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Effect of white pine blister rust (*Cronartium ribicola*) and rust-resistance breeding on genetic variation in western white pine (*Pinus monticola*)

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Abstract Western white pine (*Pinus monticola*) is an economically and ecologically important species from western North America that has declined over the past several decades mainly due to the introduction of blister rust (*Cronartium ribicola*) and reduced opportunities for regeneration. Amplified fragment length polymorphism (AFLP) was used to assess the genetic variation in northern Idaho populations of western white pine (including rust-resistant breeding stock) in relation to blister rust. A total of 176 individuals from four populations was analyzed using 163 AFLP loci. Within populations, an average 31.3% of the loci were polymorphic (*P*), and expected heterozygosity (H_e) was 0.123. Genetic differentiation values (G_{st}) showed that 9.4% of detected genetic variation was explained by differences among populations. The comparison between the rust-resistant breeding stock and a corresponding sample derived from multiple natural populations produced similar values of *P* (35% vs. 34.4%) and H_e (0.134 vs. 0.131). No apparent signs of a genetic bottleneck caused by rust-resistance breeding were found. However, a comparison of two natural populations from local geographic areas showed that the population with low pressure from blister rust had higher polymorphism and heterozygosity than the population that had experienced high mortality due to blister rust: *P* (30.7% vs. 25.1%) and H_e (0.125 vs. 0.100), respectively. In addition, the population from low blister-rust pressure had twice as many unique alleles as the blister rust-selected population. The genetic distance and Dice's similarity coefficients among the four populations indicated that the local population that survived high blister-rust pressure was genetically similar to the rust-resistant breeding stock.

Keywords AFLP · Breeding stock · Genetic variation · White pine blister rust

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Introduction

For centuries, western white pine (*Pinus monticola*; WWP) dominated the moist forest ecosystems of the northern Rocky Mountains. Rapid growth, regeneration ability, and wood quality make this species highly desirable throughout its range. Unfortunately, populations of WWP have declined precipitously in the last 70 years for a variety of reasons, foremost among them was the introduction of blister rust (caused by *Cronartium ribicola*). The introduction of blister rust to the west coast of North America in the early 1900s created a disease epidemic across most of the range of WWP (McDonald and Hoff 2001).

From 1950 to 1975, a tree improvement program was implemented to utilize naturally occurring resistance to increase the level and stability of resistance in planting stock derived from seed orchards (Bingham 1983; Fins et al. 2002). WWP mortality varies among localities, and rust-resistant WWP phenotypes were selected from areas of high hazard/mortality for a breeding program in northern Idaho (Bingham 1983). Because blister rust requires two hosts (five-needle pines and *Ribes* plants) to complete its life cycle, the number of *Ribes* plants was used as one indicator of the degree of rust hazard in WWP stands (McDonald 1979).

The effects of this rust-resistance breeding program on genetic variation are completely unknown. Some tree populations that have suffered dramatic reduction in genetic diversity may be more vulnerable to environmental stress or pest-related reduction in overall health, productivity, and fitness (Bergmann and Scholz 1987; McDonald et al. 1998; Müller-Starck 1985; Raddi et al.

1994). The impacts of blister rust on the genetic structure of local WWP populations also must be determined to assess local adaptation for WWP restoration programs. The possibility of potential genetic bottlenecks or erosion of genetic diversity has been evaluated for a few forest-tree species under different bottleneck scenarios (Bergmann and Ruetz 1991; Cheliak et al. 1988; Knowles 1985; McDonald et al. 1998; Stoehr and El-Kassaby 1997). However, the impact of white pine blister rust and other selection pressures has not been evaluated for WWP.

In the first of a series of analyses, we employed amplified fragment length polymorphisms (AFLPs) to study how the genetic resources of WWP have been impacted by blister rust in WWP populations and the rust-resistance breeding program. To our knowledge, this is the first time that AFLPs have been applied to population genetic studies of a five-needle pine species.

The objectives of this study were to: (1) assess the genetic variation of local WWP populations from high or low blister rust-hazard areas and (2) estimate the genetic variation of composite populations that form a genetic resource for regeneration efforts – F_2 progeny from the rust-resistant breeding stock, and a corresponding sample derived from multiple natural populations representing pre-blister rust conditions. These comparisons should help assess the effects of natural and artificial selection pressure or local adaptation on genetic variation in WWP.

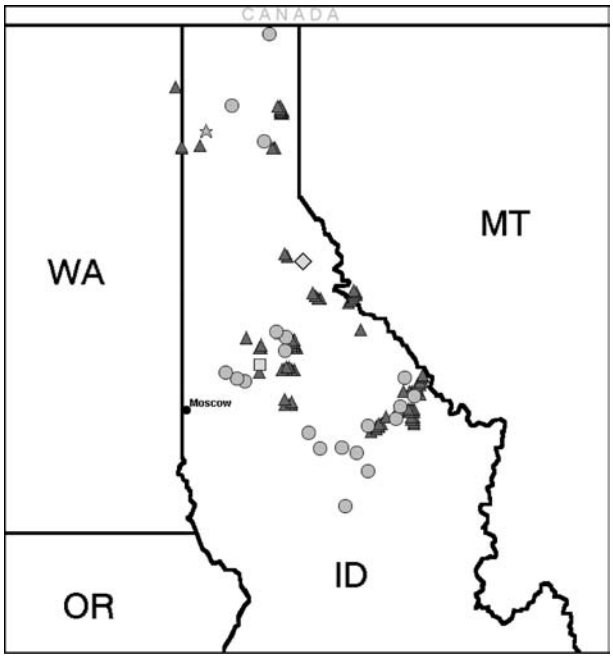

Fig. 1 Geographic origins of western white pine populations: *solid circle* Natural Variation Planting Sources (NVP) (pre-blister rust), *solid triangle* resistant parent materials for White Pine Seed Orchard (WPSO) (natural and artificial rust selection), *solid square* Emerald Creek (EmCr) (high rust hazard), and *solid diamond* Moon Saddle (MoSa) (low rust hazard)

Materials and methods

Plant materials

A total of 176 WWP individuals from four populations were analyzed in this study.

Natural Variation Planting (NVP)

A total of 49 trees were sampled from a planted stand in the Priest River Experimental Forest in northern Idaho (Fig. 1) (Steinhoff 1979). This planting was originally designed and established in 1971 for studying natural variation of WWP in Idaho. Open-pollinated seed from 225 trees was used to establish this stand (Steinhoff 1979). It is comprised of individual seeds collected in 1962 from low rust-hazard areas throughout the range of WWP in Idaho (Fig. 1). Because the NVP is located in a low rust-hazard area, almost all planted WWPs have survived. Therefore, this plantation represents a sample of the original genetic variation of WWP before it was affected by white pine blister rust.

White Pine Seed Orchard (WPSO)

In the 1950s, phenotypically rust-resistant WWP trees were identified at the early infection centers in high rust-hazard areas throughout the range of WWP in Idaho (Fig. 1). These rust-resistant phenotypes, which occurred at a frequency of approximately 0.0001 (McDonald and Hoff 1982), were used to produce seedlings (F_1) from selected crosses (Bingham 1983). F_1 seedlings were inoculated and screened for rust resistance. The WPSO in Moscow, Idaho was established in 1957 and is composed of rust-resistant, F_1 progeny from parents that displayed a general combining ability for rust resistance. Approximately 177 unique parents contributed as either female, male, or both in the crosses to produce F_1 material represented in the WPSO. Out of the 177 unique parents, 21 parents were greatly over represented in the F_1 progeny used in the WPSO. A sample of 120 F_2 seeds (bulked) was obtained from the 1991 seed harvest at the WPSO. Seeds were germinated, and 43 seedlings were randomly chosen for this study. This synthetic (composite) population represents trees that have undergone a potential breeding bottleneck, and natural and artificial selection pressure from blister rust.

Emerald Creek (EmCr) and Moon Saddle (MoSa)

Needle samples from a total of 84 trees were collected from sites near Clarkia (EmCr; 41 individuals), Idaho and Kellogg (MoSa; 43 individuals), Idaho to represent surviving natural populations after decades of heavy (EmCr) and low (MoSa) selection pressure from blister rust (Fig. 1). We categorized the level of rust hazard (heavy and low) based on tree mortality caused by blister rust. Mr. R.T. Bingham (personal communication) estimated and recorded the density of 5,000 WWP/ha at the EmCr site in the 1950s. This estimate is supported by historical pictures of the EmCr site that show a high WWP density in the 1950s. The number of WWP at the EmCr site decreased dramatically in recent years due to blister rust. This area currently contains 5.6 WWP/ha because less than 1% of WWP have survived under blister rust pressure. In contrast, the MoSa population exhibits few signs of mortality from blister rust, and this site currently contains approximately 400 WWP/ha. We sampled trees from both populations over a 12-ha area. The materials from each population represent pre- (MoSa) and post- (EmCr) blister rust selection because (1) they were naturally established before blister rust introduction into these areas, and samples were collected only from trees that were at least approximately 90 years old (i.e., the age of the MoSa stand is approx. 120 years; EmCr stand is approx. 90 years old, and blister rust was in-

troduced to northern Idaho approx. 75 years ago), and (2) two contrasting rust hazard levels (low and high) created different selection pressures on each population.

DNA extraction

Approximately 100 mg fresh or 20 mg freeze-dried WWP needles were used for DNA extraction. DNA was extracted and purified from all samples using Qiagen DNA extraction kits following the protocol of the manufacturer (Qiagen, Valencia, Calif.). DNA was quantified for AFLP analysis using a Hoefer TKO100 Fluorometer (Hoefer Scientific Instruments, San Francisco, Calif.).

AFLP analyses

The AFLP analyses were performed following the protocol of Vos et al. (1995). For the restriction digests, 500 ng genomic DNA was digested with *Eco*RI and *Mse*I to serve as the template. Resulting DNA fragments were ligated to adapters and diluted 1:10 with sterile, distilled water prior to pre-amplification. We performed pre-amplification with two-base extensions (E-AC and M-CC) instead of the typical single-base extension (E-A and M-C) primer combinations (Costa et al. 2000; Remington et al. 1999; Travis et al. 1998). With conifers, improved AFLP data have been obtained with extension of pre-amplification primer combinations (Costa et al. 2000; Remington et al. 1999; Travis et al. 1998) because of the large genome sizes (20–30 pg/C, depending on species) (Wakamiya et al. 1993). Pre-amplification reaction mixtures (total 20 µl) contained 4 µl of diluted restriction/ligation mixture as template, 10× polymerase chain reaction (PCR) buffer (Applied Biosystems, Foster City, Calif.), 25 m*M* $MgCl_2$, 2.5 m*M* dNTPs, 300 n*M* of +2 primers (E-AC and M-CC), and 1 U Ampli*Taq* DNA polymerase (Applied Biosystems). Fluorescent dye-labeled *Eco*RI (+3 primer; E-ACG) and unlabeled *Mse*I (+4 primers; M-CCAG, M-CCAT, and M-CCTA) primers (IDT DNA, Coralville, Iowa) were used for the selective amplification. For selective amplification, reaction mixtures (total 10 µl) contained 3 µl of diluted, pre-amplification products [1:40 with low TE buffer (10 m*M* Tris–HCl, 0.1 m*M* EDTA, pH 8.0)] as a template, 10× PCR buffer (Applied Biosystems), 25 m*M* $MgCl_2$, 2.5 m*M* dNTPs, 30 n*M* of +3 E primer, 125 n*M* of +4 M primers, and 1 U of Ampli*Taq* Gold polymerase (Applied Biosystems). Amplifications were performed following the method of Remington et al. (1999) with a MJ PTC-200 thermal cycler (MJ Research, Waltham, Mass.).

Selective amplification products were separated in 5% Long Ranger gels (FMC, Rockland, Me.) using an ABI 377 DNA automated sequencer (Applied Biosystems). Samples were prepared by mixing 1.5 µl of the selective amplification products with 1.3 µl of loading buffer mix containing GeneScan 500-Tamra internal standard, blue dextran/25 m*M* EDTA, and deionized formamide (Applied Biosystems). Samples were heated at 95°C for 4 min and immediately chilled on ice. Gels were run at 3,000 V for 3 h using virtual filter set "C".

Gels were tracked and extracted using GeneScan 3.1 (Applied BioSystems), and genetic markers were analyzed using Genotyper 2.5 (Applied BioSystems). Genotyper 2.5 was used to identify peaks with a fluorescent intensity greater than the threshold value (approx. 300 units) in at least one sample. Categories were made from these identified peaks for the scoring of all samples. AFLP bands were scored as present or absent.

Data analyses

We analyzed AFLP data based on both allele and phenotypic frequencies. For allele frequencies, data matrices were analyzed using TOOLS FOR POPULATION GENETIC ANALYSES (TFPGA; Miller 1997) with the assumption that the populations were in Hardy-Weinberg equilibrium. The AFLP data were treated as dominant markers in this study, since the identity of homozygotes/heterozygotes cannot be established unless breeding or pedigree studies are carried out to determine inheritance patterns of each band. With dominant loci, the variance at a single locus is increased, thereby introducing bias into the frequency measure (Lynch and Milligan 1994). We applied Lynch and Milligan's (1994) Taylor expansion estimate to circumvent this problem for calculating allele frequencies. Nei's (1978) unbiased heterozygosity was calculated from allele frequencies derived from Taylor expansion estimates and sample number. Percentage of polymorphic loci (*P*), expected heterozygosity (H_e) (Nei 1978), and genetic differentiation (G_{st}) (Weir and Cockerham 1984) were calculated as measures of genetic variation. Nei's (1978) unbiased genetic distances were also calculated by TFPGA from the allele frequency data, and distances were graphically displayed by unweighted paired-group method with arithmetic means (UPGMA). We also used a Chi-square contingency analysis to determine whether a significant difference in the relative frequencies of fixed and polymorphic fragments existed among the four populations. For phenotypic frequencies, data matrices were analyzed with POPGENE 1.32 (Yeh et al. 1997). The degree of phenotypic polymorphism was quantified using Shannon's index (I_s) of phenotypic diversity. We calculated average diversity over the four different populations ($I_{s\ pop}$) and the diversity in all populations together ($I_{s\ sp}$). Then, the proportion of diversity present within populations, $I_{s\ pop}/I_{s\ sp}$, could be compared with that between (or among) populations, $(I_{s\ sp} - I_{s\ pop})/I_{s\ sp}$. Genetic similarity was calculated as $GS_{xy} = 2a/[2a + b + c]$, where a is the number of bands common for sample x and y, b is the number of bands present only in sample x, and c is the number of bands present only in sample y (Dice 1945) using NTSYS-pc (Rohlf 1992).

Table 1 Primer combinations (showing the three and four selective nucleotides only) used to produce AFLP during selective amplification of DNA from western white pine (n = 176 individuals)

	Primer combination[a]			Totals
	1	2	3	
*Eco*RI	ACG	ACG	ACG	
*Mse*I	CCAG	CCAT	CCTA	
Number of polymorphic loci	17	30	22	69
Number of monomorphic loci	30	37	27	94
Totals	47	67	49	163

[a] Selective extension only. See Vos et al. (1995) for complete primer sequences

Results

Characterization of AFLP markers

We screened 72 selective primer combinations using seven samples. Among several promising selective primer combinations, we chose three (E-ACG/M-CCAG, E-ACG/M-CCAT, and E-ACG/M-CCTA) that produced the most manageable bands number and interpretable banding pattern. Analysis of these selective primer combinations yielded a total of 163 presumptive loci in 176 individuals from four populations (Table 1), of which 69 (42%) loci were polymorphic (Table 1). Amplified polymorphic fragments ranged in size from 76 bp to 280 bp. Each of the 176 sampled individuals had unique AFLP phenotypes.

Table 2 Genetic variability identified with analysis of 163 AFLP markers in four populations of western white pine

Population[a]	P[b]	H_e[c]	I_s[d]	Dice's similarity coefficients[e] (GS)
NVP (n = 49)	34.4	0.131	0.188	0.909
WPSO (n = 43)	35.0	0.134	0.197	0.919
EmCr (n = 41)	25.1	0.100	0.143	0.928
MoSa (n = 43)	30.7	0.125	0.177	0.902
			$I_{s\ pop}$	
Mean	31.3	0.123	0.176	
All populations	P	H_e	$I_{s\ sp}$	
	42.3	0.128	0.198	

[a] EmCr, Emerald Creek; NVP, Natural Variation Planting; WPSO, White Pine Seed Orchard; MoSa, Moon Saddle
[b] Percentage of polymorphic loci (a locus was considered polymorphic if more than one allele was detected)
[c] H_e, Expected heterozygosity (unbiased estimate) (Nei 1978)
[d] I_s; Shannon's index of phenotypic diversity
[e] Coefficients of Dice's similarity are given as the mean value of within population

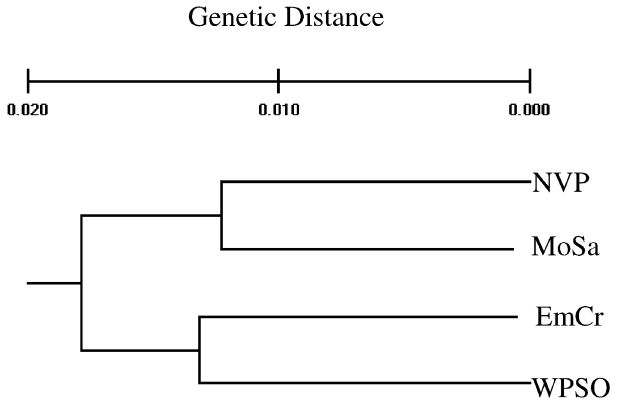

Fig. 2 UPGMA cluster of western white pine populations based on Nei's (1978) standard estimates of genetic distances. *EmCr* Emerald Creek, *NVP* Natural Variation Planting, *WPSO* White Pine Seed Orchard, *MoSa* Moon Saddle

Genetic diversity

On average, 31.3% of AFLP loci were polymorphic (P), and an expected heterozygosity (H_e) was 0.123 (Table 2). Percentage polymorphic loci at the population level ranged from approximately 25% to 35% (Table 2). Although the EmCr population (P = 25%, H_e = 0.100) appeared to have a lower level of genetic diversity than the NVP (P = 34%, H_e = 0.131), WPSO (P = 35%, H_e = 0.134), and MoSa (P = 30.7, H_e = 0.125) populations, no statistically significant differences in the proportions of polymorphic fragments were demonstrated among these four populations ($\chi^2 = 3.98$, $df = 3$, $P > 0.26$). The EmCr population also exhibited the lowest within-population Shannon index of phenotypic variability (I_s = 0.143) compared to the NVP (I_s = 0.188), WPSO (I_s = 0.197), and MoSa (I_s = 0.177) populations (Table 2).

Genetic differentiation among populations

Of the 69 polymorphic AFLP loci evaluated, seven alleles were unique to NVP, six unique to WPSO, two unique to EmCr, and one unique to MoSa. One allele in locus 39 was found only in the NVP (allele frequency 0.87) and MoSa (allele frequency 0.86) populations. When only the two local natural populations were compared MoSa had twice (16) as many unique alleles as EmCr (8). In contrast, WPSO and NVP, the two composite populations assembled from multiple sites (Fig. 1), had a similar number of unique alleles (10 and 9, respectively).

The genetic differentiation value (G_{st}) showed that 9.4% of the detected genetic variation was explained by differences among populations. The EmCr and MoSa populations showed the most differentiation (G_{st} = 0.125) (Table 3). Shannon's index provided a similar estimate of the distribution of genetic variation with an average 89% ($I_{s\ pop}/I_{s\ sp}$) distributed within and 11% [$(I_{s\ sp} - I_{s\ pop})/I_{s\ sp}$] among populations.

Genetic distances and relationships among populations

Genetic distances (Nei 1978) among populations were small and ranged from 0.0107 between NVP and MoSa to 0.0181 between EmCr and MoSa, with an average value of 0.0146 (Table 3). A cluster analysis disclosed two groups of populations (Fig. 2). The first one included populations from EmCr and WPSO, whereas the sec-

Table 3 Genetic differentiation among four populations of western white pine

Population comparisons[a]	Nei's unbiased genetic distance[b]	Dice's similarity coefficients[c] (GS)	G_{st}[d]
NVP/WPSO	0.0141	0.903 (0.818–0.984)	0.085
NVP/EmCr	0.0176	0.907 (0.814–0.976)	0.118
NVP/MoSa	0.0107	0.898 (0.804–0.906)	0.067
WPSO/EmCr	0.0116	0.917 (0.842–0.976)	0.081
WPSO/MoSa	0.0152	0.897 (0.792–0.975)	0.093
EmCr/Mosa	0.0181	0.902 (0.807–0.960)	0.125

[a] EmCr, Emerald Creek; NVP, Natural Variation Planting; WPSO, White Pine Seed Orchard; MoSa, Moon Saddle
[b] Nei's unbiased genetic distance (Nei 1978)
[c] Coefficients of Dice's similarity are given as the mean (bottom–top values)
[d] G_{st}, Genetic differentiation (Weir and Cockerham 1984)

ond group contained the NVP and MoSa populations (Fig. 2). Mean genetic similarity (GS; Dice 1945) between all possible comparisons of individuals of WWP was, on average, 0.91, and GS ranged within populations from 0.90 to 0.93. The population from EmCr was the most genetically homogeneous, with an average coefficient of similarity of 92.8% (range = 0.835–1.0). Average similarity declined slightly when comparisons were made between populations (Table 3). The most similar populations were from WPSO and EmCr (91.7%, range = 0.842–0.976) (Table 3).

Discussion

The average expected heterozygosity of WWP was 0.123, and this result was comparable to an allozyme study (H_e = 0.131) conducted by Steinhoff et al. (1983). However, a recent study of California closed-cone pine species showed that dominant biallelic markers (e.g., random amplified polymorphic DNAs, RAPDs) can predictably underestimate population diversity (Krutovskii et al. 1999; Wu et al. 1999). If this is the case for WWP, the true genetic diversity of WWP may be higher than found in this study or the previous allozyme study. AFLP markers showed that the majority (91%) of genetic diversity was contained within WWP populations with the remaining (9%) occurring among populations (Table 2). We could not directly compare our G_{st} value (Weir and Cockerham 1984) to other estimates for WWP or closely related five-needle pines because none of the other studies used AFLP markers. However, some valuable genetic variation studies of WWP were performed on the basis of geographic, ecological, elevational patterns (Rehfeldt 1979; Townsend et al. 1972), enzyme analyses (Steinhoff et al. 1983), and physiological attributes (Rehfeldt et al. 1984). These studies generally indicated that abundant genetic variability existed within populations. In addition, populations from Idaho, Montana, British Columbia (Canada), northern-interior Washington, and Oregon were genetically similar.

We also applied Shannon's index for calculating phenotypic diversity among WWP populations. The results of Shannon's measure showed very similar trends with measures using allele frequencies (Table 2).

We analyzed four groups of WWP samples; two (NVP and WPSO) can be called "composite" populations because each is composed of trees from a broad geographic area (Fig. 1), but these populations could also form the basis for regeneration plantings. The WPSO population is the combined result of natural blister rust selection (approx. 1 tree in 10,000) and artificial selection pressure in the rust-resistance breeding program (Fig. 1; Table 2). The two local natural populations had been subjected to high (EmCr) and low (MoSa) blister rust mortality. Heterozygosity and percentage polymorphic loci were very similar between the composite population (NVP) representing the pre-blister rust WWP gene pools, and the rust-selected breeding stock (WPSO). This result suggests that blister rust selection pressure has not had a major effect on genetic variability at the broad geographic scale represented within the two composite populations. Furthermore, phenotypic selection and breeding has apparently not resulted in a genetic bottleneck at this geographic scale, at least not one detectable from dominant marker data. Based on previous studies of other conifer species, genetic diversity following phenotypic selection is similar to that found in corresponding natural populations (Bergman and Ruetz 1991; Chaisurisri and El-Kassaby 1994; Cheliak et al. 1988; El-Kassaby and Ritland 1996; El-Kassaby et al. 1994; Knowles 1985; Williams et al. 1995).

Comparisons of two local natural populations revealed lower values of P and H_e at the high blister rust site (EmCr) compared to the low blister rust site (MoSa), lower in fact than any of the other three populations sampled. EmCr also had a higher GS value, reflecting its greater homogeneity. In addition, the MoSa population had twice as many unique alleles than the EmCr population. The characteristics of the EmCr and MoSa populations are very similar (e.g., naturally occurring, stand age, stand area, and geographic location, i.e., 45 km apart) except for blister rust impact. The EmCr population has been exposed to heavy blister rust pressure for approximately 75 years, resulting in an almost 1,000-fold reduction by blister rust. As in most areas where WWP was a dominant tree, the number of remaining WWP survivors in the EmCr area is tremendously small (approx. 5.6 WWP per hectare) compared to 50 years ago (approx. 5,000 WWP per hectare) (R.T. Bingham, unpublished; G.I. McDonald, unpublished). In contrast, the MoSa population exhibits few signs of mortality from blister rust. Thus, the potential reduction in the observed genetic variation and the considerably lower number of unique alleles in EmCr compared to MoSa population could be attributable to the extensive mortality caused by blister rust, although the differences in P are not statistically significant.

Genetic losses associated with bottlenecks (e.g., insect or disease mortality, harvesting, breeding, etc.) can be measured by either allelic evenness or allelic richness within population. Results from previous studies suggested that allelic richness measures (e.g., the number of alleles per locus, the number of rare alleles) may be more useful than allelic evenness measures (e.g., heterozygosity) when quantifying effects of disturbances on gene pools (Buchert et al. 1997; Leberg 1992; Marshall and Brown 1975; Rajora et al. 2000; Stoehr and El-Kassaby 1997; Tsutsui et al. 2000). Using 13 microsatellite loci, Rajora et al. (2000) showed that allelic richness measures were more sensitive than allele evenness measures for detecting genetic diversity changes caused by harvesting in a stand of old-growth eastern white pine (*P. strobus*). A significant reduction of rare (allele frequency < 0.01) and low frequency (0.25 > allele frequency ≥0.01) alleles was found after harvesting, even though heterozygosity was not substantially reduced (Rajora et al. 2000). However, Thomas et al. (1999) detected no significant differences

in the total or mean number of alleles per microsatellite locus among stands of lodgepole pine (*P. contorta* Loudon var. *latifolia*) that had been subjected to different management regimes (i.e., unharvested, planted, and harvested stand left for natural regeneration). They also measured expected heterozygosity using RAPD markers and found no significant differences among the three different stand types (Thomas et al. 1999).

In this study, we evaluated genetic variation among WWP populations based on P, H_e, and number of unique alleles. AFLP provides several excellent measures of diversity because the method gives estimates of variation at multiple loci (169 loci in this study) across the entire genome. Recently, the AFLP marker system has been commonly used for estimating genetic diversity in both cultivated and natural/rare populations (Barker et al. 1999; Kollmann et al. 2000; Lerceteau and Szmidt 1999; Paul et al. 1997; Schmidt and Jensen 2000; Sharma et al. 1996; Travis et al. 1996). However, the number of alleles per locus, a potentially more sensitive measure of genetic diversity, cannot be determined from our dominant marker data. In future studies, both dominant (e.g., amplified fragment length polymorphism) and co-dominant (e.g., microsatellite) markers are recommended for quantifying potential genetic bottlenecks caused by pathogens or other factors.

Genetic distance values (Nei 1978) among populations were small (range = 0.0107–0.0181), but much higher than those for the previously reported allozyme study of WWP (range = 0.002–0.008, northern Idaho populations) (Steinhoff et al. 1983). This may reflect a greater power of AFLP markers for detecting differences among populations. Several other studies found higher genetic distance values for DNA-based markers (RAPDs) than for allozymes in pine species (Bucci et al. 1997; Thomas et al. 1999; Wu et al. 1999).

In conclusion, the present AFLP marker data suggest that the rust-resistance breeding program (WPSO) successfully captured the representative genetic variation of WWP present in the natural populations. Furthermore, blister rust appears to be responsible for a reduction of genetic variation in local WWP populations. However, studies using co-dominant markers to examine numerous natural WWP populations are necessary to estimate the blister-rust impact at the local WWP population level. Continued application of such molecular markers can help determine the effective population sizes of WWP. An understanding of processes that determine the amount, distribution, and changes in genetic variation in natural and artificial populations is essential to the success of genetic conservation, breeding programs, management, and restoration of WWP.

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