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Allozyme inheritance, heterozygosity and outcrossing rate among *Pinus monticola* near Ladysmith, British Columbia

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Analysis of megagametophytic and embryonic allozyme variants in nine enzymatic systems encoded by 14 loci was conducted on 30 western white pine (*Pinus monticola* Dougl.) trees from a natural stand on Vancouver Island, B.C. The segregation of allozymes in megagametophytes of heterozygous trees indicated distinct, simple, Mendelian inheritance. The stand heterozygosity parameters (proportion of polymorphic loci (0·64), average number of alleles per locus (1·79), and average expected heterozygosity (0·18)) were identical to those reported from a broadly-based survey of the species (Steinhoff *et al.*, 1983). Comparison of progeny (viable embryo) and parental genotypic distributions indicated a major genetic shift between the two life-cycles phases. Among the progeny, heterozygotes occurred less frequently than expected under panmixia, while the reverse was observed for the parental population. Single-locus estimates of outcrossing rate varied between 0.751 and 1.043 and were significantly heterogeneous. Comparison between the single-locus estimates and the multilocus estimate (0·977) of outcrossing rates indicated that most of the inbreeding detected was due to consanguineous matings, rather than selfing. This form of inbreeding was consistent with the clustering of genotypes in the stand. Single-tree estimates of outcrossing rate varied considerably among trees and ranged between 0.682 and 1.207, indicating that some trees might possess high self compatibility and tolerate high selfing, or that violation of mixed-mating assumptions occurred. The implications of the apparent inbreeding on the derivation of genetic estimates from open-pollinated seeds and on seed crops for production plantations are discussed.

INTRODUCTION

Western white pine (*Pinus monticola* Dougl.) is a widespread species of mesic forests in both coastal and montane areas of western North America. Its large size, good stem form and high-quality wood have made it a desirable species since commercial forest exploitation began in its range. The accidental introduction of the white pine blister rust (*Cronartium ribicola* J. C. Fisch.: Rabenh.) threatened white pine's status as a species for commercial management, but breeding and selecting for resistance to the rust, particularly in the U. S. "Inland Empire" (Bingham, 1983) have restored it to active planting status in Oregon, Washington, Idaho and Montana (Hoff, 1982; Samman, 1982). This progress, plus the high resistance of western white pine to root diseases affecting some of its important associates (Wallis, 1976) has led to the resurrection of a tree-improvement program for the species in British Columbia (B.C.), Canada (Meagher and Hunt, 1985).

Following experience in the U.S. programs, screening of open-pollinated (OP) families from canker-free parents is proposed for B.C. as a quick and inexpensive method of identifying parents possessing useful amounts of resistance. To obtain reliable values of genetic parameters from such trials, the parents should be unrelated, should be good outcrossers and should have produced progeny equally derived from many pollen parents (Namkoong, 1966). However, relatedness and natural inbreeding have been reported for several conifers (El-Kassaby *et al.*, 1981; Park and Fowler,

1982; Shaw and Allard, 1982*a*; Roberds and Conkle, 1984), including temporal variation in the same stand (King *et al.*, 1984; Cheliak *et al.*, 1985). Hence, quantitative assessment of the outcrossing rates should be an integral part of any study of selection in plant populations (Ennos, 1981).

The objectives of this study were to determine the inheritance of enzyme markers, estimate the degree of heterozygosity, and characterise the mating system in a young white pine stand in coastal B.C. Such data are useful in designing seed orchards and in estimating the bias caused by relatedness in OP seed crops.

MATERIAL AND METHODS

Population description

The stand sampled occupies ca. 50 ha; it occurs at 800 m elevation on a N to NE aspect west of McKay Lake near Ladysmith, British Columbia (source lat. 49°01′N, long. 124°04′W). This stand originated via natural seeding following logging, which began in the late 1940s. Maximum tree age was approximately 34 years in 1984, although some white pines were 10 years younger. Associated species are Douglas-fir, (*Pseudotsuga menziesii* (Mirb.) Franco), western hemlock (*Tsuga heterophylla* (Raf.) Sarg.), western red cedar (*Thuja plicata* Donn), yellow cypress (*Chamaecyparis nootkatensis* (D. Don) Spach), amabilis fir (*Abies amabilis* (Dougl.) Forb.), red alder (*Alnus rubra* Bong.) and willows (*Salix* spp.).

The stand is reasonably open grown, with live branches at or near the ground where artificial pruning has not been conducted. White pine blister rust is present and has infected most trees, killing many.

Cone and seed material

Cones were collected from the upper $\frac{1}{3}$ of the crown in 1984 during a general, but moderate, cone crop. Thirty trees, many near a road through the stand, were selected simply on the criterion of bearing sufficient cones for collection; presence or absence of blister rust was noted for each tree. The location of each tree sampled appears in fig. 1.

Parental identity was maintained from cone collection through to seed storage. Seeds were dewinged by hand prior to cleaning in a closely-regulated air column (Edwards, 1979). All seeds were screened by soft X-ray to determine the percentage of filled and empty seeds per parent prior to winnowing. Filled deeds were stored under refrigeration (2°C) until needed.

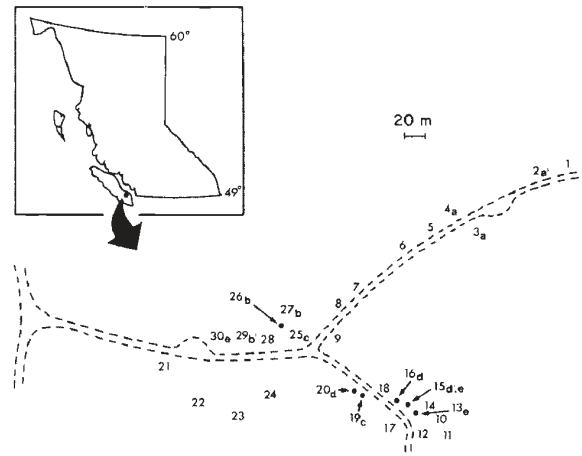

Figure 1 Location of sampled area and position of trees within the stand. 1, 2, . . . , 30 indicate position of cone parents; "a, a" denote "identical" (6-locus) genotypes; "a[1]" denotes "full-sib" tree differing from "identical" pair a, a by one band.

Electrophoretic procedures

Electrophoretic procedures, staining recipes and enzyme nomenclature used follow those of El-Kassaby *et al.* (1982). The nine enzyme systems studied were: aspartate amino-transferase (AAT) E.C.2.6.1.1; glutamate dehydrogenase (GDH) E.C.1.4.1.2; glucose-6-phosphate dehydrogenase (G6PD) E.C.1.1.1.49; isocitric dehydrogenase (IDH) E.C.1.1.1.42; malic dehydrogenase (MDH) E.C.1.1.1.37; phosphoglucose isomerase (PGI) E.C.5.3.1.9; phosphoglucomutase (PGM) E.C.2.7.5.1; 6-phosphogluconic dehydrogenase (6PGD) E.C.1.1.1.44; and superoxide dismutase (SOD) E.C.1.15.1.1. Interpretation of electroporetic banding patterns followed the method outlined in El-Kassaby *et al.* (1982). Enzyme loci were identified by abbreviation and a number. The most-anodally-migrating locus was designated as 1. Within each locus, the most-frequent band was assigned the number 1; faster and slower bands were given even and odd numbers, respectively (fig. 2).

Genetic analyses

The genotype of each tree was inferred for 14 allozyme loci by interpretation of electrophoretic assay of the haploid megagametophyte of 34 to 50 seeds per tree. The probability of misclassifying a heterozygote at a particular locus is $0{\cdot}5^{k-1}$ for k megagametophytes assayed per tree (Tigerstedt, 1973). With this large number of seeds per tree, the probability of misclassifying a heterozygote is

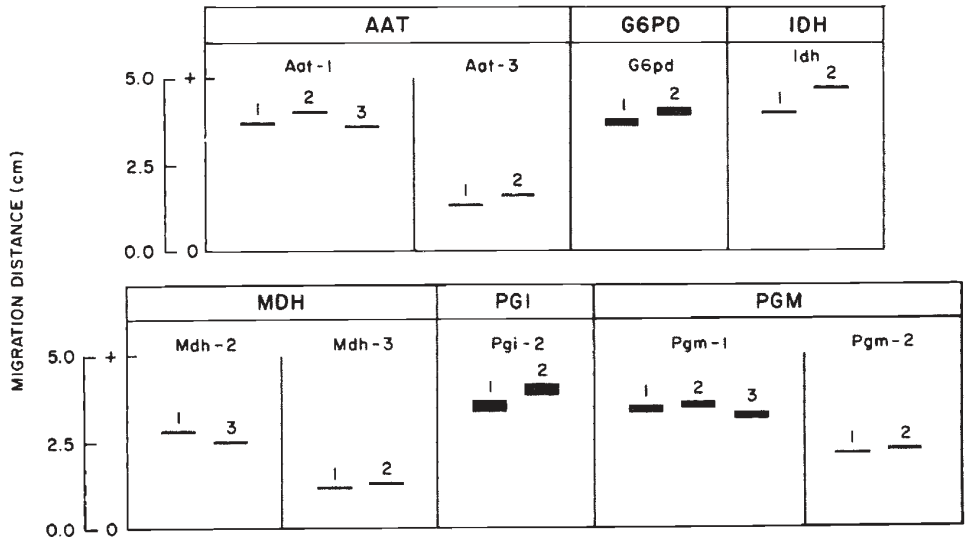

Figure 2 Banding patterns and their allelic designations for nine polymorphic loci observed in megagametophytic tissue from a natural stand of western white pine located on Vancouver Island, B.C. Number above band refers to the variant at that allozyme locus.

very close to zero. The structure of coniferous seeds (haploid megagametophyte and diploid embryo) allows detection of bands from pollen parents in the embryos.

The relationship between observed (H) and expected (h) ($h = 1 - \sum p_i^2$, where p_i is the frequency of the ith allele at the locus) frequencies of heterozygotes in both the parental generation and their progeny (viable-embryo stage) was used to measure the extent of inbreeding. Wright's (1922) fixation index (F) was calculated using the formula $F = 1 - (H/h)$.

Single- and multilocus estimates of the population outcrossing rate (t) and pollen allele frequencies (p) at six loci (*Aat*-3, *G6pd*, *Mdh*-3, *Pgi*-2, *Pgm*-1, and *Pgm*-2) were calculated using the maximum-likelihood procedure of Ritland and El-Kassaby (1985). This procedure gave estimates also of outcrossing rate and outcrossing-pollen allelic frequencies for individual trees.

Segregation analysis (1:1) was conducted after the use of the χ^2 contingency test (Steel and Torrie, 1980) on pooled data from trees sharing the same locus genotype in their gametophytic tissue.

RESULTS AND DISCUSSION

Enzyme description and inheritance

Monomorphic enzymes Three enzyme systems (Glutamate dehydrogenase (GDH), 6-phosphogluconic dehydrogenase (6PGD), and superoxide dismutase (SOD)) were monomorphic in the 30 trees studied. GDH was found to be monomorphic, but G6PD contained a second, rare, allele in Vancouver Island populations studied by Steinhoff *et al.* (1983). SOD was not studied by them.

Polymorphic enzymes Six enzyme systems were found to be polymorphic for at least one locus (table 1 and fig. 2).

Aspartate amino-transferase (*AAT*) There were four zones of activity on AAT gels: *Aat*-1, *Aat*-2, and *Aat*-3, which migrated anodally, and a cathodal zone that matched variation in *Aat*-3. The presence of this cathodal zone was reported in other pines (*P. rigida*, Guries and Ledig, 1978; *P. ponderosa*, O'Malley *et al.*, 1979; *P. taeda*, Adams and Joly, 1980; *P. strobus*, Eckert *et al.* 1981).

*Aat*1 showed three bands; the slowest one was found in only one parent (#11) while the fastest one was found only twice in embryos (*i.e.*, from the pollen pool). *Aat*-2 was monomorphic in our sample. Steinhoff *et al.* (1983) found one allele for this locus in Vancouver Island western white pine populations, but found two alleles elsewhere. *Aat*3 showed two bands in our samples. Two bands were reported for 25 of 28 populations of western white pine (Steinhoff *et al.* 1983).

Gluclose-6-phosphate dehydrogenase (*G6PD*) A single zone of activity, containing three bands, was found. One of the "fast-migrating" bands was present in only one parent (#5). It was difficult to

Table 1 Observed allozyme segregation in megagametophytic tissue of heterozygous mother trees of *P. monticola* at McKay (Ladysmith) B.C.

Locus	Allelic combination*	Observed segregation	Deviation χ^2 (1 df)	Heterogeneity† G (df)
Aat-1	1/3	17:23	0·90	—
Aat-3	1/2	285:324	2·50	7·81 (15)
G6pd	1/2	192:192	0·00	2·49 (9)
Idh	1/2	18:22	0·40	—
Mdh-2	1/3	39:41	0·05	1·25 (1)
Mdh-3	1/2	238:248	0·21	2·70 (11)
Pgi-2	1/2	402:363	1·99	5·64 (18)
Pgm-1	1/2	199:203	0·04	6·52 (9)
	2/3	20:20	0·00	—
Pgm-2	1/2	137:119	1·27	3·47 (6)

* Allozymes were numbered starting with "1" for the most common allele; faster and slower alleles were given even and odd numbers, respectively.
† None of the heterogeneity χ^2 and G-tests was significant.

differentiate this band from the most-common one, particularly in embryos, therefore, data for both bands were combined. The second "fast" band was found in 10 trees. This locus was not studied by Steinhoff *et al.* (1983).

Isocitrate dehydrogenase (*IDH*) A single zone of activity was present with two bands; the faster one was found in gametophytes of parent 19. Steinhoff *et al.* (1983) reported that the *Idh* locus was monomorphic in western white pine from Vancouver Island but that a second allele was present in Interior Washington and southern populations.

Malate dehydrogenase (*MDH*) Three zones of activity were detected on gels stained for MDH. *Mdh*-1 could be stained, but it was smeary and inconsistent; it was not included in further analyses. *Mdh*-2 and *Mdh*-3 displayed two bands. The slower band for *Mdh*-2 was found in only two parents (# 25 and 29).

One MDH locus was reported for western white pine by Steinhoff *et al.* (1983). Among other pines, the resolution of MDH band patterns varies and the number of loci reported differs (see El-Kassaby, 1981, for review).

Phosphoglucose isomerase (*PGI*) PGI gels showed two zones of activity: *Pgi*-1 was monomorphic and *Pgi*-2 was dimorphic in our population and in all 28 western white pine populations studied by Steinhoff *et al.* (1983).

Phosphoglucomutase (*PGM*) Two zones of activity were observed for PGM. Three and two bands were observed for *Pgm*-1 and *Pgm*-2, respectively. Heterozygous embryos displayed two-banded monomeric patterns. Four bands were observed for *Pgm*-1 in several western white pine populations (Steinhoff *et al.*, 1983).

In summary, no significant deviation from the expected 1:1 segregation ratio nor G-heterogeneity test was observed at any locus studied, indicating that these allozymes exhibited distinct, co-dominant expression and simple Mendelian segregation in their mode of inheritance. With the exception of *Pgm*-1 and *Pgm*-2, all loci showed triple-banded heterozygotes indicating dimeric subunit structures.

Population heterozygosity level

Individual-tree heterozygosity ranged from 0.00 to 0.36, with a mean expected heterozygosity of 0·18 (Table 2). When the McKay population heterozygosity parameters (*i.e.*, proportion of polymorphic loci, average number of alleles per locus, and average expected heterozygosity) were compared with those from the more extensive samples of Steinhoff *et al.* (1983) and, more specifically, with their Vancouver Island populations ("Coronation" and "Victoria"), the values correspond closely, in spite of differences between the two studies in the loci examined and their number (Table 2).

In summary, considerable variation in the amount of genetic polymorphism among the studied loci was observed in this population. These observations are consistent with the results of Steinhoff *et al.* (1983) and are typical of those for most coniferous trees studied by isoenzyme techniques (Mitton, 1983). Nine of the 30 trees sampled were free of blister rust. Mean heterozygosities per tree for cankered-uncankered classes were 0·206 and 0·183, respectively. The difference is not

Table 2 Summary of heterozygosity parameters in *Pinus monticola* at McKay, B.C. and comparisons to reported parameters

Parameter	McKay*	Vancouver Island†	Rangewide†
Proportion of polymorphic loci	0·64	0·67	0·65
Average number of alleles per locus	1·79	1·70	1·70
Average expected heterozygosity	0·18	0·17	0·18

* Based on 14 loci.
† Based on 12 loci (Steinhoff *et al.*, 1983).

statistically significant; thus, the presence or absence of rust cankers is not correlated with intrapopulation variability determined by this technique.

Adult-progeny allelic and genotypic frequencies

Estimates of allelic frequencies and their 95 per cent confidence intervals for ovule (maternal trees) and outcrossing-pollen gene pools for six polymorphic loci (*Aat*-3, *G6pd*, *Mdh*-3, *Pgi*-2, *Pgm*-1, and *Pgm*-2) are listed in table 3. Differences between any pairs of allelic frequencies were checked for significance ($P < 0{\cdot}05$) by comparing bounds of confidence intervals (table 3). Ovule and pollen pool allelic frequencies did not differ at the 95 per cent level, indicating that the maternal trees are representative of the local population.

Estimates of Wright's inbreeding coefficient $\hat{F}$, (Wright, 1922), which measures the excess of homozygosity above Hardy-Weinberg expectations, varied widely among loci for both adult (maternal) trees and progeny (table 4). In maternal trees, single-locus fixation indices were negative for four of six loci, while only two were negative in the progeny (table 4). The minimum-variance mean of $\hat{F}$ over loci for the maternal trees ($\bar{\hat{F}} = -0{\cdot}105$) differed significantly from $\hat{F} = 0$, indicating an excess of heterozygotes over those expected from panmixia (table 4). In contrast, the mean of $\hat{F}$ over progeny loci was positive (table 4), indicating that inbreeding has taken place in the seed crop. Similar observed variation in $\hat{F}$ among loci in both embryo samples and parent trees has been observed for Douglas-fir (Shaw and Allard, 1982b).

Positive deviation from zero (excess of homozygotes) can result from a variety of causes: a Wahlund effect (Wahlund, 1928), positive assortative mating (*i.e.*, preferential mating among similar genotypes) (Crow and Felsenstein, 1968), selection for homozygotes and family structure within restricted neighborhoods, causing mating among relatives (Levin and Kerster, 1971; 1974). Conversely, negative F values (*i.e.*, deficiency of homozygotes), can result from negative assortative mating (*i.e.*, preferential mating among dissimilar genotypes) and selection favouring heterozygotes (Brown, 1979).

Table 3 Allelic frequencies and their 95 per cent confidence intervals for six polymorphic loci in the maternal, and outcrossing pollen pools of *Pinus monticola* at McKay, B.C.

Locus	Allele*	Maternal	Outcrossing pollen
Aat-3	1	0·733 ± 0·112	0·769 ± 0·025
	2	0·267 ± 0·112	0·231 ± 0·025
G6pd	1	0·833 ± 0·094	0·807 ± 0·023
	2†	0·167 ± 0·094	0·193 ± 0·023
Mdh-3	1	0·500 ± 0·127	0·490 ± 0·029
	2	0·500 ± 0·127	0·510 ± 0·029
Pgi-2	1	0·650 ± 0·121	0·679 ± 0·027
	2	0·350 ± 0·121	0·321 ± 0·027
Pgm-1	1	0·783 ± 0·104	0·843 ± 0·021
	2†	0·217 ± 0·104	0·157 ± 0·021
Pgm-2	1	0·783 ± 0·104	0·689 ± 0·027
	2	0·217 ± 0·104	0·311 ± 0·027

* See table 1 for allelic designation.
† Synthetic allele (all alleles but the most common were bulked in one class).

Table 4 Fixation indices ($\hat{F}$) and their 95 per cent confidence intervals for 30 *Pinus monticola* parents and their progeny at McKay, B.C.*

Locus	Maternal	Progeny†
Aat-3	−0·363 ± 0·076	−0·003 ± 0·002
G6pd	−0·198 ± 0·047	−0·024 ± 0·002
Mdh-3	0·200 ± 0·165	0·028 ± 0·002
Pgi-2	−0·391 ± 0·086	−0·007 ± 0·002
Pgm-1	−0·079 ± 0·066	0·061 ± 0·002
Pgm-2	0·315 ± 0·061	0·003 ± 0·002
$\bar{\hat{F}}$	−0·105 ± 0·028	0·011 ± 0·001

* $V(\hat{F}) = (1/2N\hat{p}\hat{q})\{(1-\hat{F})[2\hat{p}\hat{q}(1-\hat{F})] + \hat{F}(2-\hat{F})(1-2\hat{p})^2\}$ (Rasmussen, 1964).
† See Table 5 for sample size.

Natural selection tends to reduce the frequency of homozygotes in natural stands (Stern and Roche, 1974; Brown, 1979; Shaw and Allard, 1982*b*). Comparison of parental and progeny genotypic distributions indicates that considerable genetic shift occurred between the two life-cycle phases. If a similar bias toward inbreeding existed in the seed crop producing the cone parents sampled here, much of the reduction in homozygosity between the two phases could be due to elimination of inbreds by competition (Brown's (1979) "heterosis for outcrosses"), to differences in inbreeding among maternal trees (see mating-system estimation section), and the presence of a Wahlund effect (Wahlund, 1928). Similar observations have been reported in both natural and experimental plant populations (Clegg and Allard, 1973; Clegg *et al.*, 1978; Shaw and Allard, 1982b; Neale, 1985).

When populations are at inbreeding equilibrium in the absence of selection, the fixation index ($\hat{F}$) defines the outcrossing frequency ($\hat{t}$) by the relationship $\hat{t} = (1-\hat{F})/(1+\hat{F})$ (Nei and Syakudo, 1958). Substituting the minimum-variance mean ($\bar{\hat{F}}$) of 0·011 (table 4) into the equation yields an expected outcrossing rate of 0·978, which is nearly the observed value for $\hat{t}_m$ (0·977) (table 5). A similar situation was reported for Douglas-fir (Shaw and Allard, 1982*b*).

Mating-system estimation

Single-locus ($\hat{t}$) and multilocus ($\hat{t}_m$) estimates of outcrossing rates are presented in table 5. Single-locus estimates showed significant departure from complete outcrossing ($t = 1{\cdot}0$) only at the *Pgm*-1 locus. Differences among single-locus outcrossing estimates have been reported for several coniferous species (Moran *et al.*, 1980; El-Kassaby *et al.*, 1981; Mitton *et al.*, 1981; Shaw and Allard 1982*a*; Epperson and Allard 1984; King *et al.*, 1984; Ritland and El-Kassaby, 1985). This observed variation is an inherent problem of all single-locus estimates due to their sensitivity to any violation of the assumptions of the mixed-mating model (Fyfe and Bailey, 1951; Brown and Allard, 1970; Ennos and Clegg, 1982; Brown *et al.*, 1984).

Table 5 Single-locus ($\hat{t}$) and multilocus ($\hat{t}_m$) estimates with 95 per cent confidence intervals of outcrossing among *Pinus monticola* at McKay, B.C.

Locus	Number of seed analysed	$\hat{t}$†
Aat-3	1113	1·027 (0·083)
G6pd	1171	1·043 (0·079)
Mdh-3	1171	0·977 (0·081)
Pgi-2	1180	0·945 (0·080)
Pgm-1	1175	0·751 (0·084)*
Pgm-2	1126	0·951 (0·083)
$\bar{\hat{t}}$‡		0·952 (0·056)
$\hat{t}_m$	1180	0·977 (0·023)

* Rejection of the null hypothesis that $\hat{t} = 1{\cdot}00$ at 5 per cent level
† The figure in parentheses gives the 95 per cent confidence interval
‡ Single-locus minimum variance mean over loci

$$(\bar{\hat{t}}) = \left[\sum_{i=1}^{n} \frac{1}{V_{\hat{t}_i}}\right]^{-1}\left[\sum_{i=1}^{n} \frac{\hat{t}_i}{V_{\hat{t}_i}}\right] \text{ where } n = \text{number of loci,}$$

$\hat{t}_i$ = single-locus estimate; $V_{\hat{t}_i}$ = variance of $\hat{t}_i$

Single-locus estimates of $\hat{t}$ are biased downward by any form of inbreeding in addition to selfing. An estimate of such possible inbreeding can be inferred by comparing values from the multilocus estimate with those from a single locus (Shaw *et al.*, 1981; Ritland and Jain, 1981). The multilocus estimate of outcrossing rate for this population (0·977) is greater than the minimum-variance, mean single-locus estimate over all loci of 0·952 (table 5), indicating that some of the inbreeding detected is due to consanguineous matings. Matings between related individuals are expected in natural stands because of the general presence of family structures and the finite limits to pollen dispersal (Stern and Roche, 1974).

The clustering of similar genotypes in the stand indicates the presence of family structure among the cone parents (fig. 1). Among the 30 parents studied, five pairs were genotypically identical for the six loci used. In two cases (trees #3–4 and #26–27), the pairs occurred within approximately 20 m of each other, in one case (#16–20) the trees were separated by 50 m and in the remaining two cases (#13–30 and #19–25) the trees were 90 and 210 m apart. In four cases, nearby trees in the stand differed by only one band from these pairs of trees, suggesting that they were siblings, while the paired trees might be selfs (fig. 1). Distances between the pairs and putative siblings varied from 20 m to approximately 340 m (the maximum separation between sampled trees is approximately 420 m). Similar clusters of genotypes have been reported by Linhart *et al.* (1981) and Mitton *et al.* (1981) for a *Pinus ponderosa* stand.

Single-tree estimates of t averaged over all loci varied among trees and ranged between 0·683 and 1·207 (fig. 3). The "biologically unrealistic" estimates of t (*i.e.*, $> 1{\cdot}0$) which were obtained could be due to negative assortative mating caused by phenological differences within and among trees (Sarvas, 1962; El-Kassaby *et al.*, 1984), to patchy allelic distribution due to pollination (Levin and

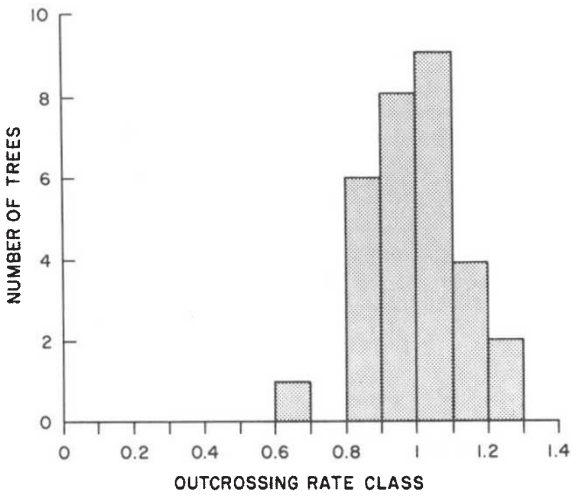

Figure 3 Frequency distribution of outcrossing rate class for 30 white pine trees sampled.

Kerster, 1968; Bradshaw, 1972; Schaal, 1974; Clegg, 1980; Hamrick, 1982), or just to sampling error. Conversely, there may be variation among single trees in their outcrossing rate as a result of genetic differences among individuals in their selfing ability.

Bingham and Squillace (1955), considered western white pine to possess self compatibility, but to differ in self fertility. However, our data indicated a non-significant correlation between percent of filled seed per tree and that tree's outcrossing rate estimate ($r = 0{\cdot}012$, $p > 0{\cdot}05$, $n = 30$). Two comparisons are worthy of comment: tree 12 gave the lowest filled-seed percentage (27·3 per cent) but displayed average outcrossing rate (1·02), while the tree with the highest filled-seed level (#29) (94·2 per cent) also showed average outcrossing rate (0·96). Furthermore, the tree (#21) showing the lowest outcrossing rate (0·68) gave near-average full seed (71·5 per cent). Tree 12 may be a poor selfer, while tree 21 could be a good selfer. Bingham and Squillace (1955) found that filled-seed yield percentage from selfing vs. outcrossing varied from near-equality to near-zero. Bingham (1973) reported an influence of inbreeding level to 0·5 (S_1 backcross or full-sib mating among selfs) vs. outcrossing on filled-seed percentage (26 per cent vs. 60 per cent). A different tendency is found here where outcrossing rate is only estimated.

The outcrossing rates of several plant species have revealed marked differences under different environmental conditions (for reviews see Clegg, 1980 and Mitton *et al.*, 1981). Therefore, the outcrossing rate estimate obtained for one population may not be applicable to other populations of that species. Highly-outcrossed species are expected to house high levels of heterozygosity and demonstrate little among-population differentiation. If the level of inbreeding found here among cone parents is common among western white pine populations, it could account for the low among-population differences reported for this species (Townsend *et al.*, 1972; Steinhoff *et al.*, 1983; Rehfeldt *et al.*, 1984).

The apparent variation in inbreeding rate among trees, and the distribution of maternal genotypes in our stand, could affect the portions of family relationships (*i.e.*, selfs, half-sibs, and full-sibs) in its OP seed crops. If so, using such partly-inbred seed crops to obtain estimates of heritability and genetic gain will produce an overestimate, since a basic assumption in heritability determined from open-pollinated seed crops is that local relatedness and inbreeding do not exist to a significant degree (Namkoong, 1966; Squillace, 1974).

Implications for seed production If similar tree-to-tree variation in their inbreeding levels occurs in other western white pine stands, the use of seed-production areas (natural stands managed for seed production during some part of their development) as seed sources may be questionable. If such a seed source is used (Hoff *et al.*, 1976), several stands should be established per planting zone, and seed crops should be mixed prior to sowing to broaden the genetic base and reduce the likely impact of intra-stand inbreeding on the resultant plantations. Furthermore, seed orchards designed to minimise matings among members of one population should produce more-vigorous offspring than obtained from OP progeny. The presence of some amount of inbreeding in the McKay white pine population as a whole (coefficient 0·011) requires adjustment of the genetic parameters drawn from it. Furthermore, the apparent, near 2-fold, variation among individual trees in their inbreeding rate necessitates a refined adjustment for each parent, if more-precise genetic information is to be obtained from OP progeny test plantations or from controlled crosses among trees with unknown genetic relatedness (Ghai, 1982; Cockerham and Weir, 1984). Otherwise, the ranking of white pine parent trees for measurable traits based on their open-pollinated progeny performance may simply reflect ephemeral differences in the proportions of inbreds, rather than genotypic superiority.

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