intensity over the landscape (Kinloch and Dulitz 1990). Genetic drift could play a major role in substructuring this metapopulation, depending on epidemiological factors and how gene flow is affected by the mating system.

Materials and methods

Sampled populations

Isolates of *C. ribicola* were obtained from infected leaves of *Ribes* spp., the alternate host, from the Coast Ranges in British Columbia to the southern Sierra Nevada, very nearly the northern and southern limits of the distribution of the rust along the western cordillera of the continent. Intermediate points were sampled in the Klamath Mountains of Siskyou County, in northern California, and the central Sierra Nevada in El Dorado County, California. Additionally, isolates from eastern North America (North Carolina and Virginia), representing a different introduction, were included as outgroups.

In the Klamath Mountains, three neighboring subpopulations were sampled. One of these, Happy Camp, was at the site where the race of rust virulent to major gene resistance (MGR) in sugar pine was discovered in a small test plantation (Kinloch and Comstock 1981). Virulence or avirulence to MGR is thought to be controlled by a single gene pair. Another subpopulation was at Thompson Ridge, about 3 km east and about 330 m higher than Happy Camp. Samples at this location were centered around three MGR trees within less than 2 km of each other, two of which had several blister rust infections. The putative allele for virulence was intermediate in frequency here; we suspected it had arrived by recent migration from Happy Camp (Kinloch et al. 1996). The third subpopulation was at Poker Flat, 10 km west of Happy Camp, 650 m higher. Despite its proximity to Happy Camp, we have not detected virulence to MGR in this subpopulation (Kinloch and Dupper 1987; Kinloch et al. 1996) and consider it as wild type.

In most cases, isolates were obtained as mature telia on infected leaves on bushes of different species of *Ribes*. About 20 bushes per site were usually sampled, one leaf each, in late summer or early autumn, after telia had matured. These were used to cast basidiospores onto sugar pine seedlings in dew chambers (Kinloch and Dupper 1987). After needle symptoms (spots) developed, individual spots, widely separated from any others, were excised and placed on special blister rust growth medium to isolate the haploid mycelium in pure culture (Kinloch and Dupper 1996). Recovery approaches 100%. Cultures were then increased rapidly by alternating them between solid and liquid media, maintaining isolate identity. Ample amounts of mycelium were thus available for extraction of enzymes or DNA for marker analyses. Isolates from North Carolina and Virginia were obtained as bulked aeciospore lots from an unknown number of sporulating eastern white pine trees. These were inoculated onto *Ribes nigrum* L. plants to produce basidiospores for inoculation of sugar pine seedlings. A sample of pure haploid isolates were then recovered, as above.

Within populations, numbers of unrelated, haploid isolates sampled were usually low and unequal in number (range 6–19; Table 2). The El Dorado population, sampled previously to study the mating system of the fungus (Gitzendanner et al.1996), was mainly a diploid population of teliospores, whose genotypes were inferred from their segregating basidiospore progeny.

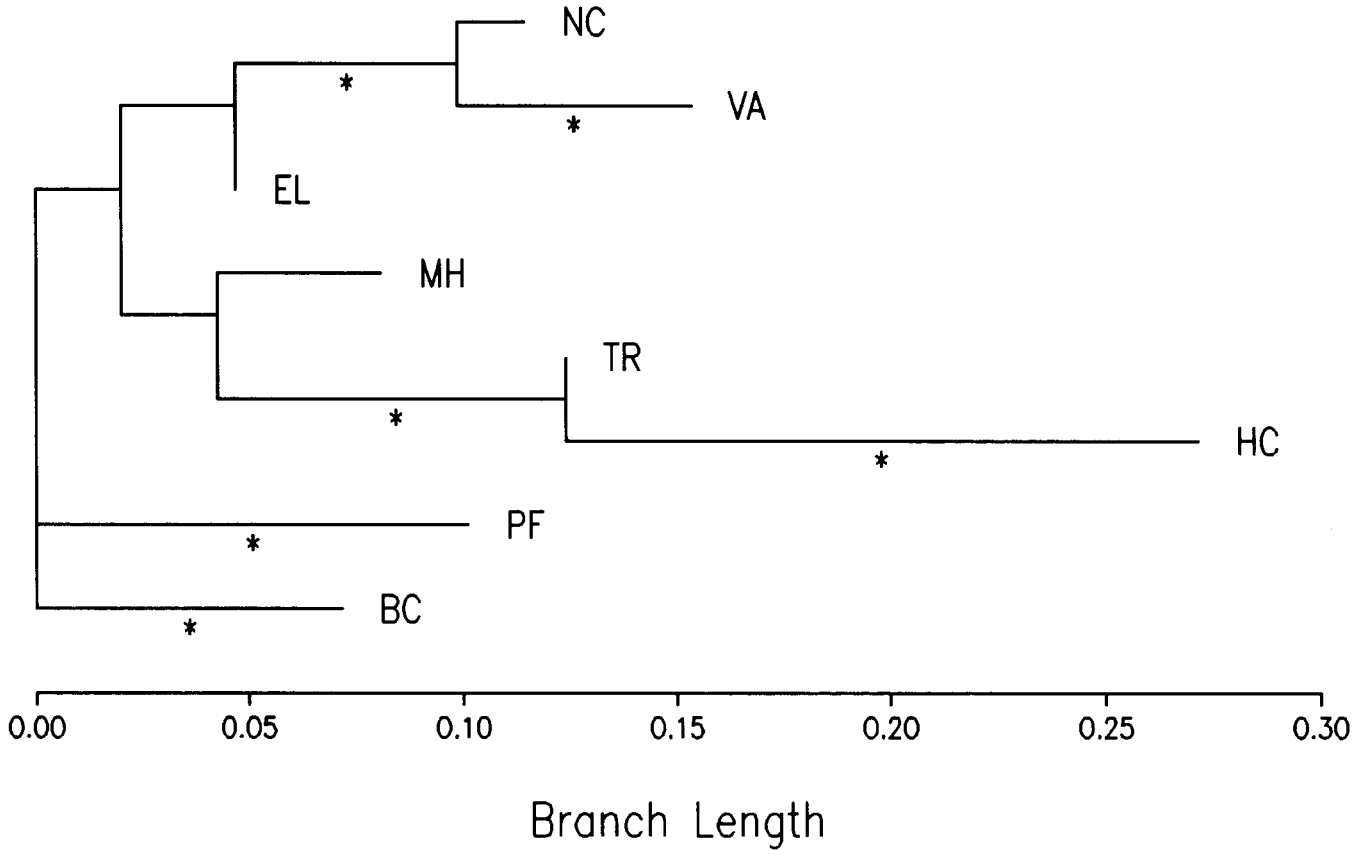
Marker loci

We used isozyme, RAPD, and RFLP loci of known inheritance (Gitzendanner et al.1996) to determine allele frequencies of polymorphic loci in each population.

For isozymes, 21 buffer–stain systems resolved 31 bands, interpretable as 28 loci, three of them polymorphic (Gitzendanner et al.1996). One of the polymorphic loci (CAT 2) was not reproducible in all populations, and was dropped from the population analysis.

The 21 RAPD primers screened amplified 90 reliable bands, of

Fig. 1. Phenetic relationships among eight populations of *Cronartium ribicola,* estimated by restricted maximum likelihood (Felsenstein 1990; see text for further details).



which 10 were polymorphic (Gitzendanner et al.1996). One of these (OPK-13) showed highly distorted segregation and also was omitted from the population analysis.

Of 94 sites identified with different restriction enzymes in the moderately repetitive fingerprint clone (BA 5; Gitzendanner et al. 1996), at least four were polymorphic with two codominant alleles each. Enzyme and DNA extraction and band staining followed conventional protocols, as described previously (Gitzendanner et al. 1996; White et al. 1996).

Analyses

Allele frequencies for each locus and population were processed by BIOSYS (Swofford and Selander 1981) to obtain estimates of genetic parameters and phenetic relationships. We also estimated phylogenetic relationships using the CONTML option in PHYLIP (Felsenstein 1990).

For greater resolution and statistical tests of differences among populations, we employed canonical variate analysis, using allele frequencies at all polymorphic loci as variables. Briefly, this procedure identifies a series of vectors, i.e., linear combinations of variables, such that differences among groups (e.g., populations) are maximized and variation within groups is minimized (Cooley and Lohnes 1971; Dunn and Everitt 1982). The strength of canonical analysis is that vectors may show substantial differences among populations, even if individual alleles do not.

To conduct the analysis on a balanced data set, we first simulated diploid genotypes (teliospore population) at each locus in each population from allele frequencies, using the harmonic mean number of individuals per locus per population. This was valid, because previous analysis (Gitzendanner et al.1996) had shown all polymorphic loci to be in Hardy–Weinberg equilibrium for the single population (El Dorado) studied. We then assigned scores to alleles at each locus according to the algorithm described by Smouse and Williams (1982). This conversion transforms each genotype into an additive value, that in linear combinations over loci approaches multivariate normality as the number of loci increase (Smouse et al. 1982; Westfall and Conkle 1992). In the canonical analysis of these scores, the first vector accounts for the greatest proportion of differences among populations. The next vector (the scores of which are uncorrelated with the first) is aligned in the direction of the next greatest variation, and so on for successive vectors. The number of vectors extracted is equal to the lesser of the number of variables in the analysis or the number of groups minus one (that is, the among-group degrees of freedom). The position of each group along any vector is denoted by canonical scores, which are transformed to a mean of zero and a within-group standard deviation of one. Canonical analysis was conducted by the

Table 1. Molecular marker variation in *C. ribicola.*

Marker type	Loci	P	A	H_e	H_s	G_{ST}
Isozymes	28	11	1.03	0.028	0.372	0.228
RAPDs	90	11	1.04	0.038	0.377	0.205
RFLPs	94	4	1.01	0.013	0.357	0.191
Combined	212	8	1.03	0.025	0.369	0.205

Note: Abbreviations are as follows: P, percent polymorphic loci; A, effective no. of alleles per locus; H_e, expected heterozygosity (including monomorphic loci); H_s, within-population diversity (polymorphic loci only); G_{ST}, coefficient of genetic differentiation.

Table 2. Allele frequencies (with number of samples given in parentheses) of molecular marker loci in eight populations of *C. ribicola*.

	Population[a]							
Locus	BC	PF	HC	TR	EL	MH	VA	NC
Isozymes								
GOT2	0.36 (11)	0.67 (9)	0.25 (12)	0.26 (19)	0.62 (110)	0.53 (15)	1.00 (11)	0.89 (9)
MPI	1.00 (12)	0.44 (9)	0.54 (13)	0.50 (19)	0.69(124)	0.87 (15)	0.18 (11)	0.56 (9)
RAPDs[b]								
D-2_{900}	0.83 (6)	1.00 (6)	0.38 (8)	0.50 (16)	0.69 (34)	0.73 (11)	0.75 (8)	0.50 (8)
D-8_{480}	0.33 (6)	0.83 (6)	0.00 (8)	0.25 (16)	0.61 (34)	0.09 (11)	0.50 (8)	0.50 (8)
D-11_{890}	1.00 (6)	0.83 (6)	0.00 (8)	0.14 (7)	0.88 (8)	0.56 (8)	0.63 (8)	0.57 (7)
D-11_{410}	0.50 (6)	0.17 (6)	0.63 (8)	0.88 (16)	0.72 (34)	0.27 (11)	0.38 (8)	0.71 (7)
K-6_{1050}	0.50 (6)	0.50 (6)	0.38 (8)	0.56 (16)	0.14 (34)	0.46 (11)	0.13 (8)	0.38 (8)
K-7_{850}	0.17 (6)	0.33 (6)	0.88 (8)	0.63 (16)	0.39 (34)	0.36 (11)	0.25 (8)	0.29 (7)
K-19_{1900}	0.71 (7)	1.00 (6)	0.50 (8)	0.81 (16)	0.67 (34)	0.46 (11)	0.75 (8)	0.38 (8)
K-20_{1600}	0.00 (6)	0.00 (6)	1.00 (8)	0.56 (16)	0.14 (34)	0.27 (11)	0.43 (7)	0.14 (7)
K-20_{1450}	0.33 (6)	0.83 (6)	0.25 (8)	0.75 (16)	0.69 (34)	0.73 (11)	0.43 (7)	0.86 (7)
RFLPs[c]								
Pvu 6.0,5.8	0.29 (7)	0.31 (8)	0.55 (11)	0.38 (8)	0.70 (10)	0.56 (9)	0.71 (7)	0.60 (10)
Pvu 8.0,5.0	0.86 (7)	0.44 (8)	0.89 (11)	0.75 (8)	0.40 (10)	0.57 (7)	1.00 (9)	1.00 (10)
Pvu 11.0,9.8	0.43 (7)	0.88 (8)	0.22 (11)	0.88 (8)	1.00 (10)	0.29 (7)	1.00 (7)	1.00 (10)
Eco 6.6,6.3	0.33 (9)	0.22 (9)	0.22 (9)	0.56 (9)	0.50 (10)	0.57 (7)	0.64 (7)	0.80 (5)

Note: Frequency of the most common allele (of two) for isozymes and RFLPs and the amplified band for RAPDs are given. Frequencies are based on haploid counts. Sample numbers are for haploid genotypes.

[a]BC, British Columbia; PF, Poker Flat; HC, Happy Camp; TR, Thompson Ridge; EL, El Dorado; MH, Mountain Home; VA, Virginia; NC, North Carolina.

[b]Alphanumeric codes are primer designations; subscripts are approximate molecular weights (bp) of amplified bands.

[c]Probes of genomic DNA digested with restriction enzyme indicated. The values given with the enzymes are fragment lengths (kb) of alternate alleles.

Table 3. Chord distances (Cavalli-Sforza and Edwards 1967) between pairs of eight populations of *C. ribicola*, average chord distance (*D*), and expected within-population heterozygosities (H_e) for each population (A) and canonical scores (CS) for the first three vectors of each population (B).

(A) Chord distances and expected heterozygosities

	BC	PF	HC	TR	EL	MH	VA	NC	*D*	H_e
BC	—	0.267	0.435	0.322	0.230	0.224	0.344	0.307	0.304	0.024
PF		—	0.484	0.314	0.240	0.290	0.296	0.311	0.314	0.022
HC			—	0.252	0.384	0.295	0.397	0.398	0.378	0.022
TR				—	0.226	0.276	0.278	0.229	0.271	0.028
EL					—	0.202	0.214	0.171	0.238	0.024
MH						—	0.299	0.296	0.269	0.029
VA							—	0.170	0.285	0.024
NC								—	0.269	0.025
Mean									0.291	0.025

(B) Canonical scores

	BC	PF	HC	TR	EL	MH	VA	NC
CS1	–5.49	4.66	–8.60	–5.51	4.94	1.95	1.69	3.37
CS2	–6.03	1.27	1.14	3.18	0.49	–3.88	2.70	–0.51
CS3	–2.51	–1.42	2.67	–1.43	0.79	2.12	–0.62	–1.25

[a]BC, British Columbia; PF, Poker Flat; HC, Happy Camp; TR, Thompson Ridge; EL, El Dorado; MH, Mountain Home; VA, Virginia; NC, North Carolina.

SAS procedure CANDISC. Orthogonal comparisons between populations or population groups of specific interest, chosen a priori, were conducted with PROC GLM, using the MANOVA and CONTRAST options (SAS Institute 1985).

Results

Most of the 212 marker loci identified were monomorphic. The proportion of polymorphic loci was similar for the three types of markers: 6% for RFLPs and 11% each for isozymes and RAPDs (average for all loci combined, 8%; Table 1). Expected heterozygosities were also low, but had a greater range, from 1.4% for RFLPs to 3.7% for RAPDs (combined mean 2.5%). With the exception of the rDNA gene cluster, all polymorphic loci had only two alleles. All were at intermediate

Fig. 2. Distances in multivariate space among eight populations of *Cronartium ribicola* along the first three canonical vectors, representing 65, 20, and 7% of the total variation, respectively. Elipsoids are one standard deviation in diameter. Shadow projections on floor and walls of box enable comparison of population differences along any pair of vectors.

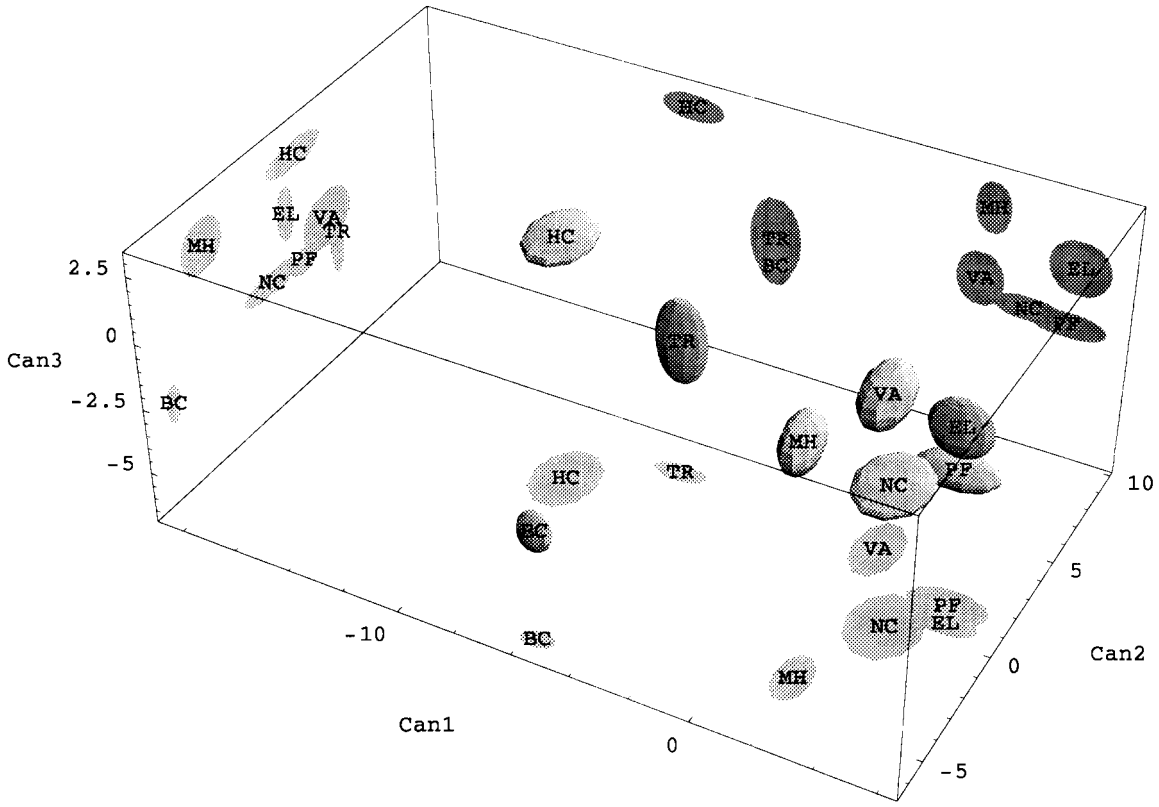


Table 4. Canonical variate analysis of molecular marker loci in western North American populations of *C. ribicola*.

Vector or comparison	Eigenvalue[a]	R^2	% of trace
1	31.73	0.97	65
2	9.61	0.91	20
3	3.46	0.78	7
4–7	4.29	—	8
Total (trace)	49.08	—	100
Orthogonal comparisons			
HC vs. TR[b]	2.79	0.74	6
(HC + TR) vs. PF	12.58	0.93	26
West vs. East	1.87	0.65	6

[a]Eigenvalues of $\mathbf{BW}^{-1}$, where **B** is the among population sums of squares and cross-products matrix, and $\mathbf{W}^{-1}$ the inverse of the within-population sums of squares and cross-products matrix, sum to the trace.

[b]West, BC, PF, EL, MH; East, VA, NC. Location codes are given in Table 2.

frequencies. No private alleles were detected in any population.

Allele frequencies of the 15 polymorphic loci used showed great variation among the eight populations (Table 2). Genetic distances among populations were estimated by both Nei's (1978) unbiased genetic distance and Cavalli-Sforza and Edward's (1967) chord distance. Both parameters gave similar relative measures among populations, but we chose the latter to represent results, because it is based on a drift model of population differentiation (as opposed to a mutation model; Weir 1990). Distances were often great, ranging from 0.484 (between Happy Camp and Poker Flat) to 0.170 (between the two eastern populations, Virginia and North Carolina; Table 3). No pattern was evident, however; for example, North Carolina was almost as close to Happy Camp (0.171) as it was to Virginia. Happy Camp had the greatest average genetic distance to any other population (0.378), and El Dorado had the least (0.238).

Different clustering algorithms were used to characterize phenetic relationships. The unrooted tree estimated from the restricted maximum likelihood method (PHYLIP; Felsenstein 1990) shows Poker Flat and British Columbia closest to the stem base and Happy Camp furthest, with Thompson Ridge branching at the same node as Happy Camp (Fig. 1). Virginia and North Carolina also branched at the same node. The distance Wagner procedure (Swofford 1981; Swofford and Selander 1981), using the Cavalli-Sforza chord distance as coefficient, and the unweighted pair-group (UPGMA) method, using Nei's unbiased genetic distance as coefficient, showed similar relationships (not shown): Happy Camp and Thompson Ridge always paired together and furthest from other populations.

In multivariate analyses, the first three canonical vectors (of a total of seven identified) accounted for 65, 20, and 7%, respectively, of the total difference (i.e., the trace) among

populations. Corresponding R^2 values were 0.97, 0.91, and 0.78. Canonical scores for each population mean in the first three vectors are listed in Table 3 and displayed graphically in three-dimensional space in Fig. 2.

The salient feature of these data is the relative isolation of the Happy Camp population on the first canonical vector. Happy Camp was significantly different statistically from other populations, including its nearest neighbors in the Klamath Mountains at Poker Flat, about 9 km west, and Thompson Ridge, only 3 km east (Fig. 2). Happy Camp and Thompson Ridge, the only populations with high to intermediate frequencies of the putative gene virulent to major gene resistance in sugar pine (Kinloch and Dupper 1987; Kinloch et al. 1996), were also significantly different, as a group, from Poker Flat (Table 4). No coherent geographic or other pattern was discernable among other populations. The second vector separated Happy Camp and Thompson Ridge from each other and all the rest, and British Columbia and Mountain Home from all the rest; the third vector separated Poker Flat from the rest (Fig. 2). Western populations (excluding Happy Camp and Thompson Ridge) did differ significantly from the two eastern outgroups (Virginia, North Carolina), but eigenvalues for these contrasts were smaller than those among the neighboring Klamath populations and accounted for only about one sixth of the overall differences among populations (Table 4). All comparisons among population groups were statistically significant at $p \leq 0.0001$.

Discussion

From this analysis of over 200 loci, including structural genes (isozymes) and molecular markers (RAPDs, RFLPs) we conclude that genetic variablity in North American populations of *C. ribicola* is low. Only 8% of loci were polymorphic, with only 1.03 effective alleles per locus. Expected heterozygosity (H_e) was only 2.5%. Yet, population differentiation was substantial; the proportion of genetic diversity attributable to differences among subpopulations (G_{ST}) was 0.205. The different marker types were consistent with each other for all of these parameters. Few data from studies of other fungi are available for comparison, partly because interest often focuses on measuring genetic differences among populations, and polymorphic loci are usually the only ones selected for study. For plant taxa as a whole, comparable statistics among populations for isozyme diversity are 34.2% polymorphic loci, with 1.15 effective alleles per locus and expected heterozygosity of 11.3% (Hamrick and Godt 1990).

Both cluster and multivariate statistical analysis indicated significant differentiation among populations, but the distribution of this variation lacked any pattern associated with geographic distance. For example, greater differences existed among western populations only a few kilometres from each other than between western and eastern populations separated by the continent. These results are similar to two other recent surveys of more regional scope but greater sampling intensity. Hamelin et al. (1995) found significant differentiation without geographic pattern among 22 subpopulations in Quebec ($F_{ST} = 0.114$; cf. our $G_{ST} = 0.205$) based on seven putative polymorphic loci (RAPDs) in dikaryotic aeciospores. Our earlier study of RFLP variation at the rDNA gene cluster in aeciospore collections from 24 populations in British Columbia also showed an essentially random distribution of variation (White et al.1996). Taken together, these surveys are extensive and portray a genetically fragmented metapopulation.

This lack of pattern suggests a population that is not in equilibrium, where founder events are frequent or even predominant. It is consistent with important phases of the life cycle and epidemiological behavior of the pathogen. The blister rust epidemic has not stabilized yet in many areas of western North America, and the distribution and intensity of infection is not uniform, especially in the Cascades, Klamath Mountains, and Sierra Nevada. Areas of intense, light, and no infection exist in a mosaic pattern over the landscape (Kinloch and Dulitz 1990). Long- and intermediate-range spread is most probably effected by aeciospores, produced on pines in the spring. Urediniospores might also migrate longer distances to establish new infection centers (Hamelin et al. 1995), but we consider this less likely. These spores tend to clump together in their pustules on the underside of *Ribes* leaves and are less adapted to long distance spread than aeciospores. Aeciospores are windborne and hardy, with documented jumps of up to 720 km from their source to initiate new infection centers on alternate hosts in *Ribes* (Mielke 1943; Smith 1996). Founders, representing a reduced sample of a source gene pool, are likely to arrive in solitary or small numbers. These genotypes are increased clonally and exponentially in the urediniospore stage during summer. But if conditions are not conducive for infection of pine in late summer or autumn (or if pine hosts are not available), this population becomes extinct.

Although highly outcrossing (Gitzendanner et al. 1996), gene flow is restricted in *C. ribicola* by the relative immobility of pycniospores (spermatia), the presumed gametes. These haploid spores are produced on pine shoots in a sticky nectar, and vectored to potential mates (other pycnia of compatible mating type) by small insects of limited foraging range (Hunt 1985). The meiotic products of these natural matings, haploid basidiospores, appear three spore stages later on *Ribes* and are responsible for initiating the disease cycle on pine. Basidiospores are windborne but thin walled and vulnerable to light and dessication; so they also have a limited range (usually only a few hundred metres), except under unusual meterological conditions (Van Arsdel 1967).

The above scenario helps to explain the apparent paradox of high outcrossing coupled with high population differentiation (with implied low gene flow). It may be more applicable to early phases of an epidemic in a new region (Kinloch and Dulitz 1990). Later, as infection centers begin to coalesce and local neighborhoods overlap, population substructuring may diminish. Population differentiation in the chestnut blight pathogen (*Cryphonectria parasitica* (Murrill) Barr), introduced into North America around the same time as blister rust, is nearly the same as for *C. ribicola* ($G_{ST} = 0.20$ and 0.21, respectively), and also was attributed to restricted gene flow in concert with genetic drift (Milgroom and Lipari 1995). In this case, however, a slightly different dynamic was operating; after virtually complete colonization of the host throughout its range, isolation of the pathogen into subpopulations was thought to have resulted from decimation of the host into patches. The importance of stochastic processes on pathogen population structure, including host patchiness, pathogen dispersal, extinction, and genetic drift were emphasized by

Burdon et al. (1989) and Jarosz and Burdon (1991). All of these factors are evident for *C. ribicola*.

The effect of genetic drift is most evident in the Happy Camp population, which stood alone from all others, including neighbors only 3 and 9 km distant. This small deme, persisting on a few hundred planted resistant sugar pines, is thought to have arisen by natural selection of a mutant race of rust with specific virulence to MGR (Kinloch and Comstock 1981). Because MGR exerts absolute selectivity on haploid basidiospore inoculum, any rust on sugar pines with this gene is of the virulent race, by definition. But accompanying neutral loci would be sampled randomly from the wild-type background (which we assume is represented by Poker Flat, on a ridge in a line of sight only 9 km west of Happy Camp).

The virulent race has so far remained virtually confined to this small area (Kinloch and Dupper 1987), except for a recent appearence on Thompson Ridge, downwind about 3 km east of Happy Camp and 330 m higher (Kinloch et al. 1996). Here, as far as we can tell, it exists on three mature MGR sugar pine that support several infections. On *Ribes*, measured frequency of the virulent race within a 25–50 m radius of these trees has fluctuated from <0.01 to 0.89 in the 8 years since its detection, reflecting the oscillations of a nascent founder population. Beyond about 1 km from the infected sugar pines, it is not detectable (Kinloch et al. 1996, and unpublished). Its derivation from the Happy Camp deme is suggested by relative proximity in both geographic and multivariate space (Fig. 2).

In spite of significant differences in gene frequencies among North American subpopulations of *C. ribicola* (Hamelin et al. 1995; White et al.1996; this study), there is no reason to suspect that all do not come from the same gene pool. No private alleles were found in any of the subpopulations. The low overall variability and basic genetic similarity reflect the epidemiological unity that has characterized the spread of this disease in Europe and North America. Following its arrival and discovery in the Baltic port city of Riga in the middle of the last century, blister rust took only about three decades to sweep across Europe (Spaulding 1929). The way had been prepared by extensive planting of non-native eastern white pine since the early eighteenth century and widespread cultivation of European black currant (*Ribes nigrum* L.). Subsequent introduction of the disease to both coasts of North America around the turn of this century on infected eastern white pine seedlings from European nurseries is well documented. The data (Hamelin et al. 1995; White et al. 1996; this study) show that the amount of variability arriving with these immigrant populations was low and has since become fragmented.

Our results cannot be extrapolated directly to the amount of variability in virulence that might exist, but the implications of low variability in the genome as a whole are encouraging for disease management programs based on resistance breeding, and are consistent with the limited evidence available. On *Ribes*, strong and apparently separate resistance mechanisms in cultivars of red and black currants have never been overcome in extensive field trials across North America and Europe or in repeated attempts to do so in artificial inoculations (Hahn 1949*a*, 1949*b*). On white pines, only two rust races with wider virulence to known or putative resistance mechanisms of white pines have been positively identified. One bears specific virulence to major gene resistance in sugar pine (Kinloch and Comstock 1981), the other to undescribed resistance mechanisms in western white pine (McDonald et al. 1984). Other races have been hypothesized (Hoff and McDonald 1993), but convincing evidence is lacking.

Continued mortality of North American white pines from the current blister rust epidemic is inevitable, but some genetic tools to mitigate this damage are being sought and gradually developed. The greatest threat to these efforts may be reintroduction of the disease from Asiatic sources. Asia is the presumed gene center of *C. ribicola*, where it exists within a poorly understood complex of forma speciales having different alternate host specificities, as well as pine-to-pine species lacking alternate hosts (reviewed in Millar and Kinloch 1991). These populations may be assumed to harbor more genetic variation generally, as well as wider virulence.

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