

## The mating system in natural and shelterwood stands of Douglas-fir\*

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**Summary.** Mating systems in two pairs of old-growth uncut and adjacent shelterwood stands of Douglas-fir (*Pseudotsuga menziesii* var. 'menziesii') were compared by estimating the proportions of viable progenies due to outcrossing ( $t$ ) with both single-locus and multilocus techniques. Single-locus population estimates ( $\hat{t}_s$ ), ranging from 0.41 to 1.16, were significantly ( $P < 0.05$ ) heterogeneous among loci in three of four stands; mean single-locus estimates for shelterwoods were not significantly different from those for uncut stands. Multilocus population estimates ( $\hat{t}_m$ ) ranged from 0.94 to 1.00; again, estimates for shelterwoods were not significantly different from those for uncut stands. Multilocus estimates were slightly higher than mean single-locus estimates for uncut stands but were nearly equivalent for shelterwoods, suggesting that related matings other than selfs may be associated with uncut stands, but not shelterwoods. Individual-tree outcrossing rates ( $\hat{t}_m$ ), estimated for six trees in each shelterwood, ranged from 0.90 to 1.10 and were significantly heterogeneous among trees at one of the two shelterwoods. Outcrossing was high ( $> 0.90$ ) in both uncut and shelterwood stands, and no evidence indicated that low parent-tree density had affected stand outcrossing rates.

**Key words:** Mating system – Outcrossing – Douglas-fir – Shelterwood – Isozymes

### Introduction

The mating system is an important determinant of the genetic structure of plant populations (Clegg 1980).

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Random mating is often assumed for cross-pollinating species, even though some form of nonrandom mating (selfing, assortative mating) characterizes many plants. Despite recent interest in tree mating systems (Moran and Brown 1980; Mitton et al. 1981; Shaw and Allard 1982; King et al. 1984; Cheliak et al. 1985), relatively little is known about the mating biology of forest trees. Two studies in natural stands of coastal Douglas-fir (*Pseudotsuga menziesii* var. 'menziesii') less than 100 years old have shown that the average rate of outcrossing is approximately 90%; the remaining 10% of viable progenies are due self-fertilization (El-Kassaby et al. 1981; Shaw and Allard 1982). However, no mating-system studies have been conducted on old-growth (200+ year) uncut or shelterwood stands.

The shelterwood is an important silvicultural tool for managing Douglas-fir. In southwest Oregon, a region of hot, dry summers, approximately 20–30 widely spaced trees (leave trees) per hectare are left after harvest of old-growth stands (preharvest stocking is typically 100 trees/ha). Leave trees provide shade for planted seedlings and, under favorable conditions, seed for natural regeneration. The wider tree spacing in shelterwoods could increase the proportion of self-fertilized offspring, as suggested by a study of a *Pinus sylvestris* seed tree stand in Sweden (Rudin et al. 1977). If some proportion of selfs survive, inbreeding in shelterwood-regenerated stands could become appreciable.

Strong inbreeding depression in the growth of selfed Douglas-fir progenies is well documented (Orr-Ewing 1957; Sorensen 1971, 1973; Sorensen and Miles 1974; Rehfeldt 1978), although survival of selfs is predicted to be very low (Sorensen 1982). However, selfs in open-pollinated seed lots may have a higher probability of surviving in highly favorable nursery environments. This is important when open-pollinated seed is collected for artificial regeneration from thinned seed-production areas.

Mating systems of individual trees are also of practical and theoretical interest. In practice, open-pollinated seed in the Pacific Northwest is collected from individual plus-trees about which competing trees have been removed to increase seed

production and facilitate cone collection. How this cultural practice affects the mating system is not known. In theory, population estimates of outcrossing (El-Kassaby et al. 1981; Shaw and Allard 1982) assume the rate of outcrossing to be constant among trees in a population, yet the validity of this assumption has been tested directly only in seed orchard populations (Shaw and Allard 1982; Omi 1983).

In this study, we used allozyme genetic markers to estimate outcrossing rates in two old-growth (200+ year) natural stands of Douglas-fir in southwest Oregon and two shelterwood stands adjoining those natural stands to (1) infer how plant density might influence the mating system of stands and (2) investigate how the mating system of individual leaf trees varies within shelterwoods.

### Seed collection and electrophoretic analysis

The two pairs of adjacent uncut and shelterwood stands (Benshell and Cutmore) studied are located within 4.2 km of each other on a large, flat plateau in the Umpqua National Forest, southwest Oregon (Neale 1984). All four stands were composed mostly of old-growth (200+ year) Douglas-fir. Spacing of leaf trees in the shelterwoods was fairly uniform, but density of leaf trees in the Benshell shelterwood (35 trees/ha) was more than twice that of the Cutmore shelterwood (15 trees/ha). Density of trees in the uncut stands was approximately 100 trees per hectare.

To analyze the mating system of each population, we first imposed a 201 m × 121 m (10 × 6 chain) sampling plot (2.4 ha in area), with intersecting grid lines (sample points) at every 20.1 m (1 chain), on each shelterwood and uncut stand. Each plot had 60 sample points in total. Shelterwood plots were separated from uncut plots by less than 200 m. In September 1980, approximately 25 cones were collected from the nearest cone-bearing tree within 10 m of each sample point. All cone-bearing trees in the shelterwood plots were included in the sample, but due to a generally poor seed crop and wide spacing of trees, the total number of trees sampled per shelterwood was 34 (Benshell) and 38 (Cutmore). Cone production was even poorer in the denser uncut stands, where only 10 fairly uniformly distributed cone-bearing trees were available for sampling within each plot. To ensure seed samples large enough for adequately estimating outcrossing in individual trees, and additional 75 cones were collected from each of six scattered leaf trees in both shelterwood plots. Self-fertilization has been shown to be higher in the lower crowns of Douglas-fir seed orchard trees (Shaw and Allard 1982; Omi 1983); therefore, all cones were collected from the mid to upper region of crowns.

Seeds were subjected to electrophoresis so that two types of information necessary for estimating outcrossing rates could be inferred: (1) the genotype of each maternal parent (seed tree) at a number of individual enzyme loci (multilocus genotype), and (2) the multilocus genotype of the pollen gamete which fertilized the ovule of each seed progeny. Multilocus genotypes of maternal parents were inferred by assaying at least 10 megagametophytes (1N) from each tree [probability of misidentifying maternal genotype (P) at a locus is less than 0.002, where  $P = (1/2)^{n-1}$  and  $n$  is the number of megagametophytes assayed]. The multilocus genotype of the pollen gamete was inferred by assaying both the megagametophyte and embryo (2N) of each seed. Because the ovule is genetically identical to the megagametophyte, the genotype of the pollen ga-

mete can be inferred from the embryo once the genotype of the megagametophyte is known. Population estimates of outcrossing for the shelterwoods and uncut stands were obtained by analyzing at least 10 progeny (megagametophyte-embryo pairs) from each tree in the population sample; individual-tree estimates were determined by assaying 46–125 (mean of 88) progeny from each leaf tree.

All seeds were assayed for 11 enzyme systems [phosphoglucumutase (PGM), phosphoglucose isomerase (PGI), leucine aminopeptidase (LAP), glutamate-oxaloacetate transaminase (GOT), glucose-6-phosphate dehydrogenase (G-6PD), glycerate dehydrogenase (GLYD), catalase (CAT), glutamate dehydrogenase (GDH), 6-phosphogluconate dehydrogenase (6-PGD), isocitrate dehydrogenase (IDH), and diaphorase (DIA)]; and a total of 13 loci were scored (Table 1). All of these loci have been confirmed by genetic segregation tests (El-Kassaby et al. 1982; Neale et al. 1984; Adams, unpublished); details of electrophoretic methods can be found in Neale (1984).

### Statistical methods

The mixed-mating model, as first described by Fyfe and Bailey (1951), classifies all matings as either selfs or outcrosses. Procedures have since been developed to estimate the mating-system parameter  $t$ , where  $t = 1 - s$  ( $t$  is the proportion of viable progeny resulting from outcrossing,  $s$  the proportion due to selfing). The mixed-mating model makes several assumptions: (1) each mating event is the result of either a random outcross (with probability  $t$ ) or a self-fertilization (with probability  $s$ ), (2) the probability of an outcross is independent of the genotype of the maternal parent, (3) outcross pollen-pool allele frequencies are homogeneous among maternal parents, and (4) no selection occurs between germination and census of seed progenies. Because the point of census is just after seed germination, the parameter  $s$  is an estimate of the proportion of self-fertilized seed which survive and are viable at census. Sorensen (1982) has shown that natural self-pollination may be high (40–60%) in Douglas-fir, but estimated proportions of viable selfed progenies are generally around 10% (Sorensen 1973; El-Kassaby et al. 1981; Shaw and Allard 1982). We used two types of procedures to estimate  $t$ : single-locus procedures, for obtaining population estimates of outcrossing, and multilocus procedures, for obtaining both population and individual leaf-tree estimates of outcrossing. All tests of significance were conducted at the 0.05 probability level.

#### Single-Locus estimation

Maximum likelihood methods for single-locus estimation of outcrossing in conifers are described in detail by Cheliak et al. (1985) and Shaw and Allard (1982). For diallelic loci, we used the method of Shaw and Allard, which allows joint estimation of  $t$  ( $t_s$ ) and  $p$  (the frequency of the  $A_1$  allele in the outcross pollen pool). For loci with more than two alleles, Shaw and Allard found  $\hat{t}_s$  for all possible diallelic combinations (i.e., 3 alleles, 3 combinations, etc.) and calculated a mean  $\hat{t}_s$  for each locus. Large differences in  $\hat{t}_s$ , however, were observed among diallelic combinations at any one locus. For this reason, we applied a triallelic extension of this model to estimate  $\hat{t}_s$ ,  $p$ , and  $q$  ( $q$  is the frequency of the  $A_2$  allele in the outcross pollen pool) whenever there were three alleles at a locus. Two loci (Got3, Idh) had more than three alleles, although the frequencies of the fourth or fifth most common alleles were rare. For these loci, the triallelic model still was applied by preserving the two

most common alleles and combining the remaining alleles into a synthetic class. Using both diallelic and triallelic models made synthetic reduction of the data virtually unnecessary.

Two computer programs were developed for single-locus estimation. Program RF004C<sup>1</sup> summarized allozyme data from progeny by tabulating the observed number of pollen gametes ( $O_i$ 's of Shaw and Allard 1982) for each of  $i$  observational classes (maternal genotypic by outcross pollen gamete genotypic class). Summarized information from RF004C was then entered into program RF004SL<sup>1</sup> where likelihood equations for the respective diallelic and triallelic models were solved numerically (Rao 1973) to estimate  $t_s$  and outcross pollen-pool allele frequencies.

#### Multilocus estimation

Multilocus procedures take advantage of information at multiple loci to estimate levels of outcrossing for populations ( $t_m$ ) and individual maternal parents ( $t_{m_i}$ ). Although a number of multilocus estimators have been described (Green et al. 1980; Ritland and Jain 1981; Shaw et al. 1981), we followed most closely the maximum likelihood estimator of Green et al., who classify all matings as either (1) detectable outcrosses or (2) ambiguous matings (some of which may be due to selfing). The multilocus genotype of a pollen gamete is compared to the genotype of the maternal parent. If the pollen gamete has an allele at any one or more loci that could not have been contributed by the maternal parent, the progeny is classified as a detectable outcross. Alternatively, if the alleles at all sampled loci in the pollen gamete could have come from the maternal parent, then the progeny may have arisen either by outcrossing to a pollen parent carrying the same alleles as the maternal parent or by self-fertilization; such a mating is classified as ambiguous.

The likelihood equation for estimating  $t_m$  is

$$L(t) = \pi (1 - G_i t_m)^{(N_i - R_i)} (G_i t_m)^{R_i}$$

where  $R_i$  is the number of detectable outcross pollen gametes observed among the  $N_i$  progeny sampled from the  $i$ th maternal parent, and  $G_i$  is the conditional probability of detecting an outcross pollen gamete in progeny of the  $i$ th maternal parent, given that an outcross has occurred (detection probability).  $G_i$ 's were estimated for each maternal parent in the population as

$$\hat{G}_i = 1 - \frac{k}{j} \hat{f}_{ij}$$

where  $\hat{f}_{ij}$  is the estimated frequency in the outcross pollen pool of alleles carried at the  $j$ th locus in the  $i$ th maternal parent, and  $k$  is the number of loci. For example, when the  $i$ th maternal parent has genotype  $A_1A_1$  at the  $j$ th locus,  $f_{ij} = p$ , where  $p$  is the frequency of the  $A_1$  allele in the outcross pollen pool. Note that in the diallelic case, a heterozygous locus ( $A_1A_2$ ) does not contribute to the detection of outcrosses (i.e., to an increase in  $G_i$ ) because  $f_{ij} = p + q = 1$ . Heterozygous loci do contribute to  $G_i$  when more than two alleles are present in the outcross pollen pool because  $f_{ij} = p + q < 1$ . Although allele frequencies in the pollen pool might be assumed to equal those observed in the maternal parents, they are directly estimated along with  $t_s$  in the single-locus estimation procedure. Direct estimates are

probably more accurate than those inferred from adult genotypes, especially when the number of maternal parents in a sample is small and the number of progeny large (Green et al. 1980). Pollen-pool allele frequencies were directly estimated in this study; thus, no more than three alleles per locus were used to calculate the  $G_i$ 's and each  $t_m$ . The estimator for  $\hat{G}_i$  assumes that alleles at the  $k$  loci are associated independently in the outcross pollen pool. Shaw et al. (1981) have shown that multilocus estimators are robust to violations of this assumption with moderate levels of linkage disequilibrium.

Computer program RF004ML<sup>1</sup> was written to solve the maximum likelihood equation for estimating  $t_m$ . RF004ML determines  $R_i$  and estimates  $G_i$  for all  $i$  maternal parents in the population. The maximum likelihood equation is then solved numerically by Fisher's method of scoring (Rao 1973). Green et al. (1980) give a large sample approximation of the variance of  $\hat{t}_m$  assuming that the  $G_i$  are known and constant. Because this assumption normally is violated (as in this study),  $\text{var } \hat{t}_m$  must be considered a lower-bound estimate.

Multilocus estimates are theoretically less sensitive than single-locus estimates to violations of assumptions of the mixed-mating model (Shaw 1980). In particular, multilocus estimates are more robust to violation of the assumption that outcross pollen-pool allele frequencies are homogeneous among maternal parents because the multilocus procedure can more powerfully discriminate between outcrosses and true selfs. If there are related matings (other than selfs) in the population, the single-locus procedure will tend to underestimate  $t_s$ . Shaw and Allard (1982) have proposed that indirect estimates of the proportion of inbred matings (other than selfs) in a population can be obtained by comparing  $\hat{t}_s$  and  $\hat{t}_m$  from the same population.

To estimate proportions of outcrossed progeny for individual trees ( $t_{m_i}$ ), the likelihood equation for  $t_m$  was solved implicitly for one maternal parent, giving  $\hat{t}_{m_i} = \hat{r}_i / \hat{G}_i$  ( $\hat{r}_i = R_i / N_i$ , the proportional of detectable outcrosses, and  $\hat{G}_i$  is the detection probability).  $\hat{G}_i$  was determined in the same fashion as  $G_i$  for the multilocus population estimator. Estimators for  $\hat{t}_{m_i}$  and  $\text{var } \hat{t}_{m_i}$  are described in detail in the Appendix. Computer program RF004ML was used to calculate  $\hat{G}_i$ ,  $\hat{t}_{m_i}$ , and  $\text{var } \hat{t}_{m_i}$ .

## Results

### Single-locus population estimates

Single-locus population estimates of the proportion of progeny due to outcrossing ( $\hat{t}_s$ ) varied widely among loci within each stand and were significantly heterogeneous except for the Benschell uncut stand (Table 1). Large differences in outcrossing estimates among loci have previously been reported in Douglas-fir (Shaw and Allard 1982) and other predominantly outcrossing forest-tree species [e.g., *Eucalyptus obliqua* (Brown et al. 1975) and *Pinus ponderosa* (Mitton et al. 1981)]. Shaw (1980) found that interlocus variation in  $\hat{t}_s$  could be attributed both to violation of assumptions of the mixed-mating model and to random error due to statistical inefficiency of the estimator. Only a few  $\hat{t}_s$  values were significantly different from  $t_s = 1.00$ . Unweighted means of single-locus estimates for the uncut stands (0.88 and 0.92) were very similar to mean single-locus outcrossing estimates previously reported for natural

<sup>1</sup> Computer programs RF004C, RF004SL, and RF004ML are written in Fortran V for a CDC-Cyber 170 computer; they are available through the Forest Science Data Bank, Department of Forest Science, Oregon State University, Corvallis, OR 97331, USA

**Table 1.** Single-locus population estimates of outcrossing ( $\hat{t}_s$ , standard errors in parentheses) for two pairs of adjacent old-growth uncut and shelterwood Douglas-fir stands

Locus	Benshell		Cutmore	
	Uncut	Shelterwood	Uncut	Shelterwood
<i>Pgm1</i>	0.98 (0.08)	0.86 (0.08) <sup>a</sup>	1.06 (0.03)	0.96 (0.05)
<i>Pgi2</i>	– <sup>b</sup>	1.01 (0.01)	–	1.01 (0.01)
<i>Lap1</i>	1.06 (0.09)	0.92 (0.05)	0.99 (0.09)	0.88 (0.05) <sup>a</sup>
<i>Lap2</i>	0.95 (0.08)	0.92 (0.05)	0.89 (0.08) <sup>a</sup>	0.91 (0.05) <sup>a</sup>
<i>Got2</i>	0.98 (0.17)	0.95 (0.07)	–	0.98 (0.05)
<i>Got3</i>	0.41 (0.39) <sup>a</sup>	1.00 (0.02)	0.66 (0.23)	0.88 (0.14)
<i>G-6pd</i>	1.11 (0.04)	1.00 (0.01)	0.94 (0.08)	1.02 (0.03)
<i>Glyd</i>	0.86 (0.14)	0.90 (0.06)	0.90 (0.17)	0.91 (0.07)
<i>Cat</i>	0.88 (0.12)	1.01 (0.07)	0.75 (0.16)	0.91 (0.06)
<i>Gdh</i>	–	1.02 (0.01)	–	–
<i>6-Pgd</i>	–	1.05 (0.03)	1.16 (0.06)	0.83 (0.08) <sup>a</sup>
<i>Idh</i>	0.86 (0.18)	0.98 (0.07)	1.08 (0.04)	1.00 (0.05)
<i>Dia</i>	0.75 (0.12) <sup>a</sup>	0.78 (0.07) <sup>a</sup>	0.79 (0.12)	0.86 (0.07) <sup>a</sup>
Unweighted mean	0.88 (0.05)	0.95 (0.01)	0.92 (0.04)	0.93 (0.02)
$\chi^2_{\text{HET}}$	16.09 (9)	28.06 (12) <sup>c</sup>	22.51 (9) <sup>c</sup>	24.76 (11) <sup>c</sup>

<sup>a</sup> Significantly different ( $P < 0.05$ ) from  $t_s = 1.00$  on the basis of  $\chi^2_{(1)}$  likelihood ratio test (Brunk 1975)

<sup>b</sup> All blanks indicate insufficient maternal genotypic classes for estimating  $\hat{t}_s$

<sup>c</sup>  $\hat{t}_s$  estimates significantly ( $P < 0.05$ ) heterogeneous over loci on the basis of Fisher's heterogeneity chi-square test (Rao 1973)

**Table 2.** Multilocus population estimates of outcrossing ( $\hat{t}_m$ , standard errors in parentheses) for two pairs of adjacent old-growth uncut and shelterwood Douglas-fir stands

Stand	No. of loci	No. of progeny	$\hat{t}_m$
Benshell uncut	10	110	0.96 (0.03)
shelterwood	12	385	0.96 (0.02) <sup>a</sup>
Cutmore uncut	9	96	1.00 (0.02)
shelterwood	11	444	0.94 (0.02) <sup>a</sup>

<sup>a</sup> Significantly different ( $P < 0.05$ ) from  $t_m = 1.00$  on the basis of  $\chi^2_{(1)}$  likelihood ratio test (Brunk 1975)

Douglas-fir stands ( $\hat{t}_s = 0.90$ , Shaw and Allard 1982; El-Kassaby et al. 1981). Mean estimates for the two shelterwood stands were slightly higher than, but not significantly different from, those for the two uncut stands.

#### Multilocus population estimates

In all cases, multilocus population estimates ( $\hat{t}_m$ ) were higher than mean single-locus estimates ( $\hat{t}_s$ ) for individual stands (Tables 1 and 2). The differences cannot be tested statistically, however, because  $\hat{t}_s$  and  $\hat{t}_m$  were calculated from the same data in each stand. Even though multilocus estimates were slightly higher than respective  $\hat{t}_s$  values, estimates from both shelterwood stands were

significantly different from  $t_m = 1.00$ . The multilocus estimate for the Benshell uncut stand was the same as that for the Benshell shelterwood (0.96), but was not significantly different from  $t_m = 1.00$  due to its larger standard error;  $\hat{t}_m$  was slightly higher in the Cutmore uncut stand than in the shelterwood, but not significantly so. Multilocus estimates from all four stands were higher than those from seven of eight Douglas-fir stands in western Oregon reported by Shaw and Allard (1982).

#### Individual-tree multilocus estimates

Individual-tree multilocus estimates ( $\hat{t}_{mi}$ ) for the six additionally sampled leave trees in each shelterwood ranged from 0.90–1.10 at Benshell and from 0.92–1.01 at Cutmore (Table 3). Estimates were significantly heterogeneous among the six trees at Benshell, but not at Cutmore. Four trees had estimates greater than 1.00, which are possible if the proportion of detectable outcrosses in the progeny sample is greater than that expected based on the detection probability of the individual tree. Mean individual-tree estimates for the six trees in each stand were slightly higher than the population estimates for the two shelterwood stands; however, the differences cannot be tested statistically because the trees used for the individual-tree estimates also were included in the samples for population estimates. Nevertheless, in both cases  $\bar{t}_{mi}$  fell within one standard devi-

**Table 3.** Individual-tree multilocus estimates of outcrossing ( $\hat{t}_{m_i}$ ; standard errors in parentheses) for six leave trees in each of two old-growth Douglas-fir shelterwood stands

Shelterwood	Leave tree	No. of loci	No. of progeny	$\hat{t}_{m_i}$
Benshell	BS08	11	125	0.92 (0.04)
	BS24	10	46	1.10 (0.11)
	BS26	10	90	0.90 (0.04)
	BS39	11	94	0.98 (0.05)
	BS57	11	95	0.93 (0.03)
	BS65	11	97	1.01 (0.01)
Unweighted mean				0.97 (0.02)
$\chi^2_{(5)}$				14.67 <sup>a</sup>
Cutmore	CS08	11	69	0.95 (0.05)
	CS31	11	90	1.01 (0.01)
	CS41	11	105	0.95 (0.05)
	CS43	11	105	0.92 (0.06)
	CS70	11	91	0.94 (0.05)
	CS80	11	87	1.01 (0.07)
Unweighted mean				0.96 (0.02)
$\chi^2_{(5)}$				6.54

<sup>a</sup>  $\hat{t}_{m_i}$  estimates significantly ( $P < 0.05$ ) heterogeneous among individual leave trees on the basis of Fisher's heterogeneity chi-square test (Rao 1973)

ation of the population estimates. Mean individual-tree outcrossing estimates were slightly higher than the mean estimate (0.93) reported by Sorensen (1973) for 19 trees scattered about western Oregon.

## Discussion

Because of the large interlocus variation in the single-locus estimates and the theoretical robustness of multilocus estimation to violation of the assumptions of the mixed mating-model (Shaw et al. 1981), multilocus estimates of  $t$  are considered more accurate and should be favored over single-locus estimates, especially in predominantly outcrossing species.

Our population estimates of outcrossing were generally higher than those previously published for coastal Douglas-fir. The mean proportion of progeny due to outcrossing was estimated to be 90% in eight 25- to 100-year-old stands in western Oregon (Shaw and Allard 1982) and in a 40-year-old open-grown stand in British Columbia (El-Kassaby et al. 1981). Differences between our estimates and those from other studies may be due to differences in the enzyme loci assayed, to estimation procedures used, to unknown-year or site-specific factors, or to chance alone. Alternatively, the higher outcrossing estimates in our stands may have been related to stand age: most trees in these stands were over

200 years old, whereas those in the previously cited studies were less than 100 years old.

Comparing single-locus and multilocus population estimates allows for inference on the amount of inbreeding other than selfing in stands (Shaw and Allard 1982). For all stands, multilocus estimates were greater than mean single-locus estimates. If we assume that  $\hat{t}_m$  and  $\hat{t}_s$  are statistically independent (which they were not in this study), the estimates for each stand would not be significantly different. However, the difference in estimates was 0.08 for both uncut stands and 0.01 for both shelterwood stands (Tables 1 and 2). One assumption of the mixed-mating model – that outcross pollen-pool allele frequencies are homogeneous among maternal parents – could be violated by the presence of localized pollen pools, possibly as a result of clustering of related individuals (family substructuring) in the population. In such a case, using the single-locus procedure would underestimate the proportion of outcrossed progeny in the population; the multilocus procedure, theoretically more robust to violation of the above-mentioned assumption, more efficiently discriminates between selfed and outcrossed matings, especially if a large number of variable loci are assayed. Therefore, the larger differences between  $\hat{t}_s$  and  $\hat{t}_m$  for the uncut stands (compared to the shelterwoods) may have been due to a higher proportion of related matings other than selfs in the uncut stands than in the shelterwoods. This could have resulted if family substructuring was present in the uncut stands but absent (eliminated by harvesting) from the shelterwood stands.

Multilocus outcrossing estimates did not differ significantly between uncut and shelterwood stands, indicating that the large reduction in density due to shelterwood harvesting had little or no impact on outcrossing rates. Furthermore,  $\hat{t}_m$  values were not significantly different between the two shelterwood stands, even though the density of leave trees at Cutmore was less than one-half that of Benshell. Mating systems should be investigated in stands of even lower density to determine the point at which reduced density does affect outcrossing rate.

Although significant heterogeneity in  $\hat{t}_{m_i}$  was observed in the Benshell shelterwood, differences in outcrossing among trees within stands were not large (range 0.90–1.00+). In the only other study of individual-tree outcrossing in natural Douglas-fir populations, Sorensen (1973) observed a wide range (72.5–100%) of outcrossing rates among the 19 trees sampled. However, by design, those trees were located over a wide geographical area, across nearly 1,500 m in elevation, and where stand structure (density, age-class distribution, species mixture) varied considerably. If the range in outcrossing rates reported by Sorensen (1973) is characteristic of the species, the ranges observed within our

two shelterwoods would indicate that as much as a third of the tree-to-tree variation in outcrossing rates can occur at the within-stand level.

The high rate of outcrossing in Douglas-fir is an important component of its adaptive strategy. If the environment is coarse grained (Levins 1962, 1963) at the time of reproduction, then outcrossing will promote the recombination of genes for successful colonization of heterogeneous environments. This is further supported by high fecundities and iteroparous reproduction. Natural self-pollination may be high (Sorensen 1982), but only 5–10% of the viable progenies are due to selfing. The decreased fitness of inbred progenies (Sorensen and Miles 1982) probably insures that only an extremely small percentage of them survives to reproductive maturity.

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## Appendix

### *Multilocus outcrossing estimation procedure for individual trees*

Green et al. (1980) presented a maximum likelihood procedure for estimating the proportion of viable outcrossed progenies ( $t$ ) from a sample of maternal parents within a population. This estimator can be modified to calculate the proportion of outcrossed progenies from individual maternal parents ( $t_{m_i}$ ) in the population:

$$L(t_{m_i}) = (1 - G_i t_{m_i})^{(N_i - R_i)} (G_i t_{m_i})^{R_i},$$

where  $R_i$  is the number of detectable outcross pollen gametes among the  $N_i$  progeny sampled from the  $i$ th maternal parent, and  $G_i$  is the conditional probability of detecting an outcross pollen gamete in the progeny of the  $i$ th maternal parent, given that an outcross has occurred ( $\hat{G}_i = 1 - \prod_{j=1}^k \hat{f}_{ij}$ , where  $\hat{f}_{ij}$  is the estimated

frequency in the outcross pollen pool of alleles carried at the  $j$ th locus in the  $i$ th maternal parent, and  $k$  is the number of loci). By setting the first derivative of the log-likelihood equation equal to zero, the estimator for  $t_{m_i}$  is solved directly:

$$\hat{t}_{m_i} = \frac{R_i}{N_i \hat{G}_i} = \frac{\hat{f}_i}{\hat{G}_i},$$

where  $\hat{f}_i = R_i/N_i$ , the observed proportion of detectable outcrosses.

A large sample approximation of the variance of  $\hat{t}_{m_i}$  is given as:

$$\begin{aligned} \text{var } \hat{t}_{m_i} = & \left[ \frac{\partial \hat{t}_{m_i}}{\partial \hat{f}_i} \right]^2 \text{var } \hat{f}_i + \left[ \frac{\partial \hat{t}_{m_i}}{\partial \hat{G}_i} \right]^2 \text{var } \hat{G}_i \\ & + 2 \left[ \frac{\partial \hat{t}_{m_i}}{\partial \hat{f}_i} \right] \left[ \frac{\partial \hat{t}_{m_i}}{\partial \hat{G}_i} \right] \text{cov}(\hat{f}_i, \hat{G}_i). \end{aligned}$$

Expressions for  $\text{var } \hat{f}_i$  and  $\text{var } \hat{G}_i$  are easily derived, but their covariance is extremely complicated, and an expression for  $\text{cov}(\hat{f}_i, \hat{G}_i)$  has not been derived. The  $G_i$  for an individual maternal parent is a function of the frequency of alleles in the total outcross pollen pool of the population. Outcross pollen-pool allele frequencies are estimated from single-locus outcrossing estimation procedures by combining progeny data from a number of maternal parents in the same population (Green et al. 1980; Shaw and Allard 1982). If progeny of the  $i$ th maternal parent for which  $t_{m_i}$  is to be estimated are included in the combined data set,  $G_i$  and  $r_i$  will not be statistically independent. Nevertheless, if the number of parents in the population sample is large, the proportion of data contributed by any one parent is small in comparison to the total data set used to estimate  $G_i$ , in which case the covariance of  $\hat{G}_i$  and  $\hat{f}_i$  is expected to be small relative to the total variance of  $\hat{t}_{m_i}$ . However, the uncertainty about the magnitude of  $\text{cov}(\hat{f}_i, \hat{G}_i)$  can be eliminated if  $\hat{G}_i$  and  $\hat{f}_i$  are made independent. This can be done simply by removing the observations on the  $i$ th maternal parent from the total data set when outcross pollen-pool allele frequencies are estimated. Thus, for each  $i$  maternal parent for which  $\hat{t}_{m_i}$  is desired, a separate set of outcross pollen-pool frequencies must be calculated. With this technique  $\text{cov}(\hat{f}_i, \hat{G}_i)$  is zero, and by substituting in expressions for the partial derivatives,  $\text{var } \hat{G}_i$  and  $\text{var } \hat{f}_i$ ,

$$\begin{aligned} \text{var } \hat{t}_{m_i} \cong & \left[ \frac{1}{\hat{G}_i^2} \right] \left[ \frac{\hat{f}_i(1 - \hat{f}_i)}{N_i} \right] \\ & + \left[ \frac{\hat{f}_i^2}{\hat{G}_i^4} \right] \left[ (1 - G_i)^2 \sum_{j=1}^k \text{var } f_{ij}/f_{ij}^2 \right]. \end{aligned}$$

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