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The population genetic structure of two Douglas-fir shelterwood stands in southwest Oregon was investigated. This study had three specific objectives: (1) assess the impacts of the shelterwood regeneration system on genetic structure of regenerated stands by comparing the levels of genic diversity at each of four life cycle stages in shelterwoods (uncut stands, shelterwood leave trees, seed crop, and seedling regeneration); (2) compare the rates of outcrossing in uncut natural stands versus shelterwood stands; and (3) develop methods to estimate effective seed and pollen dispersal distance within shelterwoods and assess the importance of seed or pollen dispersal as mechanisms for development of family structure in shelterwood regenerated stands. Allele frequencies were estimated for ten allozyme loci at each of the four life cycle stages for both shelterwoods. Allele frequencies were not significantly heterogeneous among life cycle stages for all but one locus at one shelterwood. Genic diversity measures (i.e., mean number of alleles per locus, percent polymorphic loci, and mean expected heterozygosity) were also not significantly

heterogeneous among life cycle stages for both shelterwoods. Estimates of the fixation index (F_{IS}) and chi-square tests indicated that genotypic proportions were not significantly different from Hardy-Weinberg expectation at all life cycle stages.

The outcrossing rate (t) was estimated by both single and multilocus techniques. Multilocus population ($\hat{t}_m$) and mean single locus population ($\bar{\hat{t}}_s$) estimates ranged from .88-.99 for shelterwoods and uncut stands. There was no significant differences in estimates of outcrossing rates between uncut stands and shelterwoods using either single or multilocus estimators. There was significant heterogeneity in outcrossing estimates among six individual trees ($\hat{t}_{m_i}$) in one shelterwood, but not in the other. Comparison of single versus multilocus outcrossing estimates indicated that there may be a small proportion of related matings, due to family structure, in uncut stands.

An estimation procedure was developed to infer the most likely parent among shelterwood leave trees for sampled offspring (seed, pollen, or seedlings) based on a joint likelihood of parentage and distance between parents and offspring. Distances between offspring (seed or pollen) and inferred most likely parents were used to estimate the mean seed and pollen dispersal distance within shelterwoods. Average seed and pollen dispersal distance estimates within shelterwoods were 63 m and 70 m, respectively. Estimation of maximum likelihood parents was also used to study family substructure in pollen pools and seedling regeneration.

Population Genetic Structure of the Douglas-fir Shelterwood Regeneration System in Southwest Oregon

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GENERAL INTRODUCTION

Worldwide, there has been very little research on the genetics of natural regeneration systems (Rudin et al. 1977, Tigerstedt et al. 1982). This is not surprising in that tree improvement is most often associated with artificial regeneration. However, natural regeneration is still an important silvicultural tool throughout the world and is used in reforestation of many commercially important species in North America (Minore 1973).

There is an increasing awareness that the gene pool of our forest resource must be managed properly in order to maintain the long term productivity of domesticated populations. (Adams 1981, Brown 1978, Brown and Moran 1981, Frankel and Bennett 1970). Inherent in natural regeneration systems, such as seed tree, shelterwood, or selection methods, is the potential for a reduction or reorganization of the genetic composition of natural stands with each rotation. If the maintenance of genetic diversity is desired in timber growing areas (Perry 1978), the impacts of natural regeneration systems on levels of genetic variation should be critically evaluated. Even without detectable loss in allelic diversity, inbreeding can substantially alter the genetic composition of regenerated stands. Therefore, the effect of natural regeneration methods on levels of inbreeding should also be explored.

Coastal Douglas-fir [Pseudotsuga menziesii (Mirb.) Franco var. menziesii] is the most abundant and economically important species of the mixed conifer forest in the southern Oregon Cascades. Typically, Douglas-fir grows in mixed stands with Pinus lambertiana, Pinus ponderosa, Libocedrus decurrens, and Abies concolor between 750 and 1400 m elevation (Franklin and Dyrness 1973). The two major silvicultural systems for harvesting and reforestation of Douglas-fir in this region are the clearcut and the shelterwood. Clearcuts are reforested by planting, almost without exception. Shelterwood harvesting has been limited to sites where plant moisture stress and/or susceptibility to frost may lead to poor survival of planted seedlings after clearcutting. On these sites, shelterwoods provide an environmental buffer against drought and frost mortality for underplanted seedlings. However, shelterwood harvesting followed by natural regeneration is an attractive alternative to clearcutting and planting, especially at high elevation sites or north facing slopes, where it has proven successful (Williamson 1973). Most research on Douglas-fir shelterwoods has pertained to natural or planted seedling survival or stocking following the regeneration cut (Franklin 1963, Helgerson et al. 1982, Minore 1971, Williamson 1973). There have been no studies on the genetic implications of Douglas-fir shelterwood regeneration.

Knowledge of the ecological genetics of natural populations of forest trees is important for designing efficient domestication strategies and for managing genetic resources. The major emphasis has been towards studies which describe the partitioning of genetic

variability within and between populations and the degree to which patterns of genetic variation reflect adaptation to environmental variables. There are several examples of this type of study on a macrogeographical scale (e.g., Campbell and Sorensen 1978, Ching and Hinz 1978, Irgens-Moller 1967, White et al. 1979, Yeh and O'Malley 1980). Genetic differentiation and adaptation in this species has also been studied at the microgeographical level (e.g. Campbell 1979, Hermann and Lavender 1968, Rehfeldt 1974). However, with the exception of mating system studies (Shaw and Allard 1982), very little is known about the population genetic structure within individual stands of Douglas-fir. In particular, the relative contributions of natural selection, gene flow, random genetic drift, and mating system as determinants of population structure, are poorly understood. Knowledge of these genetic parameters will improve our understanding of the evolutionary biology of Douglas-fir and contribute towards more efficient management strategies. One important consideration in both natural and artificial reforestation is the loss in productivity due to inbreeding. These losses can be minimized if forest management practices insure that a large number of parent trees are involved in matings which produce the regeneration. Furthermore, knowledge of population genetic structure will also assist in more efficient methods of plus-tree selection and progeny testing in tree improvement programs (Brown and Moran 1981, Libby et al. 1969).

The advantages of electrophoretic methods for plant population genetics research are well known (Brown 1979, Hamrick et al. 1981). This fully applies to forest trees (Brown and Moran 1981) and has

some special implications for conifers. There are two tissue types in conifer seeds, the haploid megagametophyte and the diploid embryo. The megagametophyte differentiates from the functional megaspore prior to fertilization (Allen and Owens 1972). Therefore, the megagametophyte and the egg are products of the same meiotic event and are genetically identical. Only the egg is fertilized by the pollen gamete; the megagametophyte remains haploid. Upon electrophoretic analysis of both the megagametophyte and embryo from the same seed, the genotype of both the male and female gametes can be inferred. This genetic system is potentially very powerful for population genetic studies because genotypes of the ovule and pollen gametes of all seed progenies are known and do not have to be estimated from diploid progeny arrays. In addition, various types of vegetative tissue (i.e., dormant buds, bark, needles) can also be analyzed electrophoretically, thus making it possible to study several life cycle stages in the same population.

The goal of the research described in this dissertation was to provide information on the genetics of individual stands of Douglas-fir and assess the impact of the shelterwood regeneration system on genetic structure of populations. There were three basic objectives to this study which are represented as separate chapters within the dissertation. The objective of the research described in chapter I was to assess the influence of the shelterwood regeneration system on the genetic diversity and organization of stands. This was accomplished by determining the allozyme composition of two uncut stands and in three life cycle stages (leave trees, seed crop, and

naturally regenerated seedlings) within shelterwoods adjacent to the uncut stands. In chapter II, the objective was to describe the influence of the shelterwood system on the mating system. This was achieved by comparing outcrossing rates in shelterwood leave trees with those of trees in uncut stands. Finally, the objective in chapter III was to develop statistical methods to infer the most likely parent among leave trees for individual seed, pollen, and seedling offspring in shelterwoods. These methods were then used to obtain preliminary estimates of seed and pollen dispersal within shelterwoods and infer mechanisms by which family structure might develop in shelterwood regenerated stands.

CHAPTER I. GENETIC IMPLICATIONS OF THE DOUGLAS-FIR SHELTERWOOD REGENERATION SYSTEM IN SOUTHWEST OREGON

Introduction

Shelterwood is an important silvicultural tool in the management of many forest tree species, including Douglas-fir (*Pseudotsuga menziesii* var. *menziesii*) in southwest Oregon (Minore 1973). In this region, a small number (25-35 per ha) of widely spaced leave trees are left after harvesting of old growth stands in order to provide shade for planted seedlings and, under favorable conditions, provide seed for natural regeneration. When the shelterwood is used to naturally regenerate stands, the large reduction in the number of parent trees might result in an alteration of the genetic structure of the regeneration, relative to that of stands prior to shelterwood harvesting. Artificial selection or genetic drift, as a result of the shelterwood harvest, might change allele frequencies between uncut stands and shelterwoods. A shift in allele frequencies or a loss of variability could then be transmitted to the progeny of regenerated stands. The small number of widely spaced leave trees might also lead to more self-fertilizations or consanguineous matings, and hence increase the amount of inbreeding in regenerated stands. Therefore, both non-adaptive alteration of genetic structure and loss of genetic variability are potential by-products of the shelterwood regeneration system which could effect the long-term productivity of forest tree populations. It is thus important to evaluate the impacts of natural regeneration systems, such as the shelterwood, on the gene resources of forest tree populations.

Forest trees are among the most genetically variable species of plant taxa and coastal Douglas-fir appears to be among the more variable of the North American tree species investigated to this point (Hamrick et al. 1979, Hamrick et al. 1981). Allozyme studies in conifers indicate that a large component of the total genetic variability is contained within populations (Brown and Moran 1981, Dancik and Yeh 1983, Guries and Ledig 1982, Wheeler and Guries 1982). For example, Yeh and O'Malley (1980) reported that 97% of the total gene diversity of Douglas-fir populations in British Columbia is contained within populations and only 3% is due to gene frequency differences among populations. Patterns of allozyme variation among these populations were only weakly correlated with environmental variables. Somewhat in contrast, common garden studies in Douglas-fir indicate that variability in metric traits of seedlings (height, bud set, dry weight, etc.) is often strongly correlated with environmental variables (Campbell and Sorensen 1978, Hermann and Lavender 1968, Rehfeldt 1974). However, for Douglas-fir in southern Oregon, it has been shown that the largest component of genetic variability is the within population component, whether it be for quantitative characters or allozymes (Adams and Campbell 1982). It has been proposed that the high level of genetic diversity within populations of Douglas-fir is an adaptive response to the spatial and temporal environmental heterogeneity experienced by individual populations (Adams 1981, Campbell 1979, Silen 1982). Despite the apparent significance of genetic diversity within populations, the dynamics and structure of this variability in Douglas-fir is mostly unknown.

Knowledge of within stand population genetic structure will assist in weighing the various alternatives of forest management practices. The potential for reduced vigor due to inbreeding or maladaptation due to gene frequency changes or loss of variability associated with common natural regeneration methods (i.e., shelterwood, seed tree, etc.) needs to be assessed relative to their effects on long-term productivity. It has also been proposed that naturally regenerated stands could be used for in situ genetic reserves. However, effects of natural regeneration systems on population genetic structure must be known prior to implementing this form of gene conservation. Information on stand structure will also be useful in formulating plus-tree selection strategies (Ledig 1974) and interpreting the results of wind-pollinated progeny tests (Squillace 1974). In addition, an understanding of the genetic architecture of natural stands of Douglas-fir will be helpful for designing the structure of plantation populations.

The objective of this study was to assess the impacts of the shelterwood regeneration system on the genetic structure of Douglas-fir populations. Most population genetic studies of forest trees have estimated genetic parameters at a single point in the life cycle, usually at the adult stage, even though erroneous inferences on the mechanisms responsible for population structure may occur (Prout 1965). In this study, the genetic structure at four life cycle stages in each of two Douglas-fir shelterwoods was estimated by assaying seed or vegetative bud tissue for 10 or more allozyme loci. The four stages examined were; 1) uncut stands, 2) leave trees in shelterwoods, 3) the seed crop in shelterwoods, and 4) naturally regenerated seedlings

in the understory of shelterwoods. The shelterwood system not only allows for the evaluation of the genetic impacts of this silvicultural method, it also provides an experimental system for the study of life cycle variation in genetic structure of Douglas-fir. If differences in genetic structure occur among life cycle stages it would be possible to formulate hypotheses as to how the observed changes occurred.

Materials

The Benshell and Cutmore study sites were located in June 1980 within 4.2 km of each other on a very flat plateau south of the Toketee Ranger Station, Diamond Lake District, Umpqua National Forest (Figures I.1 and I.2). Each site consisted of a shelterwood stand, in which the regeneration cut had been made 7 or 8 years previously, and an adjacent uncut stand. The sites were selected for investigation based on the following criteria: 1) both the shelterwood and uncut stands consisted primarily of Douglas-fir; 2) advance natural regeneration in the understory of shelterwood stands was insignificant prior to the shelterwood harvest; 3) stocking of natural regeneration in the shelterwoods was at least 1000 seedlings per hectare; 4) stands did not transect any abrupt environmental gradients; and 5) the majority of leave trees in the shelterwoods had seed cones in 1980. The leave trees in each shelterwood were old-growth Douglas-fir (>200 years) and had been selected prior to harvest primarily to insure even spacing, although preference was given to the largest and best formed trees within the spacing constraints (personal communication,

Silviculturist, Diamond Lake District, Umpqua National Forest). Although stand histories and site variables were very similar for the Benshell and Cutmore sites (Table I.1), there was a large difference in the density of leave trees between stands (Benshell 35 trees/ha, Cutmore 15 trees/ha). The adjacent uncut stands were similar to the shelterwoods for all site and stand variables except for density (approximately 100 trees/ha in uncut stands) and there being very little natural regeneration in the understory of the uncut stands.

In order to assess genetic variability at each of the life cycle stages, a population sample was taken for each stage. Ideally, the four stages (uncut stand, shelterwood leave trees, seed crop, seedling regeneration) should be sampled in the same stands over time. This would require sampling trees in an uncut stand prior to harvest and then again after the shelterwood cut. Seeds would then have to be collected after a seed crop was produced in shelterwoods and then, after a few years, seedlings could be sampled from the same cohort as that which produced the seed crop. At least 4 years would be needed to complete this sample, assuming the seedling regeneration was 2 years old. Because this study had to be initiated and completed within a 3 year period, it was decided to approximate these life cycle stages by sampling in an uncut stand immediately adjacent to the shelterwood and also sampling 3-5 year old seedling regeneration in 1982, even though the seed crop was sampled in 1980. Thus, in order to compare the seed and seedling samples as successive life cycle stages, it must be assumed that yearly variation in genetic composition of the seed crops in these shelterwoods is insignificant.

Sampling of all stages was achieved by imposing a 201 m by 121 m (10x6 chain) plot, with grid intersections at every 20.1 m (1 chain), in each shelterwood and uncut stand. The total plot size was 2.4 ha and there were a total of 60 sample points, one for each grid intersection. This systematic sampling scheme was chosen so that a representative population sample could be collected for each of the four life cycle stages.

Uncut Stands

The population sample for the uncut stands was obtained by sampling the nearest tree at each sample point. In the Benshell uncut stand, vegetative buds were collected from 39 trees and seeds were collected from 11 trees (approximately 25 cones per tree), for a total of 50 trees sampled (Table I.2). In the Cutmore uncut stand, buds were collected from 54 trees and seeds from 5 trees, for a total of 59 sample trees (Table I.2). Seeds from individual trees in all stands in this study were collected in September 1980 and all vegetative buds in April 1982. Trees from which seeds were collected were also used as part of the mating system analysis in chapter II. The total number of trees sampled in both uncut stands were somewhat less than the intended number of 60 due to the amount of time and difficulty of collecting these materials.

Leave Trees

The population sample for the shelterwood leave trees was obtained in a similar fashion to that of the uncut stand. The closest cone bearing tree within 10 m of each sample point was tagged and

mapped. Approximately 25 cones were collected from each sample tree for a total of 36 trees at Benshell and 43 at Cutmore (Table I.2). There were several sample points in both shelterwoods for which there were no cone bearing trees in September 1980. Therefore, to complete the population sample, vegetative buds were collected from trees at 13 sample points at Benshell and 5 sample points at Cutmore. Since not all sample points had a tree within the maximum sampling distance, the total number of leave trees sampled in each shelterwood was less than 60 (Table I.2, Figures I.3 and I.4). It should also be noted that the total area over which trees were sampled at the Cutmore shelterwood had to be expanded slightly because only 30 cone bearing trees could be found within the 201 m x 121 m plot. Thirteen trees were sampled just outside the initial plot boundary (Figure I.4).

Seed Crop

The population sample for the seed crop life cycle stage was obtained by placing 40 seed traps beneath the leave trees in each shelterwood stand (Figures I.5 and I.6) in September 1980. A seed trap is a 30.5 cm x 61 cm wooden cage with metal screens for the top and bottom. The gauge of screens was such that Douglas-fir seeds could fall into but not out of the cage. The seed traps were situated in the shelterwood stands in two ways. One trap was put at each of 10 grid intersection points to systematically sample the entire study plot. An additional 3 traps were placed about the base of 10 individual leave trees. These traps were approximately 7 m from the base of the leave tree and 120° from one another. These traps were so situated in an attempt to sample seeds beneath the crown of individual

leave trees. Because 1980 was a poor seed year for Douglas-fir in southern Oregon, very few seeds were found in the traps (Figures I.5 and I.6). Therefore, seeds collected in the traps beneath individual trees were combined with those found in the grid sample traps to form the population sample (Table I.2).

Seedling Regeneration

The population sample for the seedling life cycle stage was obtained by sampling several vegetative buds from each of four seedlings at each of the 60 sample points. Each seedling was approximately 2.4 m from the sample point and 90° from each other. Only the 60 seedlings sampled to the north of the sample point were included in analyses in this chapter, the other seedlings were used in analyses in chapter III (Table I.2). Both shelterwood stands had been underplanted with two-year-old nursery stock between 1975 and 1977. Planted seedlings were between 7 and 9 years old in April 1982. In order to insure that only naturally regenerated seedlings were included in the population sample, bud collection was restricted to 3-5 year-old seedlings. Several seedlings were aged prior to sampling by counting annual rings, in order to develop a rapid method of identifying natural seedlings based on seedling height and number of lateral whorls. Because all sampled seedlings were not aged, some seedlings less than three or greater than five years old may have been included in the population sample. However, because of the obvious physical differences between planted and natural seedlings, it was unlikely that any planted seedlings were included in the sample.

Electrophoretic Methods

Electrophoretic assay of both seed and dormant vegetative bud tissue was conducted in this study. Freezer stored seeds were soaked for 48 hours in 1% H_2O_2 at room temperature and then germinated on moist filter paper in petri dishes for 4 to 7 days at 26°C and 12 hour day length. Clearest banding patterns were obtained when the radicle had emerged 3-4 mm beyond the seed coat. Crude extracts of the megagametophyte and embryo of each seed were prepared by macerating each tissue separately in two drops of phosphate buffer (0.2 M, pH 7.5).

Dormant vegetative buds were collected by sampling lateral twigs (10-30 cm) from seedlings and mature trees. Twigs were stored in plastic bags at 4°C. Buds stored in this way could be kept for up to three months with no apparent loss of intensity or resolution of isozyme bands. On the day of analysis, buds were removed from twigs and the primordia were excised from the bud scales. Crude bud extracts were prepared by macerating three primordia per seedling or tree in an extraction buffer.[1]

Individual seed and bud extracts were absorbed onto 3.5 x 10 mm filter paper wicks (Watman No. 3 mm) and inserted into a vertical

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[1]Bud primordia extraction buffer (personal communication, W. Cheliak, University of Alberta, Edmonton, Alberta, Canada). Dissolve following in 100 mL distilled H_2O: polyvinylpyrrolidone (PVP) 7 g of 40 T and 1 g of 360 T, sucrose 10 g, EDTA (disodium) 170 mg, dithiothreitol (DTT) 15 mg, ascorbic acid 20 mg, bovine-serum albumin 100 mg, NAD 50 mg, NADP .35 mg, pyroxidal 5' phosphate 5 mg, adjust to pH 6.7 with 1 M tris solution (pH 8.0), add 5-10 drops of β-mercaptoethanol. Store refrigerated in an amber bottle.

slice in the appropriate gel. Electrophoretic buffer systems sometimes differed for seeds and buds (Table I.3.). Gels were made from 12% Sigma starch (Sigma Chemical Co., St. Louis, MO). Gels were run under refrigeration (4°C) for 15 minutes and then the wicks were removed. Electrophoresis was then continued until dye markers or fronts had migrated to appropriate distances. Gels were then sliced horizontally, stained, and incubated (37°C) in the dark. Details concerning running conditions for all buffer systems and stain recipes can be found in Neale, Weber, and Adams (in preparation) and Adams, Weber, and Doerksen (unpublished). In total, bud and seed tissue extracts were assayed for eleven enzyme systems (Table I.3.).

Seed tissues were assayed for 14 loci (Table I.3) which coded allozyme variants of 11 enzyme systems (Table I.4), whereas vegetative buds were assayed for 10 loci (Table I.3) which coded variants of 9 enzyme systems (Table I.4). The inheritance of seed tissue isozymes used in this study is described in El-Kassaby et al. (1982a) and Adams, Smith, and Doerksen (in preparation). Twelve loci controlling the expression of needle tissue isozymes in Douglas-fir have also been established (Neale, Weber, and Adams in preparation). Formal Mendelian tests involving crosses and analysis of segregating progenies have not been conducted for bud tissue isozymes. However, indirect evidence for the inheritance of allozymes for all 10 loci was obtained by D. B. Smith, Department of Forest Science, Oregon State University (personal communication) by comparing isozyme phenotypes from buds with those expected based on megagametophyte analysis for a large sample of seed orchard clones. Because of the somewhat poorer isozyme resolution in embryos and bud tissues versus that from

megagametophytes, it was necessary to bulk alleles at 5 allozyme loci (PGI2, LAP1, LAP2, G-6PD, and IDH) prior to analyses. Therefore, all analyses performed in this study were conducted using only alleles or allelic classes which were common to all tissue types involved in each analysis.

Statistical Methods

Determination of Genotypes

Allozyme genotypes of adult trees in the uncut stands and shelterwoods were inferred by assaying 10 megagametophytes per tree or were determined directly from the allozyme phenotype expressed in bud tissues. The probability of misidentifying the genotype of an individual at any one locus (p), with a sample of 10 megagametophytes, is less than 0.002 ($p < (1/2)^{n-1}$, where n = number of megagametophytes). Genotypes of seeds collected in seed traps were determined directly by assaying embryos. Megagametophytes of seed trap seeds were also analyzed so that both the maternal and pollen contribution to the seed crop could be estimated separately. Seedling genotypes were determined directly from bud tissue allozyme phenotypes.

Estimation of Gene Frequencies and Diversity Measures

Estimates of gene frequencies and several measures of genic diversity were computed using the BIOSYS-1 computer package (Swofford and Selander 1981). Four measures of genic diversity were estimated for each life cycle stage: average number of alleles per locus (A),

percent polymorphic loci (P), mean observed heterozygosity per locus ($\bar{H}_o$), and mean expected heterozygosity ($\bar{H}_e$) (unbiased estimate, Nei 1978). Mean expected heterozygosity was calculated as:

$$\hat{\bar{H}}_e = \sum_{j=1}^{\ell} \left[\frac{2N\left(1 - \sum_{i=1}^{k} X_i^2\right)}{2N-1} \right] \Big/ \ell \,,$$

where X_i is the frequency of the ith allele at the jth locus, summed over k alleles and averaged over ℓ loci, and N is the number of individuals sampled.

Deviations from Hardy-Weinberg Equilibrium and Tests of Significance

A chi-square goodness-of-fit test was performed for each locus at each life cycle stage to determine if genotypic proportions were as expected under Hardy-Weinberg equilibrium. The test statistic was calculated by BIOSYS-1 (Swofford and Selander 1981) by pooling all but the most common allele into a composite allele and then determining the number of individuals in each of three genotypic classes based on the reduced data set. An estimate of Wright's fixation index, $\hat{F}_{IS}$ (Wright 1951), was also calculated for each locus at each life cycle stage. The estimator for $F_{IS_{(ik)}}$ for the k^{th} allele is $1-H_{ik}/2p_{ik}\,q_{ik}$, where H_{ik} is the observed number of heterozygotes at the ith life cycle stage and p_{ik} and q_{ik} are the frequency of alleles or allelic classes (p_{ik} and q_{ik} represent the frequency of the kth allele and a synthetic class of all other alleles at the ith locus, respectively). A mean estimate of the fixation index for each locus

and life cycle stage ($F_{IS_{(i)}}$) was then calculated by weighting individual estimates of $F_{IS_{(ik)}}$ by the frequency of each allele. The variance of each estimate of $F_{IS_{(i)}}$ was estimated by the method of Brown (1970). This estimate of the variance of $F_{IS_{(i)}}$ can be computed using only diallelic loci, therefore all but the most common allele were bulked into a composite class for loci with more than two alleles. These estimates were used to determine the direction of deviation from expected proportions under Hardy-Weinberg equilibrium. Values of $\hat{F}_{IS}$ greater than zero (up to a maximum of 1.0) indicate an excess of homozygotes and values less than zero indicate an excess of heterozygotes.

Tests for Heterogeneity in Allele Frequencies

Tests of allelic frequency differences among life cycle stages were made by calculating chi-square heterogeneity values for each locus using the formula of Workman and Niswander (1970):

$$\chi^2 = 2N\left[\left(\sum_{j=1}^{k} \frac{\sigma^2_{\bar{p}_j}}{\bar{p}_j}\right)\right],$$

where $\bar{p}_j$ and $\sigma^2_{p_j}$ are the mean and variance of the frequency of the jth allele over all life cycle stages, k is the number of alleles, and N is the total number of individuals sampled in all stages. Chi-square values were calculated to test for heterogeneity in allele frequencies among all four life cycle stages and for several pairwise combinations of life cycle stages.

Results

Allele frequencies varied only to a minor extent among life cycle stages within each shelterwood and also between shelterwoods (Tables I.5 and I.6). The most common allele at each locus was found to be the most common at all life cycle stages in both study sites. Occasionally alleles found at low frequency in one life cycle stage were not observed at other stages. However, the failure to detect these low frequency alleles may have been due to the limited sample size, rather than the absence of alleles at any one stage. One other surprising result was that the estimated allele frequencies in the shelterwoods were very similar to allele frequency estimates based on 72 trees scattered throughout the breeding zone (North Umpqua 2500-3000 feet, Industrial Forestry Association, Progressive Tree Improvement Cooperative) within which the shelterwood stands were located (personal communication, C. Loopstra, Department of Forest Science, Oregon State University). There were a total of 32 and 36 alleles observed among the ten loci (common to seeds and buds) in the Benshell and Cutmore shelterwoods, respectively. This compares to 36 alleles detected in the breeding zone sample, which had a larger sample size. Thus, each of these shelterwoods appears to possess as much allelic diversity as can be found within the entire breeding zone.

Allele frequency heterogeneity among life cycle stages within shelterwoods was tested by contingency chi-square analyses (Tables I.7 and I.8). For only one locus (LAP2), at one study site (Benshell), was significant ($P < .05$) heterogeneity in allele frequencies found

among the four life cycle stages. The combined heterogeneity chi-square (over all loci) was significant ($P < .01$) at Benshell, but this was due primarily to LAP2, because the combined chi-square was not significant ($\chi^2_{(48)} = 60.679$, $P > .05$) when LAP2 was deleted from the analysis. Additional chi-square tests were performed to test for heterogeneity in allele frequencies among pairs of life cycle stages within shelterwoods (Appendix B). These additional tests detected significant ($P < .05$) heterogeneity for only LAP2 at Benshell, and only between the leave tree and the seed crop stages, and between the leave tree and seedling regeneration stages. Inspection of Table I.5 shows that the heterogeneity was due primarily to the higher frequency of LAP2-2 in the leave tree stage versus all other stages at Benshell. LAP2-2 was also higher at the leave tree stage at the Cutmore study site (Table I.6). Observed heterozygosities were also higher for LAP2 at the leave tree stage than at any other stage (Table I.9).

Three measures of genic diversity were estimated at each life cycle stage in the two shelterwoods (Tables I.10 and I.11). Neither the mean number of alleles per locus (A) or the expected heterozygosity ($\overline{H}_e$) differed significantly ($P > .05$) among life cycle stages at either study site. Furthermore, the mean expected heterozygosity ($\overline{H}_e = 0.237$) for the entire breeding unit was not signficantly different than expected heterozygosity estimates for the shelterwood leave tree stages at both study sites. The percent polymorphic loci did vary slightly among life cycle stages at Benshell, however these differences were due entirely to the presence or absence of rare alleles (frequencies $< .03$) at two loci (PGI2 and GDH). There was a decrease in the estimate of all genic diversity measures between the

leave tree and seed crop life cycle stages at Benshell, but this trend was not observed at Cutmore.

Genotypic proportions in all life cycle stages were very close to Hardy-Weinberg expectations. Only five (7.2%) of the 69 goodness-of-fit tests performed over the 10 loci and four life cycle stages in the two shelterwoods were significant at the five percent level (Tables I.12 and I.13). This number of significant chi-square values is only slightly greater than what might be expected by chance alone. Estimates of Wright's fixation index (F_{IS}) for individual loci within life cycle stages were not consistently in the direction of either heterozygote excess or heterozygote deficiency (Tables I.14 and I.15). Mean estimates across loci were very close to zero for all life cycle stages, with the exception of the seedling stage at Benshell ($\hat{F}_{IS}$ = 0.113).

The fixation index (F_{IS}) is a measure of the total deviation in heterozygosity from Hardy-Weinberg proportions as a result of any or all evolutionary forces, including the mating system. An estimate of the equilibrium value of the expected inbreeding coefficient ($\hat{F}_e = \frac{1-\hat{t}}{1+\hat{t}}$, where t is the estimated outcrossing rate and var $\hat{F}_e = \frac{4}{(1+\hat{t})^4}$ var $\hat{t}$) is a measure of the deviation in genotypic proportions from Hardy-Weinberg assuming that the mating system alone affects genotypic proportions. The estimate of F_e for each shelterwood can be compared with estimates of F_{IS} for the seed crop and seedling regeneration stages. Differences between F_e and F_{IS} can be used to assess the similarity of expected genotypic proportions based on the mating system versus observed genotypic proportions as a net result of all evolutionary forces operating on the regeneration of

these stands. Estimates of F_e were not significantly ($P > .05$) different than mean estimates of F_{IS} for both the seed crop and seedling regeneration in both shelterwoods (Table I.16), although $\hat{F}_{IS}$ for the seedling stage was nearly significantly ($P < .10$) different from $\hat{F}_e$ in the Benshell shelterwood. Based on the mating system alone, a slight deficiency of heterozygotes (i.e., $\hat{F}_{IS} > 0$) should have been observed for both the seed crop and seedling regeneration stages. Although none of the mean estimates of F_{IS} were significantly different from estimates of F_e, there was a consistent trend for heterozygote excess ($\hat{F}_{IS} < 0$) in seed crops and heterozygote deficiency in seedling regeneration ($\hat{F}_{IS} > 0$) (Table I.16).

Discussion

Shelterwood harvesting and regeneration of two Douglas-fir stands in southwest Oregon does not appear to have significantly impacted the genetic structure of the natural regeneration of these stands. There was no detectable loss in genic diversity among the four life cycle stages sampled, and with the exception of LAP2 at Benshell, allele frequencies were homogeneous among life cycle stages. However, these results may not be strictly applicable to shelterwood regeneration in other species, or even for other Douglas-fir shelterwoods in southwest Oregon. The Benshell and Cutmore stands were comprised of mostly old-growth trees and were located on a high elevation (915 m) plateau on the west slope of the Cascades. Environmental conditions for regeneration and survival on these sites may be quite different from some of the more xeric sites commonly found in southwest Oregon. Therefore, impacts on genetic structure of shelterwood regenerated

stands should also be evaluated for a variety of site types, particularly those sites which are difficult to regenerate in this region (i.e., hot, dry, skeletal soils).

It is also possible that the way in which these stands were sampled could have lead to a failure to detect real differences in genetic structure among life cycle stages, especially if the shelterwood stands prior to harvest were genetically different than the adjacent uncut stands, or if the seed crop sample was not representative of the genetic composition of the seed crops which produced the seedling regeneration sample. However, if either of these conditions were true, one would expect to observe genetic differences between the sampled life cycle stages, and not the invariance of genetic structure observed in this study.

Several hypotheses can be made as to why there were no observed differences in genetic structure among life cycle stages in the two shelterwood stands. For both shelterwood stands, mean expected heterozygosities were only slightly less than that estimated for the entire breeding zone within which these stands were located. The large component of within stand variability observed in this study and others (Yeh and O'Malley 1980) and the high heterozygosities of individual trees within stands would be expected to help maintain diversity in shelterwood regenerated stands, even when the number of parent trees has been drastically reduced.

The density of parent trees in shelterwoods was reduced by approximately 85 percent as a result of the shelterwood harvest. This large reduction in the number of parent trees might potentially lead to a loss of allelic diversity or a chance fluctuation in gene

frequencies due to genetic drift. There was little evidence for gene frequency shifts due to genetic drift in these shelterwoods, with the possible exception of LAP2 at Benshell. Although the increase in the frequency of LAP2-2 in the leave tree stage at Benshell may have been due to sampling drift during the shelterwood harvest, this shift in gene frequency was transient because the frequency of LAP2-2 in both the seed crop and seedling regeneration was very close to that of the uncut stand. Apparently, large effective population sizes are maintained in these shelterwoods, even though parent tree densities are much less than uncut stands. The effective population sizes (N_e) were estimated to be no less than 31 trees (see chapter III). Nei et al. (1975) have shown theoretically, for neutral alleles, that much of the heterozygosity will be retained within populations even when the effective population size is 10 or less.

Natural selection must certainly be operative in natural populations of Douglas-fir (Campbell 1979, Hermann and Lavender 1968), but there is little evidence from this study that the shelterwood system has imposed a drastically different set of selection pressures during the harvesting and regeneration of these stands. The slight increase in the frequency of the LAP2-2 allele at the leave tree stage could possibly have been due to some form of selection for this allele, or other gene(s) closely linked to LAP2, in the process of selecting leave trees for the shelterwood, although it is highly unlikely that the increased fitness of a single gene is associated with superior phenotype of shelterwood leave trees. There is also little evidence to suggest that selection pressures on the seed and seedling regeneration in shelterwoods are any different than those under which

Douglas-fir naturally regenerates after fire or catostrophe. However, it is possible that the 10 allozyme loci assayed are neutral or do not mark differentially selected portions of the genome and therefore shifts in allele frequencies at differentially selected loci were undetected.

Gene flow would have the potential to change the population genetic structure of the seed crop or seedling regeneration relative to the uncut stand or leave trees in these shelterwoods. In order for a gene to immigrate into a population it must first be incorporated into the population and survive to produce offspring. The relative invariance of allele frequencies between the leave tree stage and the seed crop or seedling regeneration stages and the near absence of new alleles in the regeneration would indicate that there has been little gene flow into these stands during one generation of shelterwood harvesting and regeneration. However, dispersal analyses conducted in chapter III showed that a minimum of 20 percent of the seed and pollen sampled within the shelterwood study area came from trees outside the study area. This large amount of seed and pollen immigration into the study plot, without an associated shift in gene frequencies, would indicate that trees surrounding the shelterwood study areas were genetically similar to trees within the study area, or that trees outside and within the study area were members of the same panmictic unit. However, the observed lack of allozyme differentiation among coastal Douglas-fir populations (personal communication, W. T. Adams, Department of Forest Science, Oregon State University; Yeh and O'Malley 1980) would argue that gene flow must be important in the macroevolution of Douglas-fir.

The mating system, in part, determines genotypic proportions in the seed crops and seedling regeneration of shelterwoods. Outcrossing rates were approximately 95 percent in these shelterwoods (see chapter II) and the observed genotypic proportions (measured by the estimation of F_{IS}) in the seed crops and seedling regeneration were not significantly different from those expected by the equilibrium value of the inbreeding coefficient (F_e), and were also not significantly different than Hardy-Weinberg proportions. However, both shelterwoods displayed a trend towards increased heterozygosity in the seed crop and decreased heterozygosity in the seedling regeneration.

The difference in predicted versus observed inbreeding in the seed crops may reflect fertility differences in the parent population and may be suggestive of some selection against selfed progenies between cone ripening and the collection of the seed crop. However, this would have to be followed by selection for homozygous progenies at the seedling stage in order to explain the observed genotypic proportions at the seedling regeneration stage. These hypotheses are speculative and it is best to conclude that both the seed crop and seedling regeneration have genotypic proportions that are not significantly different than Hardy-Weinberg proportions.

The shelterwood regeneration system did not appear to impact the genetic structure of natural regeneration in two stands in southwest Oregon, even with the large reduction in the parent population prior to regeneration. There was little evidence for differences in allelic frequencies or levels of genic diversity among four life cycle stages in these stands. These results can be attributed to several factors; 1) maintenance of the effective population size after the reduction in

density of the parent population, 2) high within stand and individual tree heterozygosities, and 3) a high rate of outcrossing within stands. These forces preserve genetic diversity in these stands under the current management conditions. However, if the density of leave trees was reduced substantially more than the reductions at the Benshell or Cutmore stands, then a loss of genetic variability or an increase in inbreeding in the regeneration of these stands could result from genetic drift or by an increase in selfed or related matings due to the wide spacing among leave trees. Douglas-fir stands with a small proportion of within stand variability, possibly due to narrow adaptation or stand history, may also not be suitable for shelterwood harvest and regeneration. Therefore, many factors should be considered when assessing the impacts of the shelterwood regeneration system for the long-term management of the Douglas-fir gene pool in southwest Oregon.

Table I.1. Site and stand history information for the Benshell and Cutmore shelterwood stands.

	Benshell	Cutmore
Latitude	43°14'	43°16'
Longitude	122°21'	122°32'
Elevation (m)	975	975
Aspect	N	SW
Slope (%)	5	5
Average age (yr) of leave trees	200+	300+
Date of shelterwood harvest	1973	1972
Leave tree basal area (m^2/ha)	23	10
Leave trees per ha	35	15
Stocking, natural regeneration (seedlings/ha)	1212	1082

Table I.2. Numbers of individuals sampled at each life cycle stage (by tissue type) in two Douglas-fir study sites.

Stand	Life Cycle Stage	Tissue		
		Bud	Seed	Total
Benshell	Uncut Stand	39	11	50
	Leave Trees	13	36	49
	Seed Crop	--	69	69
	Seedling Regeneration	60	--	60
Cutmore	Uncut Stand	54	5	59
	Leave Trees	5	43	48
	Seed Crop	--	64	64
	Seedling Regeneration	60	--	60

Table I.3. List of enzyme systems assayed in seed and bud tissues of Douglas-fir and their corresponding electrophoretic buffers.

	Buffers[1]		
	Seed		Bud
Enzyme System	megagametophyte	embryo	
PGM (phosphoglucomutase)	A	A	D
PGI (phosphoglucose isomerase)	A	A	A
LAP (Leucine aminopeptidase)	A	A	A
GOT (glutamate-oxaloacetate transaminase)	B	B	B
G-6PD (glucose-6-phosphate dehydrogenase)	B	B	B
GLYD (glycerate dehydrogenase)	B	B	-
CAT (catalase)	B	B	-
GDH (glutamate dehydrogenase)	B	B	B
6PGD (6-phosphogluconate dehydrogenase)	C	C	C
IDH (isocitrate dehydrogenase)	C	C	D
DIA (diaphorase)	C	C	C

[1]Electrophoretic buffers and gel running amperages
A = Lithium borate pH 8.3 - Tris citrate pH 8.3 (Scandalious 1969), 75 mA.
B = Tris citrate pH 8.8 - Sodium borate pH 8.6 (Adams and Joly 1980), 70 mA.
C = Morpholine citrate pH 6.1 (Clayton and Tretiak 1972), 50 mA.
D = Histidine - citrate pH 8.0 (Fildes and Harris 1966), 35 mA.

Table I.4. List of allozyme loci resolved in seed and bud tissues in Douglas-fir.

Locus	Tissue		
	Seed		
	Megagametophyte	Embryo	Bud
PGM1	+	+	+
PGI2	+	+	+
LAP1	+	+	+
LAP2	+	+	+
GOT1	+	+	-
GOT2	+	+	+
GOT3	+	+	-
G-6PD	+	+	+
GLYD	+	+	-
CAT	+	+	-
GDH	+	+	+
6PGD	+	+	+
IDH	+	+	+
DIA	+	+	+

+Isozymes resolved clearly and scored in the designated tissue.

-Isozymes not resolved in the designated tissue or too poorly resolved to be scored.

Table I.5. Estimated allele frequencies for 14 allozyme loci at four life cycle stages in the Benshell shelterwood.

		Life Cycle Stages					
				Seed Crop			Seedling
Locus	Allele	Uncut Stand	Leave Trees	Ovule	Pollen	Embryo	Regeneration
PGM1	1	.140	.071	.159	.118	.140	.100
	2	.810	.868	.797	.824	.809	.842
	3	.050	.061	.044	.058	.051	.058
	N[a]	50	49	69	68	68	60
PGI2	2	.990	.980	1.000	1.000	1.000	.975
	3	b	.020				.017
	4	.010					.008
	N	50	49	69	68	68	60
LAP1	2	.400	.458	.492	.514	.529	.525
	5	.360	.195	.116	.265	.192	.283
	7	.240	.319	.334	.221	.279	.192
	8	(N)[c]	.028	.058	(N)	(N)	(N)
	N	50	36	69	68	68	60
LAP2	2	.040	.125	.014	.029	.022	.008
	3	.940	.847	.971	.942	.956	.950
	5	.020	.028	.015	.029	.022	.042
	N	50	36	69	68	68	60

Table I.5 (continued)

Locus	Allele	Life Cycle Stages					
				Seed Crop			Seedling
		Uncut Stand	Leave Trees	Ovule	Pollen	Embryo	Regeneration
GOT1	1	--[d]	1.000	1.000	1.000	.993	--
	2	--				.007	--
	N		36	69	69	69	
GOT2[e]	1	.113	.051	--	--	--	.092
	2	.867	.918	--	--	--	.908
	3	.020	.031	--	--	--	
	N	49	49				60
GOT3	1	--	.055	.101	.000	.052	--
	2	--	.903	.836	.735	.904	--
	3	--	.014	.029	.265	.022	--
	4	--	.014	.029		.015	--
	5	--		.015		.007	--
	7	--	.014				--
	N		36	69	65	68	
G6PD	1	.980	.949	.927	1.000	.961	.942
	3	.010	.010	.059		.031	.008
	4	.010	.041	.014		.008	.050
	N	50	49	66	68	65	60
GLYD	1	--		.014		.007	--
	2	--	.680	.609	.735	.676	--
	3	--	.306	.377	.265	.317	--
	4	--	.014				--
	N		36	69	68	68	

Table I.5 (continued)

Locus	Allele	Life Cycle Stages					
				Seed Crop			Seedling
		Uncut Stand	Leave Trees	Ovule	Pollen	Embryo	Regeneration
CAT	1	--	.278	.191	.265	.228	--
	2	--	.722	.809	.735	.772	--
	N		36	68	68	68	
GDH	1	1.000	.986	1.000	1.000	1.000	.975
	2		.014				.025
	N	50	36	69	68	68	60
6PGD	1	.010	.020	.014		.007	
	2	.950	.939	.899	.941	.918	.950
	3	.040	.041	.087	.059	.075	.050
	N	50	49	68	68	67	60
IDH	1	.140	.235	.159	.279	.220	.158
	2	.860	.755	.826	.706	.766	.825
	3				.015	.007	
	5		.010	.015		.007	.017
	N	50	49	69	68	68	60
DIA	1	.270	.255	.130	.132	.132	.192
	3	.730	.745	.870	.868	.868	.800
	4						.008
	N	50	49	69	68	68	60

Table I.5. (continued)

Footnotes

[a]Number of gametes (seed crop megagametophyte and pollen) or genotypes (uncut stand, leave trees, seed crop embryos, seedling regeneration) in sample.

[b]Blank indicates allele was not detected in sample.

[c]Allele LAP1-8 was a null allele that could only be scored in megagametophytes.

[d]--indicates locus not expressed in tissue type used at specific life cycle stage under electrophoretic conditions in this study.

[e]GOT2 allele frequency estimates in the seed crop are missing due to a fault in the eletrophoretic procedure while assaying the seed crop seeds.

Table I.6. Estimated allele frequencies for 14 allozyme loci at four life cycle stages in the Cutmore shelterwood.

		Life Cycle Stages					
				Seed Crop			Seedling
Locus	Allele	Uncut Stand	Leave Trees	Ovule	Pollen	Embryo	Regeneration
PGM1	1	.136	.063	.063	.117	.075	.142
	2	.796	.885	.859	.866	.883	.817
	3	.068	.052	.078	.017	.042	.041
	N[a]	59	48	64	60	60	60
PG12	2	.992	.980	.985	1.000	.992	.975
	3	.008	.010	.015		.008	.017
	4	b	.010				.008
	N	59	48	64	62	62	60
LAP1	2	.553	.512	.360	.515	.444	.483
	5	.245	.232	.312	.235	.278	.267
	7	.202	.233	.297	.250	.278	.250
	8	(N)[c]	.023	.031	(N)	(N)	(N)
	N	57	43	64	64	63	60
LAP2	2	.034	.083	.078	.016	.048	.033
	3	.941	.885	.891	.984	.935	.934
	5	.025	.032	.031		.017	.033
	N	59	48	64	62	62	60

Table I.6. (continued)

Locus	Allele	Life Cycle Stages					
				Seed Crop			Seedling
		Uncut Stand	Leave Trees	Ovule	Pollen	Embryo	Regeneration
GOT1	1	--[d]	1.000	1.000	1.000	1.000	--
	2	--					--
	N		43	64	64	64	
GOT2	1	.062	.063	.078	.097	.092	.075
	2	.912	.917	.922	.903	.908	.925
	3	.026	.020				
	N	57	48	64	62	60	60
GOT3	1	--	.023	.016	.016	.017	--
	2	--	.977	.968	.984	.975	--
	3	--		.016		.008	--
	4	--					--
	5	--					--
	N		43	64	62	60	
G6PD	1	.923	.938	.984	.984	.984	.958
	3	.034	.031				
	4	.043	.031	.016	.016	.016	.042
	N	59	48	64	62	61	60
GLYD	1	--					--
	2	--	.607	.613	.452	.533	--
	3	--	.393	.387	.548	.467	--
	N		42	62	62	61	

Table I.6. (continued)

Locus	Allele	Life Cycle Stages					
				Seed Crop			Seedling
		Uncut Stand	Leave Trees	Ovule	Pollen	Embryo	Regeneration
CAT	1	--	.326	.281	.323	.311	--
	2	--	.674	.719	.677	.689	--
	N		43	64	62	61	
GDH	1	1.000	1.000	1.000	1.000	1.000	1.000
	2						
	N	59	48	64	64	64	60
6PGD	1	.034	.010		.016	.008	.025
	2	.924	.927	.984	.903	.943	.950
	3	.042	.063	.016	.081	.049	.025
	N	59	48	64	62	61	60
IDH	1	.135	.156	.234	.250	.246	.200
	2	.797	.823	.750	.718	.730	.775
	3		.010				.008
	5	.068	.010	.016	.016	.016	.017
	8				.016	.008	
	N	59	48	64	64	63	60
DIA	1	.229	.240	.297	.250	.275	.259
	3	.771	.760	.703	.750	.725	.741
	N	59	48	64	60	60	60

Table I.6. (continued)

Footnotes

[a]Number of gametes (seed crop megagametophyte and pollen) or genotypes (uncut stand, leave trees, seed crop embryos, seedling regeneration) in sample.

[b]Blank indicates allele was not detected in sample.

[c]Allele LAP1-8 was a null allele that could only be scored in megagametophytes.

[d]--indicates locus not expressed in tissue type used at specific life cycle stage under electrophoretic conditions in this study.

Table I.7. Contingency chi-square analysis for the test of heterogeneity in allele frequencies among four life cycle stages in the Benshell Shelterwood.

Locus	No. of alleles	Chi-square	D.F.	P
PGM1	3	3.489	6	.745
PGI2	3	6.458	6	.373
LAP1	3	11.400	6	.076
LAP2	3	20.317	6	.002
GDH	2	5.593	3	.133
G6PD	3	8.753	6	.187
6PGD	3	4.651	6	.589
IDH	4	9.973	9	.352
DIA	3	10.380	6	.109
(Combined)		81.013	54	.010

Table I.8. Contingency chi-square analysis for the test of heterogeneity in allele frequencies among four life cycle stages in the Cutmore Shelterwood.

Locus	No. of alleles	Chi-square	D.F.	P
PGM1	3	6.416	6	.378
PGI2	3	2.661	6	.850
LAP1	3	3.855	6	.696
LAP2	3	4.289	6	.637
GOT2	3	6.988	6	.321
G6PD	4	11.250	9	.258
6PGD	3	4.374	6	.626
IDH	4	15.057	9	.089
DIA	2	.864	3	.834
(Combined)		55.754	57	.521

Table I.9. Observed heterozygosities (H_o) for LAP2 at four life cycle stages in two shelterwood stands.

		Life Cycle Stage		
Stand	Uncut Stand	Leave Trees	Seed Crop	Seedling Regeneration
Benshell	.120	.224	.092	.067
Cutmore	.085	.167	.143	.100

Table I.10. Summary of estimated genic diversity measures (standard errors in parentheses) for 4 life cycle stages in the Benshell shelterwood.

Life Cycle Stage	Mean Sample Size Per Locus	Loci	Alleles Per Locus (A)	Polymorphic Loci % (P)	Heterozygosity[a] Observed (H_o)	Heterozygosity[a] Expected (H_e)
Uncut Stand	50.0	9	2.4 (0.2)	88.9	.211 (.072)	.211 (.073)
Leave Trees	49.0	9	2.7 (0.2)	100.0	.215 (.050)	.244 (.069)
Seed Crop	65.0	9	2.4 (0.3)	77.8	.197 (.061)	.205 (.066)
Seedling Regeneration	60.0	9	2.8 (0.1)	100.0	.180 (.049)	.213 (.062)

[a]Estimates of H_o and H_e were not significantly ($P>.05$) heterogeneous among life cycle stages based on Fisher's chi-square test (Rao 1973).

Table I.11. Summary of estimated genic diversity measures (standard errors in parentheses) for 4 life cycle stages in the Cutmore shelterwood.

Life Cycle Stage	Mean Sample Size Per Locus	Loci	Alleles Per Locus (A)	Polymorphic loci % (P)	Heterozygosity[a] Observed (H_o)	Heterozygosity[a] Expected (H_e)
Uncut Stand	58.6	10	2.7 (0.3)	90.0	.200 (.048)	.225 (.058)
Leave Trees	48.0	10	2.9 (0.3)	90.0	.217 (.055)	.220 (.057)
Seed Crop	56.0	10	2.5 (0.2)	90.0	.223 (.067)	.219 (.065)
Seedling Regeneration	60.0	10	2.6 (0.3)	90.0	.210 (.060)	.214 (.062)

[a]Estimates of H_o and H_e were not significantly ($P>.05$) heterogeneous among life cycle stages based on Fisher's chi-square test (Rao 1973).

Table I.12. Chi-square goodness-of-fit values (1 degree of freedom) for deviation of genotypic frequencies from expected Hardy-Weinberg proportions in 4 life cycle stages in the Benshell shelterwood.

	Life Cycle Stage			
Locus	Uncut Stand	Leave Trees	Seed Crop	Seedling Regeneration
PGM1	0.032	1.146	0.104	0.231
PGI2	0.005	0.021	--[a]	0.039
LAP1	3.624	12.250**	0.118	3.213
LAP2	0.204	1.361	0.152	5.337*
GDH	--[a]	0.005	--[a]	0.039
G6PD	0.021	0.142	0.104	3.498
6PGD	0.139	0.208	0.555	0.166
IDH	1.325	0.002	2.122	1.081
DIA	0.065	0.020	0.066	1.667

[a]Monomorphic locus

* significant at the .05 probability level
** significant at the .01 probability level

Table I.13. Chi-square goodness-of-fit values (1 degree of freedom) for deviation of genotypic frequencies from expected Hardy-Weinberg proportions in 4 life cycle stages in the Cutmore shelterwood.

Locus	Life Cycle Stage			
	Uncut Stand	Leave Trees	Seed Crop	Seedling Regeneration
PGM1	1.570	0.804	0.023	0.768
PGI2	0.004	0.022	0.005	0.039
LAP1	6.053*	0.333	0.492	0.000
LAP2	3.417	3.798	0.331	12.934**
GDH	--[a]	--[a]	--[a]	--[a]
GOT2	0.863	0.397	0.481	0.394
G6PD	0.506	0.297	0.077	0.113
6PGD	0.402	0.297	0.249	5.337*
IDH	0.125	2.223	1.493	2.275
DIA	0.004	0.973	0.029	1.194

[a]Monomorphic locus

* significant at the .05 probability level
** significant at the .01 probability level

Table I.14. Estimated fixation indices, F_{IS}[a], (standard errors in parentheses) for 9 loci at each of 4 life cycle stages in the Benshell shelterwood.

		Life Cycle Stage		
Locus	Uncut Stand	Leave Trees	Seed Crop	Seedling Regeneration
PGM1	.068(.153)	-.111(.085)	-.087(.105)	.041(.137)
PGI2	-.010(.131)	-.021(.120)	--[b]	-.020(.023)
LAP1	-.011(.141)	.375(.155)	.084(.121)	.204(.127)
LAP2	-.049(.074)	.102(.192)	-.306(.067)	.303(.257)
GDH	--[b]	-.010(.021)	--[b]	-.026(.055)
G6PD	-.015(.075)	-.045(.060)	-.033(.060)	.247(.230)
6PGD	-.044(.063)	-.050(.073)	-.084(.035)	-.053(.018)
IDH	-.163(.043)	.019(.145)	.188(.132)	.150(.150)
DIA	.036(.144)	-.020(.141)	-.032(.111)	.175(.145)
Mean[c]	-.024(.039)	.027(.040)	-.038(.036)	.113(.050)

[a] F_{IS} values were calculated as a weighted mean over estimates for individual alleles.

[b] Monomorphic locus.

[c] Unweighted mean estimate across loci.

Table I.15. Estimated fixation indices, F_{IS}[a], (standard errors in parentheses) for 9 loci at each of 4 life cycle stages in the Cutmore shelterwood.

	Life Cycle Stage			
Locus	Uncut Stand	Leave Trees	Seed Crop	Seedling Regeneration
PGM1	.159(.146)	-.094(.092)	-.028(.118)	-.124(.098)
PGI2	-.009(.116)	-.016(.115)	-.009(.043)	-.020(.079)
LAP1	.261(.128)	.033(.152)	-.012(.126)	.078(.129)
LAP2	.252(.233)	.199(.191)	-.062(.032)	.211(.210)
GOT2	.140(.183)	-.073(.067)	.093(.166)	-.081(.045)
G6PD	-.064(.074)	-.053(.085)	-.028(.043)	-.043(.140)
6PGD	-.061(.078)	-.068(.054)	-.058(.046)	.134(.217)
IDH	-.039(.124)	-.188(.077)	-.138(.113)	-.161(.103)
DIA	-.009(.129)	.142(.155)	.023(.131)	.141(.136)
Mean[b]	.070(.047)	-.013(.039)	-.024(.034)	.015(.046)

[a] F_{IS} values were calculated as a weighted mean over estimates for individual alleles.

[b] Unweighted mean estimate across loci.

Table I.16. Comparison of $\hat{F}_e$ with $\bar{\hat{F}}_{IS}$ (standard errors in parentheses) for two life cycle stages in each of two shelterwoods.

Shelterwood	$\hat{t}_m$ [a]	$\hat{F}_e$ [b]	$\bar{\hat{F}}_{IS}$ Seed Crop	$\bar{\hat{F}}_{IS}$ Seedling Regeneration
Benshell	.959(.018)	.021(.009)	-.038(.036)	.113(.050)
Cutmore	.945(.020)	.028(.011)	-.024(.034)	.015(.046)

[a] Proportion of progeny due to outcrossing, see Table II.3.

[b] $\hat{F}_e = \dfrac{1 - \hat{t}_m}{1 + \hat{t}_m}$

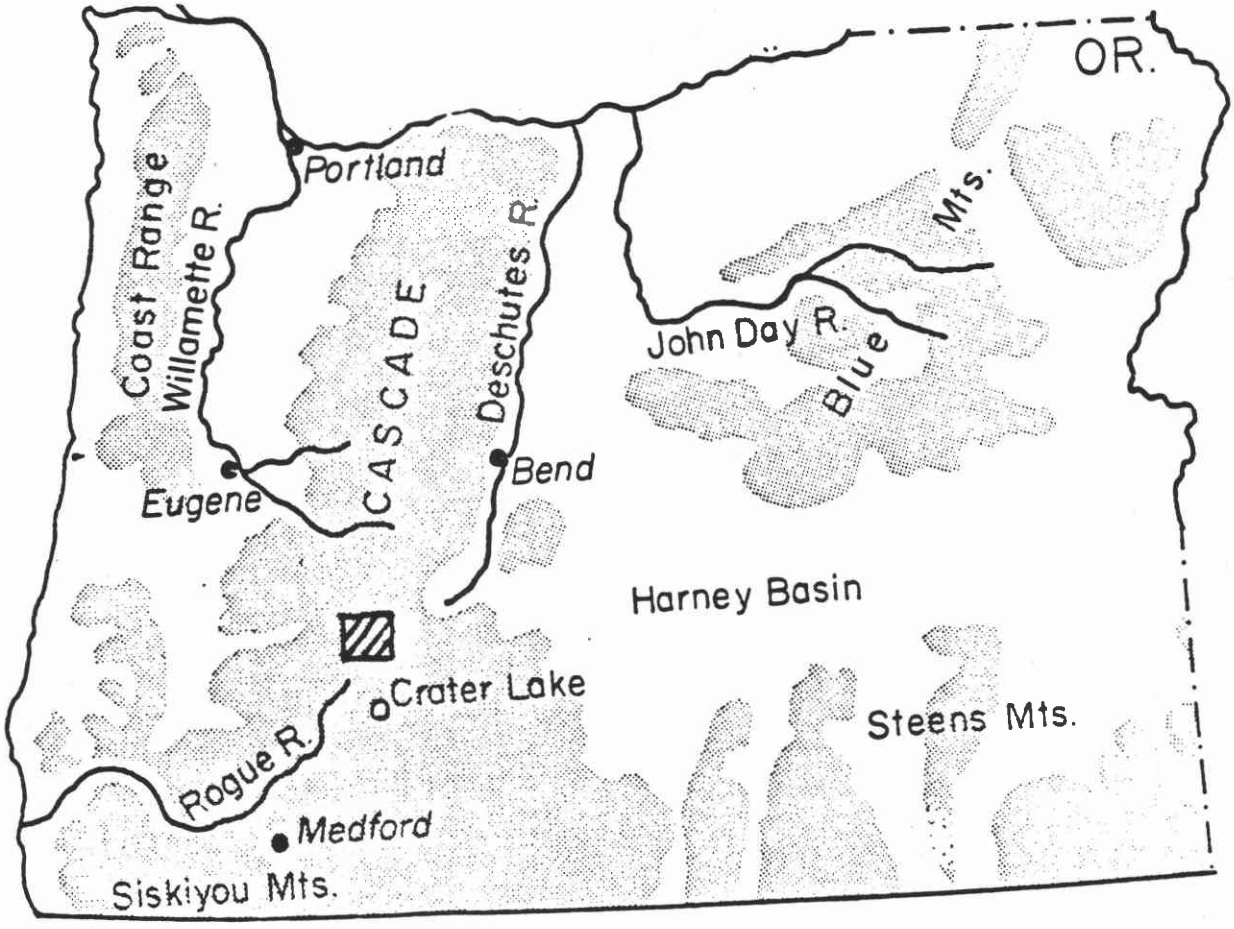

Figure I.1. Location of shelterwood study sites (▨) in southwest Oregon.

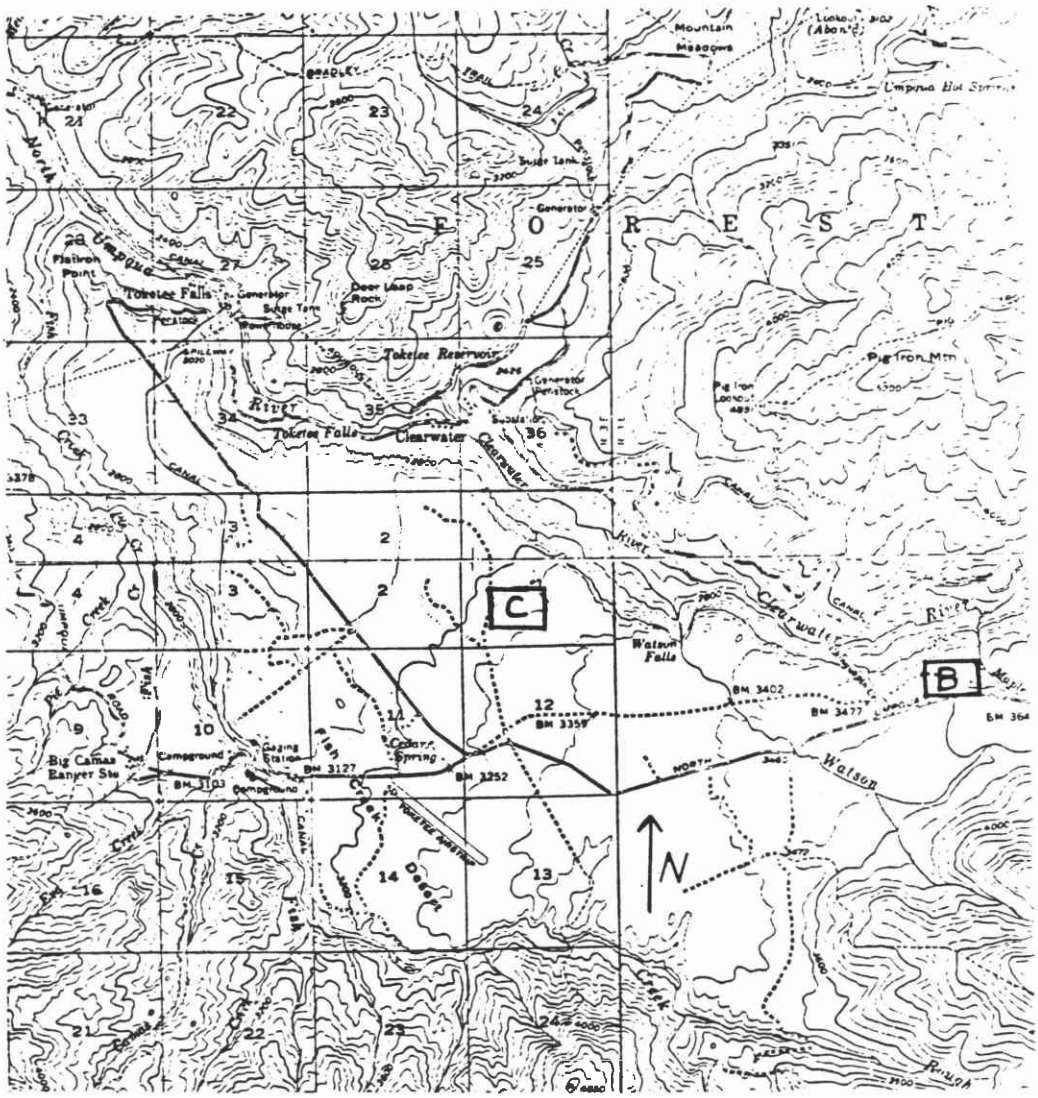

Figure I.2 Approximate locations of Benshell (B) and Cutmore (C) shelterwood study sites on the Diamond Lake District, Umpqua National Forest, Oregon (composite map of Toketee Falls and Garwood Butte 15' series, USGS).

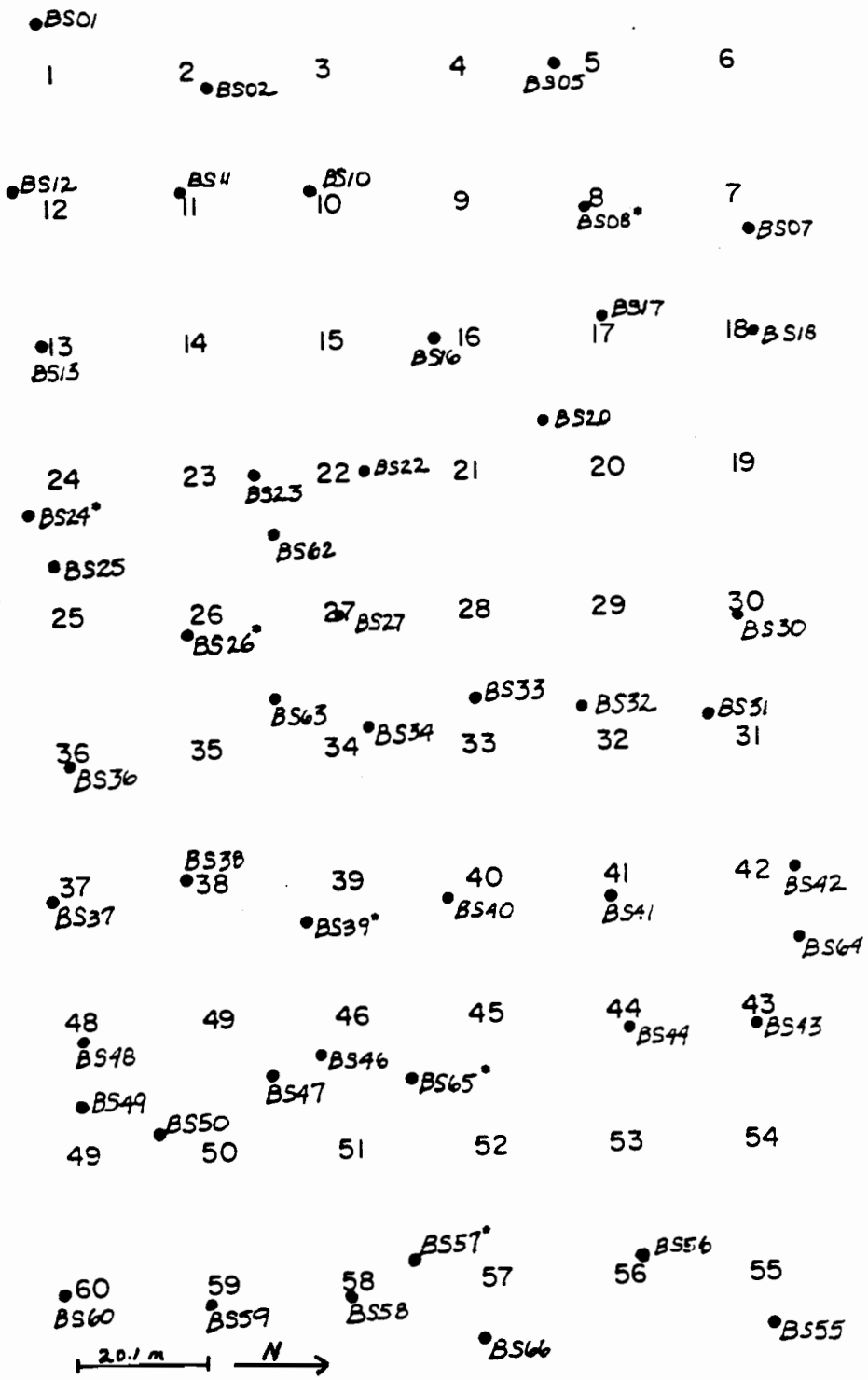

Figure I.3 Location of leave trees in the Benshell shelterwood. • indicates position of leave tree and boldface numbers are sample points.

* indicates leave trees used for individual tree mating system estimation (Chapter II).

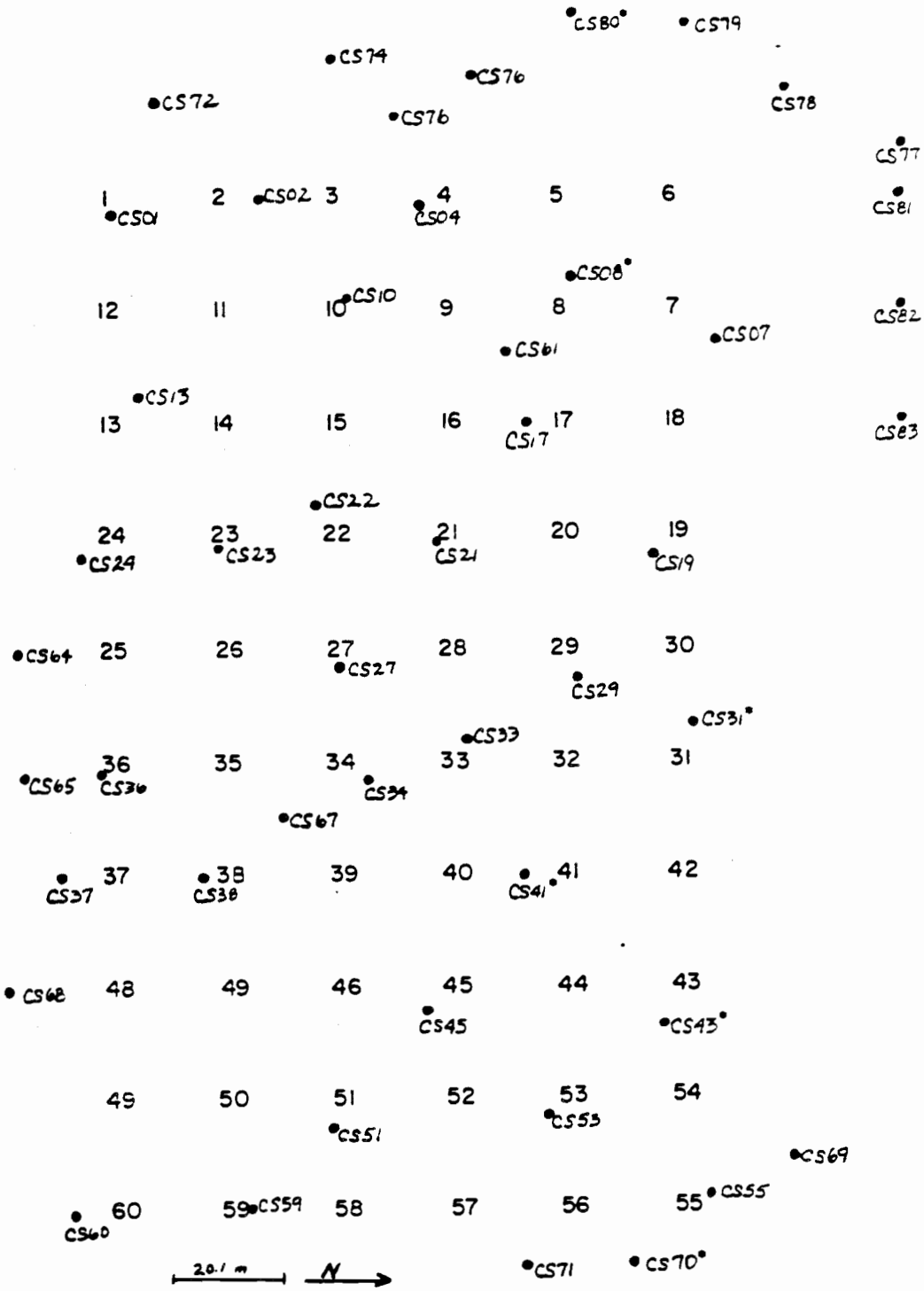

Figure I.4 Location of leave trees in the Cutmore shelterwood. • indicates position of leave tree and boldface numbers are sample points.

* indicates leave trees used for individual tree mating system estimation (Chapter II).

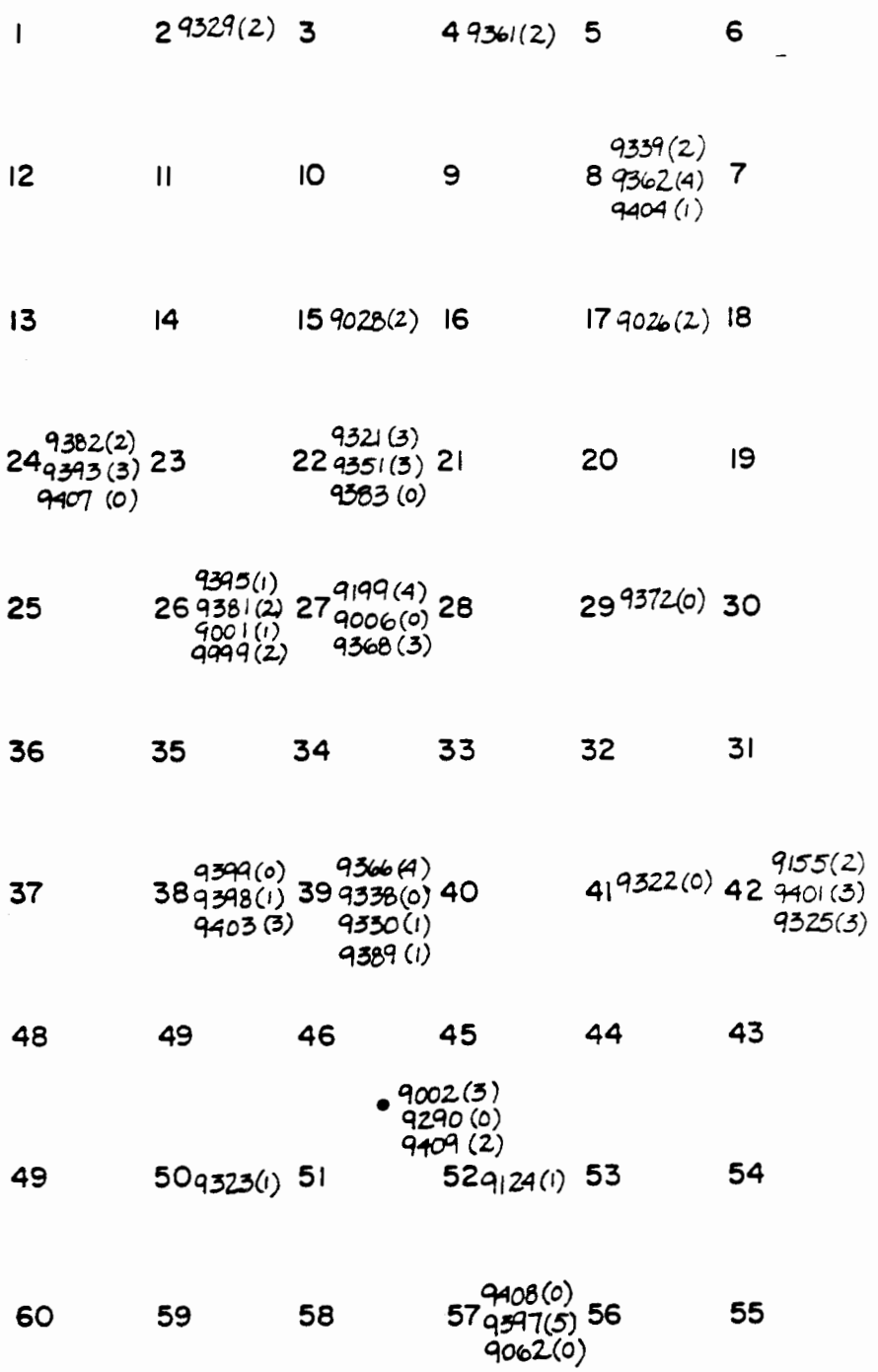

Figure I.5 Location and 4 digit identification number of seed traps and numbers of seeds caught per trap (in parentheses) in the Benshell shelterwood. Boldface numbers are sample points.

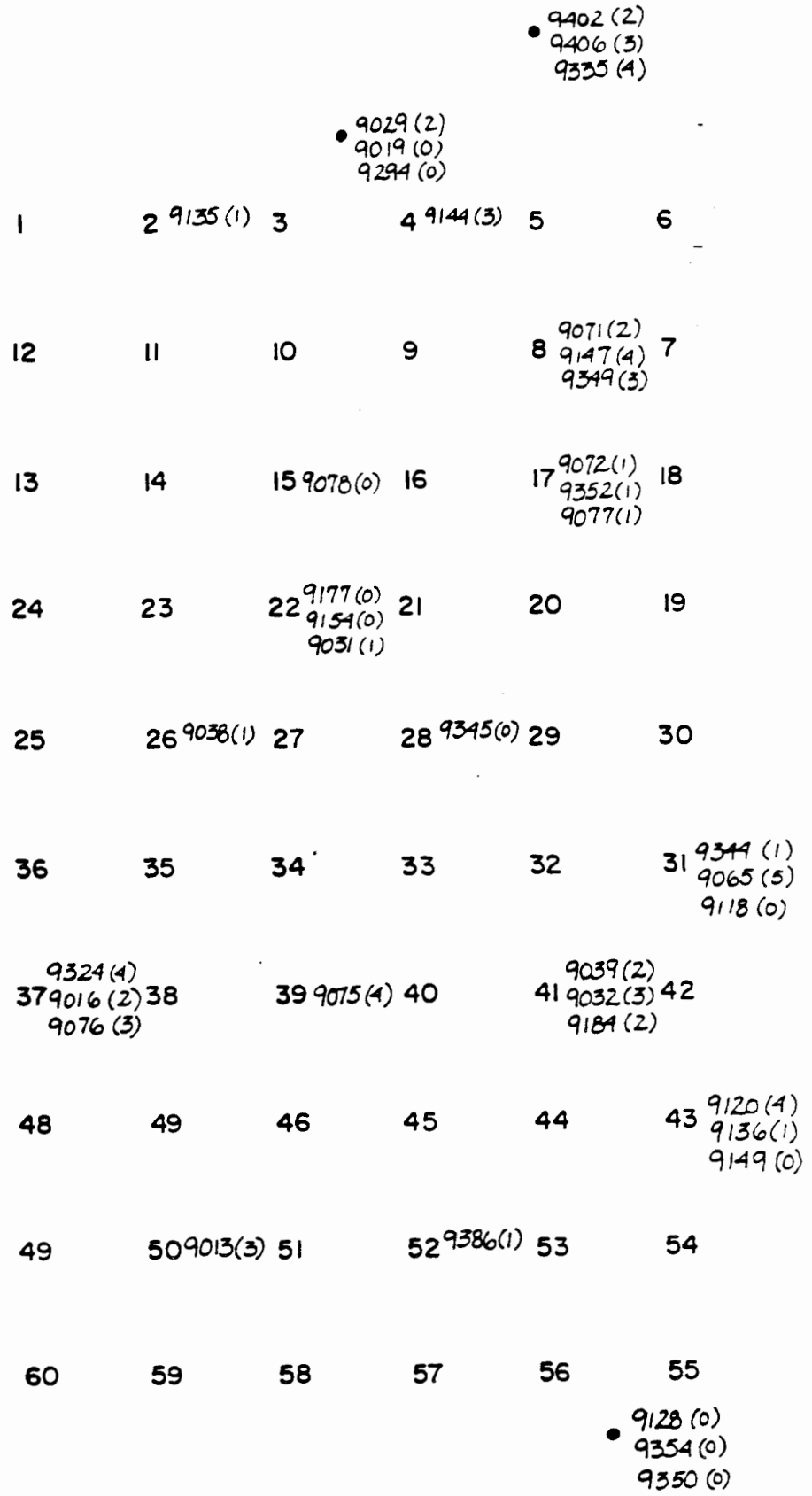

Figure I.6 Location and 4 digit identification number of seed traps and numbers of seeds caught per trap (in parentheses) in the Cutmore shelterwood. Boldface numbers are sample points.

CHAPTER II. THE MATING SYSTEM IN NATURAL AND SHELTERWOOD STANDS OF DOUGLAS-FIR

Introduction

The mating system is an important determinant of the genetic structure of plant populations (Clegg 1980). Random mating is often assumed for cross-pollinating species, even though some form of non-random mating (selfing, assortative mating) is characteristic of many plants. Although there recently has been more interest in studying mating systems (see Table II.1), the understanding of the mating biology of forest trees is still very incomplete. Two studies in natural populations of Douglas-fir (*Pseudotsuga menziesii* var. *menziesii*) have shown that the average rate of outcrossing is approximately 90%, the remaining 10% being due to self-fertilization (El-Kassaby et al. 1981, Shaw and Allard 1982). Both of these studies were conducted in stands of less than 100 years in age. There have not been any studies on the mating system of old-growth (200+ yr) stands or of shelterwood stands. In this study, the mating systems of old-growth natural stands and shelterwood stands of Douglas-fir in southwest Oregon were compared.

There are many reasons why the study of mating systems in shelterwoods is of interest. A typical Douglas-fir shelterwood has approximately 20-30 trees per hectare, whereas an old-growth natural stand has about 100 trees per hectare. The large increase in the spacing between trees in shelterwoods may potentially increase the proportion of self-fertilized progenies, as has been suggested by a study of a *Pinus sylvestris* seed tree stand in Sweden (Rudin et al.

1977). Inbreeding in the progeny of shelterwood regenerated stands might become appreciable, especially if a large proportion of self-fertilized progenies survive.

Inbreeding depression in Douglas-fir is well documented (Orr-Ewing 1957, Rehfeldt 1978, Sorensen 1971, 1973, Sorensen and Miles 1974), although survival of selfed progenies is predicted to be very low (Sorensen 1982). However, selfs from open-pollinated seed grown in nurseries may have a higher probability of surviving if selection against selfs in the nursery environment is relaxed. This is especially important when open-pollinated seed for artificial regeneration is collected from thinned seed production areas or where thinning around selected seed trees is practiced.

There is some evidence that the outcrossing rate in Douglas-fir varies among individual trees. Average outcrossing rates ranged from 72.5 to 100% among 19 trees scattered across western Oregon (Sorensen 1973). It was therefore of interest to determine if outcrossing varies among individual open-grown trees within a single shelterwood stand. Variation in the mating system among trees within the shelterwood could potentially impact the population genetic structure of the natural regeneration in a different way than if all trees displayed the same rate of outcrossing. Likewise, it is important to be aware of mating system variation if open-pollinated seed is collected from individual open-grown trees.

There were two objectives in this study. The first was to use allozyme genetic markers to estimate the rate of outcrossing in two 200+ year old natural stands and in two shelterwood stands

immediately adjacent to the natural stands. The effect of plant density on the mating system of Douglas-fir could then be inferred. The second objective was to estimate the rate of outcrossing at six individual leave trees in both shelterwoods to investigate individual tree variability in mating system in shelterwood stands.

Materials and Methods

Two Douglas-fir shelterwood stands (Benshell and Cutmore), each adjacent to an uncut natural stand, were located within 4.2 km of each other on the Diamond Lake District of the Umpqua National Forest in southwest Oregon. Both shelterwoods and uncut stands were comprised of mostly old-growth (200+ yr) Douglas-fir and were situated on a flat plateau south of the Toketee Ranger Station (see Figures I.1, I.2 and Table I.1, chapter I). Spacing of leave trees in shelterwoods was fairly uniform, but the density of leave trees in the Benshell shelterwood (35 trees/ha) was more than twice that of the Cutmore shelterwood (15 trees/ha). The density of trees in uncut stands was approximately 100 trees/ha.

Trees were sampled for mating system analysis by first imposing a 201 m x 121 m (10 x 6 chains) sampling plot, with intersecting grid lines (sample points) at every 20.1 m (1 chain), on each shelterwood and uncut stand. The plot areas were 2.4 hectares and there were a total of 60 sample points per plot. The shelterwood sample plots were separated from uncut stand 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 in the shelterwood stands. Due to the wide spacing of leave trees in

shelterwoods and the poor cone production in 1980, all cone bearing trees within the shelterwood plots were included in the sample. Cone-bearing trees were not found at all sample points, so the total number of trees sampled in each shelterwood was 34 (Benshell) and 38 (Cutmore).

In order to estimate outcrossing in individual shelterwood trees, an additional 75 cones were collected from 6 leave trees in each shelterwood plot. The 6 trees per shelterwood were chosen so as to sample over the entire plot, but with the restriction that they had enough cones with seed to permit the analysis. The location within shelterwoods of all sampled trees is shown in Figures I.3 and I.4, chapter I.

It was also planned to sample cones from one tree at each sample point in the uncut stands. Because 1980 was a poor cone year and because cone production is normally very light in closed-canopy, old-growth stands, cones were collected from only 10 trees in each uncut stand. These trees were distributed fairly uniformly about the sample plot. Self-fertilization has been shown to be higher in the lower crowns of Douglas-fir seed orchard trees (Omi 1983, Shaw and Allard 1982), therefore cones were collected from the mid to upper region of crowns whenever possible.

Two types of information, necessary for mating system estimation, can be inferred from electrophoretic analysis of seed progenies of parent trees: (1) the multilocus allozyme genotype of the maternal parents (leave trees); and (2) the multilocus allozyme genotype of the pollen gamete which fertilized the embryo of each seed progeny. The multilocus genotype of the maternal parents was inferred

by assaying 10 megagametophytes (1N) from each tree (probability of misidentifying maternal genotype (p) at a locus is less than .002, where $p = 1/2^{n-1}$ and n is the number of megagametophytes assayed). The multilocus genotype of the pollen gamete can be 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 gamete 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 assaying at least 10 megagametophyte-embryo pairs from each tree in the population sample. Individual tree outcrossing estimates required the assaying of 46-125 (mean of 88) megagametophyte-embryo pairs from each tree. All seeds were assayed for 13 allozyme loci (PGM, PGI2, LAP1, LAP2, GOT2, GOT3, G-6PD, GLYD, CAT, GDH, 6-PGD, IDH, and DIA). Details of electrophoretic methods can be found in chapter I.

Statistical Methods

The mixed-mating model, as first described by Fyfe and Bailey (1951), classifies all matings as either selfs or outcrosses. Estimation 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 and s is 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) there is no selection between germination and census of seed progenies. Two types of estimation procedures (single locus and multilocus) were used in this study to estimate the mating system parameter t. Single locus procedures were used to obtain population estimates of outcrossing for the shelterwoods and uncut stands. Multilocus procedures were used for both population estimates of outcrossing and for estimates from individual leave trees.

Single Locus Population Estimation

Statistical procedures for the estimation of t with single locus data were first described by Fyfe and Bailey (1951). Brown and Allard (1970) presented a two-step procedure for the single locus estimation of maternal genotypes in the population and the joint estimation of t and outcross pollen pool frequencies. This method was modified for use in conifers to a simpler one-step procedure by Shaw and Allard (1982), where maternal genotypes and pollen gametes successful in fertilization can be inferred directly from megagametophyte-embryo pair data. The one-step procedure (see Shaw (1980) for a more detailed explanation) requires diallelic data for the single locus estimation of t ($\hat{t}_s$). Therefore, two parameters can be estimated using this method, t and p (p = frequency of the A_1 allele in the outcross pollen pool). For loci with more than two alleles, Shaw and Allard estimated t_s for all possible diallelic combinations (i.e., 3 alleles, 3 combinations, etc.) and a mean estimate of t_s was calculated for each locus.

Shaw (1980) observed large differences in the estimates of t_s within any one locus, depending upon how the data were reduced for estimation with the diallelic model. For this reason a triallelic model was applied in this study whenever three alleles were observed at a locus. A few loci (LAP1, GOT3, IDH, GLYD) had more than three alleles, although the frequencies of the fourth or fifth most common alleles were very rare. For these loci, a triallelic model was still applied by preserving the two most common alleles and bulking the remaining alleles into a synthetic class. By using both diallelic and triallelic models, synthetic reduction of the data was rarely necessary in this study. The maximum likelihood procedures for the joint estimation of t and the outcross pollen pool allele frequencies using a diallelic model were given in Shaw and Allard (1982). The maximum likelihood estimator for a triallelic model is given in appendix C. Also given in appendix C is a description of the computer programs used for solving the maximum likelihood estimator. There must be a minimum of two or three maternal genotypic classes for estimation of t_s when a diallelic or triallelic model, respectively, is employed. The minimum requirement of two maternal genotypic classes was not met in all cases, therefore t_s could not be estimated for all 13 loci in all populations.

Multilocus Population Estimation

Multilocus population estimates of outcrossing (t_m) for the uncut and shelterwood stands were obtained using the maximum likelihood estimator of Green et al. (1980). The essence of the multilocus method is to classify each seed

progeny of a maternal parent as either: (1) a detectable outcross, or (2) ambiguous (resulting from either a self or outcross). Progenies are classified by comparing the multilocus genotype of the pollen gamete to that of the maternal parent. If the pollen gamete has an allele at any locus, which is not carried by the maternal parent, then that progeny is classified as a detectable outcross, otherwise the progeny is classified as ambiguous. This is done for all progenies of all maternal parents in the population. The multilocus estimator of Green et al. (1980) includes an estimate of the detection probability for each maternal parent. The detection probability is defined as the conditional expectation of detecting an outcross given that an outcross has occurred at that maternal parent. In this study, the detection probability for each maternal parent was estimated from the frequency of alleles in the outcross pollen pool (outcross pollen pool allele frequencies are estimated jointly with t_s in the single locus procedure). An estimate of t_m is obtained by solving the likelihood equation numerically, since each maternal parent in the population has an associated number of detectable outcrosses and a detection probability.

Green et al. (1980) derived an expression for the variance of t_m which assumes that the detection probabilities are known and constant. This assumption was violated in this study, therefore the variance of t_m should be considered a minimum estimate. The estimators for the detection probability, t_m and var t_m are described in detail in appendix C.

Multilocus estimates are theoretically less sensitive to violations of assumptions of the mixed mating model than single locus estimates (Shaw 1980). Multilocus estimates are more robust to the assumption of homogeneity of allele frequencies in the outcross pollen pools of 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, then the single locus procedure will tend to underestimate t_s. Therefore, Shaw and Allard (1982) have proposed that estimates of t_s can be compared with an estimate of t_m from the same population, to indirectly estimate the amount of inbred matings, other than selfs, in the population. Multilocus estimates are also more robust than single locus estimates to the assumption of independence between the rate of outcrossing and the genotype of the maternal parent and the assumption of no selection between germination and census of seed progenies. Single locus estimates are more prone to violation of these assumptions because cross incompatabilities or differential selection are more likely to be marked by specific loci, thus biasing frequencies in the progeny arrays at individual loci only. The multilocus procedure requires one additional assumption that is not made using the single locus procedure, that being that there are no associations among loci in the outcross pollen pool. Shaw et al. (1981) have shown with simulations that the potential biases in multilocus estimation are small if loci are not in strong linkage disequilibrium. For this reason, PGI2 was excluded from the multilocus analysis because PGI2 and GOT2 have been shown to be closely linked in Douglas-fir (El-Kassaby et al. 1982b).

Individual Tree Multilocus Estimation

Estimates of outcrossing for individual trees can also be obtained using multilocus techniques. The maximum likelihood estimator of Green et al. (1980) can be solved implicitly for the multilocus outcrossing rate of an individual tree (t_{m_i}), where $t_{m_i} = \frac{r}{G}$, and r is the proportion of detectable outcrosses and G is the detection probability (see appendix C). If the parameters r and G are estimated from the same data, the variance of t_{m_i} is a function of the individual variances of the parameters and the covariance of r and G (see appendix C). Expressions for the variances of r and G are easily derived, but their covariance is complex and an expression cannot be easily derived. If r and G could be estimated independently, the covariance term can be eliminated from the estimate of the variance of t_{m_i}. The parameters r and G can be estimated independently be removing the information on the tree for which t_{m_i} is to be estimated when the G for that tree is estimated. The estimators for t_{m_i} and var t_{m_i} are given in detail in appendix C.

Tests of Significance

Several tests of significance were performed on the estimates of t. All single locus and multilocus population estimates were tested with a likelihood ratio test (Brunk 1975) to determine if the estimates were significantly different from t = 1.0. Fisher's heterogeneity chi-square tests were performed on single locus estimates within a stand and on individual tree multilocus estimates within stands (Rao 1973). Chi-square tests were also used to test for

differences in outcrossing between uncut and shelterwood stands (Rao 1973). These tests were performed with mean single locus estimates and with multilocus population estimates.

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 ($P < .05$) heterogeneous in three of the four stands (Table II.2). The estimates were not significantly ($P > .05$) heterogeneous at the Benshell uncut stand, which may have been in part due to the large standard errors of the individual estimates, even though the range of estimates was very large (.410-1.114). Large differences in outcrossing estimates among loci have been previously 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. 1981b)). Only a few of the individual locus estimates of t_s were significantly ($P < .05$) different from $t_s = 1.0$. Unweighted means of single locus estimates for the uncut stands ($\bar{\hat{t}}_s = 0.884$ and 0.923) were similar to mean single locus outcrossing estimates previously reported for natural stands of Douglas-fir ($\bar{\hat{t}}_s = 0.90$, Shaw and Allard 1982; $\bar{\hat{t}}_s = 0.90$, El-Kassaby et al. 1981). At both the Benshell and Cutmore study sites mean single locus estimates for the shelterwood stands were slightly higher, but not significantly ($P > .05$) different, from mean estimates for the uncut stands.

Multilocus Population Estimates

In all cases, the multilocus population outcrossing estimates ($\hat{t}_m$) were higher than mean single locus estimates for individual stands (Table II.3). The differences cannot be tested statistically, however, because $\bar{\hat{t}}_s$ and $\hat{t}_m$ were estimated from the same data in each stand. Despite the slightly higher estimates of outcrossing with the multilocus technique, estimates from both the shelterwood stands were significantly ($P < .05$) different from $t_m = 1.0$. The multilocus outcrossing estimate for the Benshell uncut stand was exactly the same as that for the Benshell shelterwood ($\hat{t}_m = .959$), but was not significantly ($P > .05$) different from $t_m = 1.0$ due to its larger standard error. The estimate of t_m was slightly higher in the Cutmore uncut stand than the shelterwood, but this difference was not significant ($P > .05$). Multilocus estimates from both uncut and shelterwood stands were higher than multilocus estimates from 7 out of 8 Douglas-fir stands in western Oregon reported by Shaw and Allard (1982) ($\bar{\hat{t}}_m$ for 8 stands was .90).

Individual Tree Multilocus Estimates

Single tree multilocus outcrossing estimates ($\hat{t}_{m_i}$) among the 6 trees in each shelterwood ranged from .904 to 1.100 at Benshell and .922 to 1.014 at Cutmore (Table II.4). Estimates were significantly ($P < .05$) heterogeneous among the 6 trees at Benshell, but were not so at Cutmore. A total of 4 trees had estimates of t_{m_i} greater than 1.0. Individual tree multilocus outcrossing estimates greater than 1.0 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 (see appendix C for a discussion of the estimator). Mean individual tree outcrossing estimates ($\bar{\hat{t}}_{m_i}$) for the 6 trees in each stand were slightly higher than the multilocus population estimates ($\hat{t}_m$) for the two shelterwood stands. The differences cannot be tested statistically because the 6 trees used for the individual tree estimates were included in the population samples used in the estimation of t_m. Even if $\bar{\hat{t}}_{m_i}$ and $\hat{t}_m$ were statistically independent, it is unlikely that they would be statistically different based on their respective standard errors. Mean individual tree outcrossing estimates were slightly higher than the mean estimate ($\bar{\hat{t}}$ = .93) reported by Sorensen (1973) for 19 trees scattered about western Oregon.

Discussion

Population estimates of outcrossing in this study were generally higher than previously published population estimates for coastal Douglas-fir. The mean proportion of progeny due to outcrossing was estimated to be 90 percent in eight 25-100 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). In addition, 90 percent of the progeny of clonal seed orchards in western Oregon were found to be the result of outcrossing (Omi 1983, Shaw and Allard 1982). Differences in outcrossing estimates between this study and other studies may reflect differences in the allozyme loci assayed, estimation procedures, unknown year or site specific factors, or may be just due to chance. However, the higher outcrossing rates in these stands may have been related to stand age. The uncut and

shelterwood stands in this study were comprised of mostly 200+ year old trees, while trees in the previously cited studies were less than 100 years old. Higher outcrossing estimates in these 200+ year old stands might be a function of age or other environmental or physical factors associated with old-growth stands (i.e., tree height, density, proximity of male and female flowers). Although mating systems have been shown to vary temporally (Moran and Brown 1980) and over generations (Kahler et al. 1975), there have been no reports of age-related mating system variation in long lived conifers.

Comparison of single locus and multilocus population estimates allows for inferences on other forms of inbreeding other than selfing (Shaw 1980). For all stands, the multilocus estimates ($\hat{t}_m$) were greater than the mean single locus estimates ($\bar{\hat{t}}_s$). Assuming that the estimates of $\hat{t}_m$ and $\bar{\hat{t}}_s$ were statistically independent (which they are not in this study), the estimates for each stand were not significantly ($P > .05$) different. However, the average difference in the estimates was approximately .07 for uncut stands and .01 for shelterwood stands (Tables II.2 and II.3). One assumption of the mixed-mating model is that there is no difference in the outcross pollen pool among maternal parents. This assumption could be violated by the presence of localized pollen pools, possibly as a result of family structuring in the population. If there are many related matings due to family substructuring, single locus estimation results in underestimation of the proportion of outcrossed progeny in the population. This bias is theoretically less severe with multilocus estimation because the multilocus procedure more efficiently discriminates between selfed and outcrossed matings, especially if a large

number of variable loci are employed. Therefore, the larger differences between $\bar{\hat{t}}_s$ and $\hat{t}_m$ for the uncut stands, compared to the shelterwoods, may have been due to a higher proportion of related matings in the uncut stands than in the shelterwoods. The higher proportion of related matings may be a function of family substructuring in uncut stands, whereas family substructuring may have been eliminated in shelterwood stands as a result of harvesting.

Plant density can influence the mating system. For insect pollinated species, it has generally been observed that outcrossing is inversely related to plant density (Ellstrand et al. 1978). However, it has been assumed that a positive association exists between plant density and outcrossing for wind-pollinated species. Rudin et al. (1977) presented indirect evidence that outcrossing decreases with lower plant density in Pinus sylvestris. In this study, there were no significant differences in the multilocus population outcrossing estimates ($\hat{t}_m$) between the uncut stands and shelterwoods, indicating that the large reduction in density due to the shelterwood harvesting of these stands had little or no impact on the mating system. For the Benshell study site, t_m was equal to .959 for both the uncut stand and shelterwood. For the Cutmore stand, $\hat{t}_m$ was 5.5 percent higher for the uncut stand. Although this difference was not significant, it may be more related to factors other than density, such as crown position of sampled cones. At the Cutmore uncut stand, a large proportion of cones in this stand were located in the tops of the closed-canopy trees. If outcrossing is higher in upper crowns, as has been suggested by studies in Douglas-fir seed orchards (Omi 1983, Shaw and Allard 1982), then this may explain the slightly

higher estimate of t_m for the Cutmore uncut stand. Estimates of t_m were not significantly ($P > .05$) different for the two shelterwood stands, even though the density of leave trees at Cutmore was less than one half that of Benshell. Mating systems need to be investigated in stands of low density to determine the point at which density effects the rate of outcrossing.

The results of the single tree outcrossing estimation indicate that although the mixed mating model assumption of invariance of outcrossing among maternal parents is not valid, the differences among trees were not large. All estimates were within a range of .90-1.00+. In the only other study of individual tree outcrossing in natural populations of Douglas-fir, Sorensen (1973) observed a wide range (72-100%) of outcrossing rates among trees. However, by design, the 19 trees sampled for Sorensen's study were located over a wide geographical area, across nearly 1500 m in elevation, and were sampled from a range of stand structure conditions (density, age-class distribution, and mixture of other species). Furthermore, outcrossing estimates were based on the frequency of single gene mutant markers in the seedling progenies of open-pollinated seed. If the range in outcrossing reported by Sorensen (1973) is characteristic of the species, the ranges observed within two individual shelterwood stands would indicate that the within stand component of individual tree mating system variability is fairly large. Large differences in outcrossing among individual trees have been reported for Pinus sylvestris (67-89%, Rudin et al. 1977) and Picea abies (75-93%, Lundkvist 1979), as well as among clones in two Douglas-fir clonal seed orchards (Omi 1983, Shaw and Allard 1982).

The high rate of outcrossing in Douglas-fir is an important component of the adaptive strategy of this species. 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 viable selfed progenies are on the order of 5-10%. The decreased fitness of inbred progenies (Sorensen and Miles 1982) probably insures that an extremely small percentage of these progenies survive to reproductive maturity.

Table II.1. Examples of outcrossing (t) estimates in natural populations of forest trees based on allozyme polymorphisms.

Species	Estimation procedure[a]	No. loci	$\hat{t}$ Range	$\hat{t}$ mean	Source
Abies balsamea	M	3	.78-.99[b]	.89	Neale and Adams (in preparation)
Eucalyptus delegatenis	M	3	.66-.85[b]	.77	Moran and Brown 1980
Eucalyptus obliqua	S	3	.42-1.05[b]	.76	Brown et al. 1975
Eucalyptus pauciflora	S	4	.30-.85[b]	.63	Phillips and Brown 1977
Picea abies	R	1	.74-1.00[c]	.89	Lundkvist 1979
Picea abies	R	1	.84-.93[c]	.88	Müller 1976
Pinus contorta	M	7	.32-.78[b]	.66	Carlson (unpublished)
Pinus ponderosa	S	1	--	.96	Mitton et al. 1977
Pinus ponderosa	S	6	.60-1.15[d]	.96	Mitton et al. 1981b
Pinus sylvestris	R	1	.91-.97[c]	.94	Müller 1977
Pinus sylvestris	S	4	.73-.95[d]	.83	Rudin et al. 1977
Pseudotsuga menziesii	M	11	.86-.96[b]	.90	Shaw and Allard 1982
Pseudotsuga menziesii	S	4	.62-1.12[d]	.90	El-Kassaby et al. 1981

[a]M = multilocus, S = single locus, R = rare allele
[b]Range of estimates across populations and/or years.
[c]Range of estimates across individual trees.
[d]Range of estimates across loci within a single population.

Table II.2. Single locus population estimates of outcrossing ($\hat{t}_s$, standard errors in parentheses) for two pairs (Benshell and Cutmore) of adjacent uncut and shelterwood stands of Douglas-fir in southwest Oregon.

	Benshell		Cutmore	
Locus	Uncut	Shelterwood	Uncut	Shelterwood
PGM1	.984(.081)	.857(.082)[a]	1.065(.028)	.961(.053)
PGI2	--[b]	1.011(.008)	--[b]	1.014(.008)
LAP1	1.057(.091)	.925(.048)	.995(.095)	.880(.049)[a]
LAP2	.947(.079)	.920(.053)	.894(.078)[a]	.911(.048)[a]
GOT2	.976(.168)	.948(.068)	--	.976(.051)
GOT3	.410(.387)[a]	1.004(.021)	.659(.234)	.885(.136)
G6PD	1.114(.045)	1.005(.007)	.936(.077)	1.018(.026)
GLYD	.858(.144)	.904(.064)	.897(.167)	.908(.070)
CAT	.883(.117)	1.009(.075)	.746(.158)	.912(.064)
GDH	--[b]	1.016(.009)	--[b]	--[b]
6PGD	--[b]	1.053(.031)	1.161(.058)	.833(.084)[a]
IDH	.859(.183)	.978(.066)	1.084(.044)	1.003(.048)
DIA	.753(.125)[a]	.779(.068)[a]	.792(.124)	.860(.070)[a]
mean[c]	.884(.053)	.955(.015)	.923(.039)	.930(.019)
χ^2_{HET}	16.09(9)	28.06(12)[d]	22.51(9)[d]	24.76(11)[d]

[a]Significantly different ($P < .05$) from $t_s = 1.0$ based on $\chi^2_{(1)}$ likelihood ratio test (Brunk 1975).

[b]Insufficient maternal genotypic classes for estimation of $\hat{t}_s$.

[c]Unweighted mean.

[d]$\hat{t}_s$ estimates significantly ($P < .05$) heterogeneous over loci.

Table II.3. Multilocus population estimates of outcrossing ($\hat{t}_m$, standard errors in parentheses) for two pairs (Benshell and Cutmore) of adjacent uncut and shelterwood stands of Douglas-fir in southwest Oregon.

Stand	Loci[a]	N[b]	$\hat{t}_m$	χ^{2c}
Benshell uncut	10	110	.959(.035)	1.74
Benshell shelterwood	12	385	.959(.018)	7.06[d]
Cutmore uncut	9	96	.997(.023)	0.01
Cutmore shelterwood	11	444	.945(.020)	9.70[d]

[a]Number of loci used in multilocus estimation.

[b]Total number of progeny in sample.

[c]Likelihood ratio test of $H_o : t_m = 1.0$, χ^2 with 1 degree of freedom.

[d]Significantly different than 1.0 at 5% probability level.

Table II.4. Individual tree multilocus outcrossing estimates ($\hat{t}_{m_i}$, standard errors in parentheses) for 6 leave trees in each of two Douglas-fir shelterwood stands (Benshell and Cutmore) in southwest Oregon.

Shelterwood	Leave tree	Loci[a]	N[b]	$\hat{t}_{m_i}$
Benshell	BS08	11	125	.917(.040)
	BS24	10	46	1.100(.109)
	BS26	10	90	.904(.041)
	BS39	11	94	.984(.054)
	BS57	11	95	.934(.035)
	BS65	11	97	1.011(.011)
mean[c]				.975(.023)
Heterogeneity[d]				14.67[e]
Cutmore	CS08	11	69	.947(.048)
	CS31	11	90	1.014(.012)
	CS41	11	105	.954(.048)
	CS43	11	105	.922(.062)
	CS70	11	91	.945(.049)
	CS80	11	87	1.009(.066)
mean[c]				.965(.021)
Heterogeneity[d]				6.54

[a]Number of loci used in multilocus estimation.

[b]Total number of progeny in sample.

[c]Unweighted mean.

[d]Test for heterogeneity among $\hat{t}_{m_i}$'s, χ^2 with 5 degrees of freedom.

[e]Significant at the 5% probability level.

CHAPTER III. GENETIC STRUCTURE OF DOUGLAS-FIR SHELTERWOOD STANDS

Introduction

Shelterwood harvesting is a common silvicultural practice for the regeneration of Douglas-fir (*Pseudotsuga menziesii* var. *menziesii*) stands in southwest Oregon. As an alternative to clearcutting, a small (25-35 per ha) number of uniformly distributed trees (leave trees) are left after logging to produce seed for the next generation. Although shelterwood harvesting imposes a management system on natural populations of old-growth Douglas-fir, the reproductive ecology of the shelterwood stand may be quite similar to that under which Douglas-fir regenerates after fire or other catastrophies. Therefore, the shelterwood provides an experimental system that can be used to study the development of stand structure in a forest tree population which has been reduced to a small number of parent trees prior to reproduction.

In the two previous chapters, genic diversity at several life cycle stages was inferred from isozyme genetic markers and the rate of outcrossing was estimated in two Douglas-fir stands in southwest Oregon. The goal of this portion of the study was to compare the genetic structure of the parental population to that of its offspring to evaluate the relative influence of evolutionary forces, particularly gene flow, on the genetic structure of shelterwood regenerated stands. Four specific objectives were identified: (1) develop techniques to estimate effective seed and pollen dispersal using allozyme markers and obtain preliminary estimates of dispersal distances in shelterwoods; (2) estimate the effective population size in

shelterwoods based on the preliminary dispersal distance estimates; (3) determine if there is genetic substructuring among groups of seedling regeneration and pollen pools within shelterwoods; and (4) explore mechanisms for the development of family substructure in shelterwood regenerated stands based on preliminary estimates of distances between parents and their offspring.

Coastal Douglas-fir is a long-lived (400+ year) tree with a very large and continuous distribution encompassing a range of highly heterogeneous environments (Silen 1978). It has high fecundity (Campbell 1979) and a wind-pollinated, primarily outcrossed mating system (Shaw and Allard 1982, chapter II). Furthermore, seeds (Isaac 1930) and pollen (Silen 1962) can travel considerable distances. These ecological and life history parameters would seem to be characteristic of a species where high amounts of gene flow maintain large population size and prevent population subdivision. However, the results of common garden studies in Douglas-fir provide evidence for microgeographical adaptation of populations to physiographic and environmental variables (Campbell 1979, Campbell and Sorensen 1978). These results would argue that strong selection is the primary determinant of population genetic structure and that gene flow (whether it be large or small) has only a limited role in determining patterns of variation among populations of Douglas-fir.

Family substructuring within forest tree populations could occur as a result of restricted seed dispersal about individual parent trees, especially if the density of parent trees is low. Knowledge of family substructuring will be useful for interpreting the results of mating system studies (Shaw and Allard 1982, chapter II) and would

also assist in designing efficient plus-tree selection schemes for tree improvement programs (Ledig 1974). Presently, evidence for family structure in natural populations is limited. Sakai and Miyazaki (1972) determined that genetically related individuals of Thujopis dolabrata may be clustered about a 20-25 m radius in natural stands. Linhart et al. (1981) have shown that a population of Pinus ponderosa in Colorado was subdivided into six groups which differed in allozyme frequencies. Rudin et al. (1977) have found some evidence for family structuring among leave trees in a Pinus sylvestris seed tree stand in Sweden. Finally, Tigerstedt et al. (1982) detected allele frequency heterogeneity among 37 regeneration subpopulations in a stand of Pinus sylvestris in Finland. Guries and Ledig (1977) were unable to detect family structuring within four stands of Pinus rigida.

Heterogeneous pollen pools among individual trees within stands could occur if small subsets of the pollen parents in the population contribute pollen to individual maternal parents. Allele frequency heterogeneity among pollen pools has been demonstrated for Abies balsamea (Neale 1978) and for Pinus ponderosa (Mitton et al. 1981b). Knowledge of pollen pool heterogeneity will be important for assessing the role of pollen dispersal in the development of family substructuring in natural populations and for determining the breadth of pollen parents in open-pollinated seed used in progeny testing.

The study of gene flow in forest trees has been largely ignored, with a few noteworthy exceptions (see Levin and Kerster 1974 for review). There have been a few attempts to measure the vehicles for gene flow (seed and pollen dispersal) in Douglas-fir (Isaac 1930,

Silen 1962, Wright 1952). Isaac (1930) counted seeds at varying distances from the edge of a clearcut and found that at 30 m there were still as many as 20,000 seeds/ha. Silen (1962) measured pollen dispersal at varying distances from isolated Douglas-fir trees (heights 29-37 m) and determined that the bulk of the pollen from the source trees was dispersed within 100 m. However, he also found that much of the pollen captured could not have come from the source tree, but had to have come from distant stands. Wright (1952) also estimated pollen dispersal distance from a point source by trapping pollen from a single Douglas-fir tree (height 8.3 m) in Indiana. He found that 66 percent of the pollen was dispersed within 20 m of the source tree. Even though pollen dispersal from individual parent source trees may be somewhat restricted, Douglas-fir pollen is still capable of travelling great distances (Silen 1962). Unfortunately, there have been no empirical studies in Douglas-fir to determine the proportions of pollen received by individual trees that came from nearby versus very distance sources. Until better estimates of the effective seed and pollen dispersal distance become available, it will be difficult to assess the relative importance of gene flow as a determinant of population genetic structure in Douglas-fir. Once seed and pollen dispersal and the mating system can be quantified, then it will be possible to derive accurate estimates of the effective population size. This information will not only be useful to tree breeders, but will also assist in delineating _in situ_ gene conservation areas.

Measurements of gene flow in plants have for the most part relied on indirect estimates of seed and pollen dispersal (see Levin 1981 and Levin and Kerster 1974 for reviews). These studies have been of three

major types; (1) marking of seeds or pollen prior to dispersal and capture of marked offspring (Levin and Kerster 1968, Price and Waser 1979), (2) measurement of pollinator flight distances (Beattie and Culver 1979, Levin and Kerster 1968, Schaal 1980), and (3) introduction of marker genes into experimental populations (Handel 1983, Levin 1981, Schaal 1980). These studies as such do not provide actual estimates of gene flow and may in fact under-estimate gene flow (Levin 1981). In this study, methods were developed to estimate effective seed and pollen dispersal within stands of Douglas-fir. The estimation procedure determines the most likely parent for a sampled seed or pollen gamete based on a multilocus likelihood of parentage and the physical distance between the parent and offspring. Once most likely parents are assigned to a large sample of seeds and pollen, then effective dispersal distances can be estimated. Assignment of most likely parents for a large number of individuals in a pool of offspring can also be used for inferences on family structure in regenerated stands.

Materials and Methods

Two Douglas-fir shelterwoods (Benshell and Cutmore) were located within 4.2 km of each other on the Diamond Lake District, Umpqua National Forest, Oregon. The Benshell and Cutmore stands were harvested in 1973 and 1972, respectively. Both stands were very similar in terms of site variables (slope, aspect, elevation) and stand histories (average age, age class distribution) except that the density of leave trees at Benshell (35 trees/ha) was much greater than Cutmore (15 trees/ha). Both stands had very little advance

regeneration, but had an average stocking of 1212 (Benshell) and 1082 (Cutmore) natural seedlings per hectare 8 years after harvest (personal communication, Silviculturist, Diamond Lake District, Umpqua National Forest). The age distribution of seedlings was not known, although most seedlings were between 1-10 years old.

Four classes of materials (leave trees, pollen pool, seed crop, and seedling regeneration) needed to be sampled in order to meet the objectives of this study. Allozyme genotypes and map positions within stands had to be determined for individuals sampled from each of these classes. A 121 m x 201 m (10 x 6 chain) plot, with grid intersections (sample points) at every 20.1 m (1 chain) was laid out prior to sampling in each shelterwood. The plot was 2.4 ha in size and there were a total of 60 sampling points.

Leave trees to be included in the sample were chosen by selecting the nearest cone bearing tree within a 10 m radius of each sample point, and approximately 25 cones were sampled from each tree in September 1980 (Table III.1). Since 1980 was a fairly poor cone year in these stands and because of the wide spacing of leave trees, no more than one cone bearing tree was located at each sample point and all cone bearing trees in the plot were sampled. An additional 75 cones were collected from 6 leave trees in each shelterwood. A sample of seed from these trees (mean 88, range 46-125) was to be used in a pollen pool analysis (Table III.1). The six trees were chosen so as to sample systematically over the entire plot, with the restriction that they had enough cones with seed to permit the analysis. If there were no cone bearing trees at a sample point, the nearest non-cone

bearing tree to the sample point was chosen for sampling and dormant vegetative buds were collected from it in April 1982 (Table III.1).

The 1980 seed crop was sampled in two ways. Three seed traps were placed surrounding (approximately 120° from one another) and approximately 7 m from the base of 10 leave trees in each shelterwood. These 10 leave trees were selected in order to sample across the entire plot, but also had to have a large number of cones in September 1980. Six of the ten trees were the same as those from which an additional 75 cones were sampled. An additional 10 seed traps were placed systematically about the study plot. It was originally planned to analyze the systematic and leave tree seed crop samples separately, but because of the poor seed crop in 1980 only a small number of seeds were collected in each trap, and the leave tree seed crop analysis had to be abandoned. Instead, the seeds collected in the leave tree seed crop sample were combined with those of the systematic sample to form a single population sample (Table III.1).

The seedling regeneration in each shelterwood was also sampled in two ways. A systematic sample of seedlings was obtained by selecting 4 seedlings at each of the 60 sample points (total of 240 seedlings per shelterwood) (Table III.1). One seedling was chosen at each of the four cardinal directions and approximately 2-4 m from each sampling point. The leave tree seedling sample was obtained by selecting 50 seedlings about a 10 m radius of the base of 6 leave trees in each shelterwood (total of 300 seedlings per shelterwood) (Table III.1). The leave trees were the same as those used for the pollen pool and seed crop analyses. All seedlings sampled were approximately within the 3-5 year old class. Several seedlings were

aged by counting annual rings prior to sampling in order to develop a method of sampling seedlings based on seedling height and number of lateral whorls.

Seed tissues (megagametophyte and embryo) were scored for 14 loci (PGM, PGI2, LAP1, LAP2, GOT1, GOT2, GOT3, G-6PD, GLYD, CAT, GDH, 6-PGD, IDH, and DIA) coding allozyme variants of 11 enzyme systems. Bud tissues were also scored for 14 loci (PGM1, PGM2, PGI2, LAP1, LAP2, GDH, GOT2, GOT4, G-6PD, SDH, 6PGD, IDH, DIA, and MDH3) coding variants of 11 enzyme systems. Inheritance of seed tissue isozymes has been reported by El-Kassaby et al. (1982a) and Adams, Smith, and Doerksen (in preparation). Inheritance of bud tissue isozymes was indirectly established by comparing isozyme band pattern phenotypes in buds to those expected based on genotypes of a large number of seed orchard clones determined from megagametophytes (personal communication, D. B. Smith, Department of Forest Science, Oregon State University) for all loci except SDH and GOT4. Band patterns for SDH in buds corresponded exactly to those in needle tissues for which inheritance has been established with Mendelian tests (Neale, Weber, and Adams, in preparation). GOT4 in buds and GOT1(N) in needles (see Neale, Weber, and Adams, in preparation) have been observed to be the same locus (personal communication, G. F. Moran, Department of Forest Science, Oregon State University). Details of electrophoretic procedures were described in chapter I.

Genotypes of leave trees were determined by assaying at least 10 megagametophytes per tree or were determined directly from the allozyme phenotypes expressed in bud tissues. The probability of misidentifying the genotype of an individual at any one locus (p), with a

sample of 10 megagametophytes, is less than 0.002 ($p < (1/2)^{n-1}$, where n = number of megagametophytes). Genotypes of pollen gametes were inferred from embryo genotypes by assaying megagametophytes and embryos simultaneously. The genotype of the maternal contribution to seeds (ovules) collected in seed traps was determined by assaying megagametophytes. Seedling genotypes were determined directly from bud tissue allozyme phenotypes.

Statistical Methods

Seed and Pollen Dispersal Estimation Procedure

Genetic markers have been used to model seed and pollen dispersal in experimental plant populations (Levin 1981). In the absence of unique genetic markers in natural populations, an estimation procedure was developed which takes advantage of genetic variability contained among loci in the parent population. If a large number of electrophoretic loci are variable in a population, it is possible to infer the true parent of seeds or pollen dispersed within the population with a fairly high likelihood based on the number of genes held in common between parents and offspring (seed or pollen). In conifer seeds, the genotype of the ovule is identical to that of the haploid megagametophyte. Therefore, inference of the true parent of dispersed seeds is based on the comparison of genotypes of megagametophytes of seeds and diploid genotypes of parent trees. By assaying both megagametophytes and embryos of individual seeds, it is also possible to infer the genotype of the pollen gamete effective in fertilization. The inference of the true parents of pollen gametes is then based on

collecting seed from individual trees, inferring the genotype of the pollen gametes from embryos, and then comparing to diploid genotypes of parent trees. Furthermore, the physical distance between parents and dispersed offspring (seed or pollen) can also be utilized in inferring true parents of seeds or pollen. In this section, procedures will be described which were used to infer the most likely maternal parents among shelterwood leave trees for a sample of seeds collected in seed traps, and most likely pollen parents for pollen gametes sampled by leave trees within the two shelterwood stands. Once distances between seeds or pollen and most likely parents were determined, preliminary estimates of seed and pollen dispersal within shelterwoods were obtained. This procedure could be employed to estimate dispersal in many conifer species, for which there is very little information on effective gene flow.

The statistical inference of the most likely parent of a seed or pollen gamete is a two-step procedure. In the first step, a multilocus likelihood of parentage is calculated for all possible parents in the population based on the multilocus genotype of the parent and the offspring. The second step utilizes information on distance between possible parents and the offspring to estimate a likelihood of parentage based on distance. The most likely parent is then inferred by calculating a joint likelihood of parentage based on the two individual likelihoods for each possible parent.

Prior to the inference of most likely parents for a sample of offspring, the multilocus genotype of all possible parents in the population must be known, as well as the multilocus genotype of each gamete (ovule or pollen). A genetic model was then developed to

determine the likelihood of parentage based on the multilocus diploid genotype of possible parents and the multilocus haploid genotype of ovule or pollen gametes. The model is as follows,

$$P(A_{ij}) = \prod_{\ell=1}^{k} g_{ij\ell} \ , \qquad (1)$$

where $P(A_{ij})$ = conditional probability of producing the multilocus genotype of the jth haploid offspring (ovule or pollen), given the offspring has come from the ith parental genotype.

$g_{ij\ell}$ = 1.0 if the ith parent is homozygous at the ℓth locus for the allele found at the ℓth locus in the jth offspring.

$g_{ij\ell}$ = 0.5 if the ith parent is heterozygous at the ℓth locus for the allele found at the ℓth locus in the jth offspring.

$g_{ij\ell}$ = 0 if the ith parent and the jth offspring have no alleles in common at the ℓth locus.

k = number of loci.

The derivation of $P(A_{ij})$ assumes that all loci used in the analysis are independent (i.e., no linkage). It is also assumed that there is no selection among parents in the production of seeds or pollen and that there is no differential selection among offspring (seed or pollen) genotypes.

If $P(A_{ij})$ is equal to zero for all i parents in the population, then the jth offspring is classified as an immigrant (i.e., the

offspring had to have come from a parent outside the sample population). If a large number of variable loci can be assayed from parents and offspring, then it is possible that there would be only one possible parent among the array of all possible parents in the population for a particular offspring (i.e., one possible parent with a value of $P(A_{ij}) > 0$). More likely, individual offspring will have several possible parents with different values of $P(A_{ij})$. In this situation, the information on distance between possible parents and offspring can be utilized to help infer the most likely parent from the array of possible parents.

In order to utilize the information on distance between parents and offspring, it is assumed that dispersal of offspring about a point source can be approximated by a circular normal distribution. This assumption is valid based on empirical distributions for seed and pollen dispersal reported in the literature (see Levin and Kerster 1974). The probability that an offspring is dispersed to a point on a segment of any concentric circle about the center is a function of two parameters; r, the radius of the concentric circle and θ, the angle of the segment of the concentric circle. If it can be assumed that dispersal is the same over all intervals of the angle θ for a given value of r, then the conditional probability of an offspring being dispersed distance r, given angle θ, can be written as; $f(r|\theta) \propto \exp[-r^2/2\sigma_\theta^2]$. The marginal density function of r, over any interval of θ for which σ_θ^2 is constant, is then; $f(r) \propto r \exp[-r^2/2\sigma_\theta^2]$. Now let X equal r^2. By changing the variable, f(X)

is proportional to $e^{-X/\beta}$, which is the familiar form of the negative exponential distribution[1] (Figure III.1).

The distribution function of a negative exponential has the form,

$$F(X) = 1 - e^{-X/\beta} , \tag{2}$$

where X is the squared distance between an offspring and a possible parent and β is the mean squared dispersal distance (i.e., σ_θ^2). By taking the first derivative of this function the probability density is,

$$f(X) = \frac{1}{\beta} e^{-X/\beta} . \tag{3}$$

The probability density can be used to estimate the likelihood that any seed or pollen gamete was dispersed a squared distance X from any possible parent in the population.

Before calculating f(X), an initial estimate of the mean squared dispersal distance ($\hat{\beta}_i$), must be determined to specify the density function. In this study, β_i was estimated as,

$$\hat{\beta}_i = \sum_{ij}^{n} r_{ij}^2/n, \tag{4}$$

where r_{ij}^2 is the squared distance between the ith genetic putative parent (i.e., the possible parent with the highest value of $P(A_{ij})$)

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[1]This derivation was provided by a personal communication with S. Overton, Department of Statistics, Oregon State University.

and the jth offspring, and n is the number of offspring. If more than one possible parent had the highest value of $P(A_{ij})$ then this genetic putative parent-offspring pair was not included in the estimation of β_i. By this method, the probability density function is specified by an estimate of β_i based on a subset of the data for which there was a single possible parent with the highest likelihood of parentage based on the multilocus genotype of the parent and the offspring.

Two likelihoods, $P(A_{ij})$ and $f(X_{ij})$, can then be assigned to all possible parent-offspring pairs for all j offspring in the population sample. The most likely parent of the jth offspring can then be inferred from the joint likelihood of parentage given as,

$$P(A_{ij}, X_{ij}) = P(A_{ij})f(X_{ij}), \tag{5}$$

where $P(A_{ij})$ and $f(X_{ij})$ are given in (1) and (3), respectively[2]. The possible parent with the highest value of (5) is the maximum likelihood estimate of the true parent of the jth offspring among all possible parents in the sampled population.

Once the most likely parents are inferred for all j offspring, the mean squared dispersal distance can be re-estimated as

$$\hat{\beta} = \sum_{ij} r_{ij}^2/n, \tag{6}$$

where r_{ij}^2, in this case, are the distances between inferred most likely parents and their offspring. The variance of $\hat{\beta}$ is

[2]Inference of the most likely parent with a joint likelihood was suggested by S. Overton, Department of Statistics, Oregon State University.

approximately equal to $\hat{\beta}^2/n$. This estimate of β represents the average dispersal distance throughout the study plot of many offspring from several parents. If there are large differences in dispersal distance depending on cardinal direction, due to prevailing winds etc., then these differences cannot be detected if only a single β is estimated. One way to visualize differences in dispersal due to direction would be to plot the position of offspring relative to their most likely parent. A composite plot, with the most likely parent at the center, can be used to visualize average dispersal patterns in study populations. Another way to investigate dispersal patterns would be to compute an estimate of β for different quadrants of the study plot depending on cardinal direction (i.e., north, south, east, and west), if there are enough data points to estimate four different β's. Both of these techniques will be used to study seed and pollen dispersal patterns in the two shelterwood stands.

The maximum likelihood procedure for the inference of most likely parents of seeds or pollen gametes can be modified to infer the most likely maternal parents of seedlings in the regeneration of shelterwoods. Unlike the model for seeds or pollen, the most likely maternal parent must be inferred using the diploid genotype of the offspring (seedling). Brown and Allard (1970) presented the conditional probabilities of obtaining a single locus progeny genotype, given the genotype of the maternal parent (Table 2 of their paper). The product of conditional probabilities across loci for any possible maternal parent-seedling pair can be used to infer the most likely maternal parent for a seedling offspring and is given as,

$$P(B_{ij}) = \prod_{\ell=1}^{k} C_{ij\ell}, \qquad (7)$$

where $C_{ij\ell}$ is the conditional probability of producing the jth seedling genotype at the ℓth locus, given the genotype of the ith maternal parent at the ℓth locus, and k is the number of loci. If it can be assumed that all progenies are the result of outcrossing, then the conditional proabilities given by Brown and Allard (1970) for a diallelic model reduce to those shown in Table III.2. Inference of most likely parents of seedlings also requires the same assumptions as the inference of parents for seed or pollen offspring.

Most likely parents for individual seedlings can be inferred using (5) by substituting $P(B_{ij})$ for $P(A_{ij})$ into the joint likelihood function. The estimate of β determined from the distances between seeds and their most likely parents was used as an initial estimate of β (β_i) in order to specify the density function for distance, equation 3. This estimate of β_i is justified because the mean squared distance between seedlings and their parents is likely to be equivalent to the mean squared dispersal distance for seeds.

Estimation of Effective Population Size

Wright (1946) showed that effective population size (N_e) can be estimated as a function of variance of dispersal distance (σ^2) and effective plant density (d):

$$\hat{N}_e = 4\pi d\sigma^2 \ . \qquad (8)$$

This formulation assumes that all individuals in the population are hermaphrodites, dispersion of male and female gametes is equivalent, mating is at random, and the distribution of offspring from individual parents follows a Poisson distribution. Levin and Kerster (1971) modified this formula to take into account differences in dispersal variance between seed and pollen and also added a correction for the amount of outcrossing in the population:

$$N_e = 4\pi dt \left(\frac{\sigma_2^2 + \sigma_p^2}{2}\right) , \qquad (9)$$

where t is the outcrossing rate and σ_s^2 and σ_p^2 are the variances of seed and pollen dispersal, respectively.

Estimates of leave tree density ($\hat{d}$) were made by foresters at the Diamond Lake Ranger District (Benshell $\hat{d}$ = 35 trees/ha, Cutmore $\hat{d}$ = 15 trees/ha). Multilocus estimates of outcrossing ($\hat{t}_m$) were determined in chapter II (Benshell $\hat{t}_m$ = .959, Cutmore $\hat{t}_m$ = .945). Empirical estimates of the variance of seed and pollen ($\hat{\sigma}_s^2$ and $\hat{\sigma}_p^2$) were determined using the formula:

$$\hat{\sigma}^2 = \frac{\sum_{i=1}^{n} (x_i - \bar{x})^2}{n-1} , \qquad (10)$$

where x_i is the dispersal distance of the ith offspring (either seed or pollen), $\bar{x}$ is the mean dispersal distance, and n is the total number of offspring. The effective population size (N_e) and the neighborhood area ($NA = N_e/d$) could then be estimated for both shelterwood stands. σ_s^2 and σ_p^2 were estimated using only dispersal distances between seed or pollen and their most likely parents within

shelterwoods using equation (5). If seeds or pollen were dispersed large distances beyond the study plot boundaries then these offspring would not have been sampled. Thus, σ_s^2 and σ_p^2 and hence N_e are considered to be minimum estimates.

Pollen Pool and Seedling Regeneration Substructure Analysis

Pollen pool and seedling regeneration substructure were each investigated in two ways. In the first method, allele frequencies in the 6 pollen pools and 6 groups of 50 seedlings within each shelterwood were tested for heterogeneity by calculating the chi-square statistic of Workman and Niswander (1970). F-statistics (Nei 1977) were also computed for the seedling groups to investigate population subdivision of seedlings. All analyses were performed using the BIOSYS-1 computer program (Swoffard and Selander 1981).

Substructure in pollen and seedling pools was also investigated by inferring most likely parents for all pollen gametes and seedlings within pools by calculating the joint likelihood of parentage for all pairs of offspring (pollen or seedlings) and possible parents.

Once the maximum likelihood parents were inferred then the number of parents which contributed offspring to a given pool (pollen or seedlings) at increasing distances from pools could be tabulated. The data presented in this way allows for an interpretation of the relative effect of seed and pollen dispersal within stands as a determinant of population substructure.

Results

Efficiency of Dispersal Estimation Procedure

The likelihood of parentage, based on the multilocus genotypes of the parent and offspring, was determined for possible parents of samples of seed, pollen, and seedlings in the two shelterwoods. Likelihoods were calculated by using equation (1) for seed and pollen, and equation (7) for seedlings. It is theoretically possible to assign individual offspring to just one possible parent given a very large number of polymorphic loci for the discrimination of the true parent. However, of the 18 loci (assayed from either seed or bud tissues) used in this study, only 3 loci had 3 or more alleles with frequencies greater than .05. Most loci had one common allele and 1 to 5 alleles in low frequencies. Furthermore, no parent tree was polymorphic at more than 6 loci. Therefore, individual offspring (seed, pollen, or seedlings) could not always be assigned to just one possible parent.

Seed offspring had anywhere from 1 to 11 possible parents (Figure III.2). For seeds with multiple possible parents, the likelihood of parentage based on genetics (equation (1)) ranged from 0.0156 to 1.00. These represent possible parents with 0 to 6 heterozygous loci. Pollen offspring generally had slightly more possible parents per offspring than seeds due to the necessity of bulking alleles in scoring embryos, from which pollen genotypes are inferred. Likelihoods for possible parents of pollen gametes also ranged from 0.0156 to 1.00. Seedlings had as few as 3 and as many as all (49 Benshell, 48 Cutmore) of the leave trees as possible parents.

Likelihoods for possible parents of seedlings ranged from .0000001 to .20. The lower level of precision associated with inferring most likely parents of seedlings was due to seedlings being diploid, whereas inference with seeds (ovules) and pollen was based on haploid genotypes.

Estimation of the joint likelihood of parentage based on genetics and distance (equation (5)) for possible parents of offspring improves the estimation of most likely parents in two ways. For the seed and pollen analyses, there were often two or more possible parents with the highest, but exactly the same, value of $P(A_{ij})$ (equation (1)). In this situation, the parent with the largest value of the joint likelihood would be the one nearest to the point where the offspring was sampled. The choice of the most likely parent of seeds using the joint likelihood (equation (5)) was different from that parent chosen by the likelihood of parentage based on genetics alone (equation (1)) approximately 20 percent of the times. For pollen, a different most likely parent was chosen by the joint likelihood 15 percent of the time. Inference of the most likely parent in these cases was made in favor of parent trees which were closer to the offspring but had a smaller value of the likelihood of parentage based on genetics. For seedlings, approximately 38% of the outcomes of inferred most likely parents changed. The larger amount of changed outcomes associated with seedlings was presumably due to the smaller differences in the values of the likelihoods of parentage based on genetics (equation (7)) among possible seedling parents as compared to differences in likelihoods (equation (1)) among seed or pollen parents. Therefore, the second way in which calculation of the joint likelihood improves

estimation is that it eliminates possible parents which are very distant from the offspring, but still have a high likelihood of parentage based on genetics alone. Because many parent trees can produce the same multilocus gametes or zygotes with equal likelihoods, the inference of most likely parents was improved by including the information on distance between possible parents and offspring.

Estimates of Seed and Pollen Dispersal

Seed Dispersal

Seed dispersal distance estimates were based on small samples of seeds (Table III.1), therefore estimates should be considered as preliminary. All cone bearing trees within the shelterwood study plots were sampled and genotyped, therefore all possible seed parents of the seed crop, within the study plot, were known. At the Benshell stand, 18 of 65 (26.6%) and at Cutmore, 7 of 42 (16.7%) of the seeds collected in seed traps were classified as immigrants. These estimates represent the minimum proportion of the seed crop which had maternal parents outside the boundary of the study plot. The proportion of seeds which had maternal parents just outside the boundary versus those that were dispersed great distances could not be determined from this analysis. In retrospect, it would have been prudent to genotype trees outside the study area to get a better estimate of the amount of long distance seed immigration into the study area. Seeds collected in seed traps which were dispersed from parent trees outside of the study plot would have been classified as immigrants or erroneously assigned to a parent tree within the plot. In so doing, seeds that

were dispersed great distances were not included in the estimation of the mean squared dispersal distance. However, if seeds are dispersed approximately as a negative exponential, this proportion of misclassified seeds should be small. Nevertheless, estimates obtained from this study should be considered minimum estimates.

The initial estimate of the mean squared dispersal distance ($\hat{\beta}_i$) was computed using 46 genetic putative parent-offspring pairs at Benshell and 30 pairs at Cutmore (i.e., seeds for which one possible parent had the largest value of $P(A_{ij})$). $\hat{\beta}_i$ was 6046.00 m at Benshell and 11901.81 m at Cutmore. A chi-square goodness-of-fit test was also performed to determine if the dispersal distances of the genetic putative parent-offspring pairs fit the negative exponential distribution. The test statistic was calculated by dividing the data into 10 classes, each representing a decile of the frequency distribution of dispersal distances. The expected dispersal distances for each decile of the frequency distribution were determined by rearranging equation (2) such that the expected distance, r, was equal to $[-\hat{\beta}_i \ln (1 - F(X))]^{1/2}$. The expected number of observations at each decile of the frequency distribution was n/10, where n is the number of genetic putative parent-offspring pairs. The observed number of pairs at each of the 10 values of r was then determined. The chi-square tests were non-significant ($P > .05$) for both Benshell ($\chi^2_{(8)} = 13.56$) and Cutmore ($\chi^2_{(8)} = 8.33$), therefore the null hypothesis that the distances between genetic putative parents and seed offspring in these shelterwoods are distributed as a negative exponential could not be rejected.

Most likely parents were then inferred for all 47 (Benshell) and 35 (Cutmore) seeds based on the joint likelihood of parentage (equation (5)). The mean squared dispersal distance (β) was then estimated for each stand. $\hat{\beta}$ was 2991.03 m (S.E. = 436.39) at Benshell and 7503.92 m (S.E. = 1268.39) at Cutmore. At the Cutmore stand, all but three most likely parent-seed offspring pairs had joint likelihoods ranging from .0004-.0066 (mean = .0027). The other three pairs had values less than .0002, which were substantially outside the range of all other pairs. The small joint likelihoods were due both to small likelihoods of parentage based on genetics and the maximum likelihood parent being a great distance from the seed. For these three seeds, it appeared as though the inferred most likely parent was not the true parent, the true parent being outside the study plot. A second estimate of the mean squared dispersal distance for Cutmore was then determined after eliminating these pairs ($\hat{\beta}$ = 5268.64 m (S.E. = 931.37)). This estimate of β was considerably less because the three eliminated pairs had large dispersal distances and because β was estimated from a sample of only 35 seeds. The elimination of the three pairs reduces the mean dispersal distance at Cutmore from 86 m to 72 m.

Composite plots of seed dispersal about a central point (i.e., seeds plotted relative to their inferred most likely parents) illustrate the average dispersal patterns in these stands (Figures III.3 and III.4). As was indicated by the $\hat{\beta}$'s, seeds appear to be dispersed greater distances at Cutmore. For both stands, more seeds are dispersed towards the north than the south. Also, seeds appear to be dispersed greater distances in the northeast direction. The

concentric circle drawn on each figure represents the area within which 50 percent of the seeds would be dispersed, on average, in each stand based on the estimate of β.

The number of seeds contributed by individual most likely parents was tabulated to determine if there were any obvious differences in seed production among shelterwood trees. At Benshell, 19 of 43 trees were among the most likely parents (mean = 1.84 seeds/tree, range = 1-4 seeds/tree) and there were 23 of 47 at Cutmore (mean = 2.04 seeds/tree, range = 1-5 seeds/tree). These results would indicate that estimates of β are not biased to any large extent by large differences in seed production among shelterwood leave trees.

Pollen Dispersal

All cone bearing trees in shelterwoods were sampled and were used as possible parents in the pollen dispersal analysis. Because pollen production records were not kept in this study, it was not known if some of the leave trees that were sampled for buds only, and were not included as possible parents, may have produced pollen in the spring of 1980. There is evidence from a Douglas-fir clonal seed orchard, that individual clones produce either both male and female flowers or no flowers at all (personal communication, W. T. Adams, Departent of Forest Science, Oregon State University). Therefore, it was assumed that all possible pollen parents within the study plot were included in the analysis, although it is possible that a small number were not.

Multilocus genotypes of a large sample (Benshell 547, Cutmore 574) of pollen gametes were inferred from embryos in the two shelterwoods. The minimum proportion of pollen gametes that came from trees

outside the study plot (immigrants) was 26.6% for Benshell and 20.4% for Cutmore.

Initial estimates of the mean squared pollen dispersal distance were determined by choosing a subset of 25 genetic putative parent-pollen offspring pairs from each of the 6 leave tree pollen pools per shelterwood (i.e., total sample was 150 pollen gametes per stand). $\hat{\beta}_i$'s were 6228.19 m for Benshell and 11782.75 m for Cutmore. A chi-square goodness-of-fit test was performed to determine if distances between genetic putative parents and pollen offspring were distributed approximately as a negative exponential. This test was performed in the same way as in the seed dispersal analysis except quintiles instead of deciles were used. Quintiles were used because pollen was sampled at only six locations within stands; therefore fewer actual dispersal distances were recorded as opposed to seeds which were sampled at 20 locations in the stand. The chi-square test was non-significant ($P > .05$) for Benshell ($\chi^2_{(3)} = 2.87$), but was significant ($P < .05$) at Cutmore ($\chi^2_{(3)} = 10.28$). Despite the lack of fit in the Cutmore pollen dispersal data, the negative exponential distribution was still used to infer maximum likelihood pollen parents at both shelterwoods, based on the good fit at Benshell and the results of previous theoretical and empirical studies of pollen dispersal (Bateman 1950, Gleaves 1973, Muller 1977).

Most likely parents were then inferred for 399 (Benshell) and 437 (Cutmore) pollen gametes, based on the joint likelihood of parentage (equation (5)). The estimated mean squared dispersal distance ($\hat{\beta}$) was 3425.80 m (S.E. = 171.50) for Benshell and 6821.03 m (S.E. = 326.29) for Cutmore. If the most likely pollen parent-offspring pairs

which were inferred as self pollen were eliminated from the data, the estimates of β were 3775.95 m (S.E. = 198.46) and 7662.70 m (S.E. = 388.51), for Benshell and Cutmore, respectively. The exclusion of selfs increases the mean pollen dispersal distance by approximately 5 percent. Mean pollen dispersal distance $\left(\sqrt{\hat{\beta}}\right)$ was approximately 25 m farther at Cutmore (82.6 m) than at Benshell (58.5 m). The distances between inferred most likely parents and pollen gametes were divided into four classes, depending on the cardinal direction in which the pollen gamete was dispersed (i.e., north, south, east, and west). A separate estimate of the mean squared dispersal distance was then calculated for each quadrant (Table III.3). The largest estimates of β were found for the two easterly quadrants, with the southeast being the largest in both cases.

Distance Between Seedlings and Parent Trees

An analysis of the distance between seedlings and their most likely parents was performed to determine if seed dispersal patterns are reflected in the seedling regeneration of the shelterwoods. The inference of most likely parents of seedlings was based on electrophoretic loci which could be assayed from bud tissues. Therefore, all 49 (Benshell) and 48 (Cutmore) leave trees genotyped at these stands were used in the analysis. These trees represent almost all leave trees within the study plots. However, the seedling regeneration is a product of several seed crops. Therefore, if a few trees were not genotyped in shelterwoods this might lead to additional error in the inference of most likely parents of seedlings.

The likelihood of parentage based on genetics (equation (7)) was estimated for all possible parents of the 240 systematically sampled seedlings in each stand. Most likely parents were then inferred by estimating the joint likelihood of parentage. The probability density function for distance in the joint likelihood analysis was specified by using the estimate of β from the seed dispersal analysis. Estimated mean squared distance between seedlings and their most likely parents (β) was 2059.99 m (S.E. = 135.83) for Benshell and 2869.61 m (S.E. = 188.40) for Cutmore. The estimate of β was again larger for Cutmore, but the magnitude of difference in the estimates between shelterwoods was not as large as for seeds. Separate estimates of β were also calculated for each of the 4 quadrants in order to investigate patterns of dispersal between seedlings and most likely parents as a function of cardinal direction (Table III.4). The estimates of β among quadrants are fairly consistent with observations from the seed dispersal analyses (Figures III.3 and III.4), i.e., seeds are probably dispersed greater distances to the north and northeast direction in these stands.

Estimates of Effective Population Size

Estimates of the variance of seed and pollen dispersal ($\hat{\sigma}_s^2$, $\hat{\sigma}_p^2$), plant density ($\hat{d}$), and outcrossing rate ($\hat{t}_m$) were used to estimate effective number of males, females, and total population size in the two shelterwoods (Table III.5). The effective number of males (N_m) was greater than females (N_f) for both stands. Effective population sizes were slightly higher for Benshell, the denser stand. However, there was a two-fold difference in the neighborhood area (NA) between

stands, the more dense stand (Benshell) having the smaller neighborhood area. Estimates of N_m are similar to a previous estimate ($N_m = 25$) by J. W. Wright (1952), but both $\hat{N}_m$ and $\hat{N}_f$ are much less than estimates by Campbell (1979) ($\hat{N}_m = 147$, $\hat{N}_f = 1169$). The estimates in this study are minimum estimates, but are within the range of those previously published.

Pollen and Seedling Pool Gene Frequency Analysis

Pollen Pool

Differences in allele frequencies among the 6 pollen pools in each shelterwood were small, although a few loci had allele frequency differences of .10-.20 between individual pollen pools (Tables III.6 and III.7). For all loci, the same allele was in highest frequency in all pollen pools. Allele frequencies were significantly ($P < .05$) heterogeneous among the pollen pools of the 6 leave trees for 2 loci (LAP2, CAT) at Benshell and 4 loci (GOT1, G-6PD, CAT, 6-PGD) at Cutmore (Tables III.8 and III.9). Chi-square values combined over all 14 loci were significant ($P < .05$) for both shelterwoods.

Seedling Pools

In general, genotype frequencies were fairly uniform among seedling subpopulations; the widest range in genotypic frequencies among subpopulations was .30 (Tables III.10 and III.11). Allele frequencies were significantly ($P < .05$) heterogeneous among subpopulations at only one locus at each stand (PGI2 Benshell, MDH3 Cutmore) (Tables III.12 and III.13). Analysis of population structure

with F-statistics also illustrates that subdivision of seedling populations within shelterwoods is extremely limited ($\hat{F}_{ST}$ = .009 Benshell, $\hat{F}_{ST}$ = .011 Cutmore) (Tables III.14 and III.15). Furthermore, values of $\hat{F}_{IS}$ and $\hat{F}_{IT}$ show that genotype frquencies in subpopulations and the population as a whole, respectively, are very close to those expected under Hardy-Weinberg proportions.

Analysis of Pollen and Seedling Substructure Based on Dispersal Estimates

Pollen Substructure

Maximum likelihood parents were inferred for a sample of 399 (Benshell) and 437 (Cutmore) pollen gametes as part of the pollen dispersal analysis. Once the distance between the pollen pool and the inferred most likely parent was determined for all pollen gametes, then the proportion of the total pollen pool contributed by leave trees at varying distances from the pollen pool was tabulated (Tables III.16 and III.17). For example (Table III.16), it was estimated that 8 percent of the pollen contributed to the pollen pool of leave tree BS08 was due to selfing (i.e., from a tree 0 distance away) and 18.4 percent was contributed by leave trees .01-20.12 m away (2 leave trees contributed pollen). In total, approximately 6 pollen parents (including selfs) contributed almost 50 percent of the effective pollen at BS08 and these pollen parents were within 60.4 m of BS08. On the average, 9.9 leave trees within 60 m of the pollen pool contributed 50 percent of the pollen to individual pools at Benshell. At

Cutmore, approximately 50 percent of the pollen was contributed by 11.8 leave trees, within 80 m of the pollen pool.

In order to investigate the contributions of individual trees at varying distances from pollen pools, the mean percent pollen accounted for by each distance class was divided by the number of trees contributing pollen within that distance class. For example (Table III.16), at the Benshell stand the average tree at 0 distance contributed 6.3 percent of the pollen and the average tree at .01-20.02 m contributed 5.1 percent (11.8/2.3 = 5.1) of the pollen. These values were calculated for all distance classes in both stands and plotted (Figure III.5). It appears that individual trees at distances 60-140 m at Cutmore (the less dense stand) contribute proportionately more pollen to pollen pools than individual trees at these distances in the Benshell shelterwood.

One further tabulation was constructed in order to determine the relative contribution of individual pollen parents to the pollen pools of leave trees, without regard to distance (Figure III.6). Instead of tabulating the proportion of the pollen contributed by leave trees as a function of distance (as in Tables III.16 and III.17), the cumulative proportion of pollen contributed by individual trees was calculated, beginning with the pollen parent which contributed the most pollen to an individual pool. This tabulation shows that although a large number of parent trees contribute pollen to individual pollen pools, as few as 5 or 6 trees may contribute a large proportion of the pollen.

Seedling Substructure

Maximum likelihood maternal parents were also inferred for each of the 50 seedlings in 4 of the subpopulations in each shelterwood. Two seedling subpopulations in each shelterwood were not included in this analysis because they were close to the study plot boundaries and thus might have a larger number of true parents outside the study plot relative to the other seedling subpopulations. Once most likely parents were inferred, then the percent of seedlings contributed by individual leave trees as a function of distance from the seedling subpopulation could be tabulated (Tables III.18 and III.19). This tabulation was done in a similar fashion as that described for the pollen substructure analysis. On the average, 51 percent of the seedlings in the Benshell subpopulations were contributed by 9 maternal parents which were within a 40 m radius of the seedling subpopulations (Table III.18). At Cutmore, an average of 64 percent of seedlings were contributed by just 6 trees which were within a 40 m radius (Table III.19). A comparison between shelterwoods of the mean cumulative percent of seedlings contributed by leave trees within 40 m of the subpopulation (Tables III.18 and III.19) indicates that a smaller number of trees within 40 m contribute more seedlings at Cutmore (the less dense stand) than do a larger number of trees within the 40 m at Benshell. Mean proportions of seedlings contributed to subpopulations by individual trees as a function of distance were also calculated by distance class and plotted (Figure III.7). These plots also illustrate that individual trees at 0-40 m from subpopulations at

the Cutmore shelterwood contribute proportionately more seedlings than individual trees at these distances in the Benshell shelterwood.

Discussion

Population genetic structure within individual stands of forest trees and evolutionary determinants of this structure have received very little attention. One reason for this is the lack of suitable methodology for quantifying some of the more important parameters which in part determine population genetic structure (i.e., gene flow and effective population size). In this study, methods were developed to quantify gene flow through seed and pollen dispersal in two Douglas-fir shelterwood stands. The statistical inference of the most likely parent of seed and pollen gametes is potentially very powerful if there is a complete enumeration of parental genotypes. Unfortunately, this was not possible in these large shelterwood stands. Because potential parent trees outside the study plot were not genotyped, an unknown proportion of the offspring sample were probably assigned a most likely parent from within the study plot, when in fact the true parent was outside the study plot. Dispersal estimates should then be considered as minimum estimates. However, the statistical procedures used do offer a methodology to quantify effective seed and pollen dispersal within natural plant populations. These methods offer an alternative to the experimental manipulations often used in the study of seed and pollen dispersal (see Levin and Kerster 1974).

Seed and pollen dispersal in forest trees has most often been studied by collecting seed or pollen from isolated point source trees

(Ford et al. 1983, Silen 1962, Wright 1952), by collecting seed or pollen at varying distances from a forest (Isaac 1930, Koski 1970), or by marking and recapturing seed or pollen (Colwell 1951). The relationship between estimates from these types of studies versus actual dispersal distances of seeds or pollen from individual trees within natural stands is unknown. The dispersal estimates in this study were determined under natural conditions by utilizing the genetic information contained in progenies and the parent population and the physical distances between parents and offspring.

Average estimated seed and pollen dispersal distances over both stands were 63 m and 70 m, respectively. Isaac (1930) showed that most Douglas-fir seeds fall within 60 m of their source but could be found up to 300 m from the source. The mean seed dispersal estimate of the present study is slightly greater than that of Isaac (1930) (mean = 54 m). Pollen dispersal has been studied for a variety of conifer species (see Colwell 1951, Müller 1977, Wright 1952). Silen (1962) and Wright (1952) determined that most Douglas-fir pollen is dispersed within 100 m and 20 m, respectively, of a point source. The estimates in this study are within the range of those previously reported.

Estimated mean seed and pollen dispersal distances at Cutmore were 33 and 41 percent greater, respectively, than at Benshell. The greater dispersal distances at Cutmore may be related to site variable differences between shelterwoods. Both shelterwoods were located on relatively flat terrain, but the Benshell shelterwood was bordered to the south by a large uncut stand which could have potentially moderated high winds, whereas the Cutmore shelterwood was at a slightly higher location than surrounding stands and thus less protected from

the wind. The higher density of leave trees at Benshell may have also contributed towards moderating wind velocities. However, the maximum distance between any seed or pollen gamete and a possible parent was greater at Cutmore (232 m) than at Benshell (196 m). This difference between stands is probably not large enough to account for the large differences in mean squared dispersal distance (seed or pollen) between shelterwoods especially since very few seeds or pollen gametes dispersed large distances are included in the estimation of β. Directional patterns for seed and pollen dispersal were similar for both shelterwoods. Seeds appeared to disperse greater distances to the northeast, while the highest estimated pollen dispersal distances were to the southeast. Dispersal in an easterly direction is consistent with prevailing wind directions in Oregon and the differences between seed and pollen may reflect differences in wind patterns during the seed fall (September-November) and pollination (April-May) periods.

The mean squared distance between seedlings and their inferred most likely parents and the mean squared seed dispersal distance were both greater at Cutmore than at Benshell. However, the magnitude of difference was less for seedlings. Seed dispersal distances were generally greater towards the northeast (Figures III.3 and III.4), as were distances between seedlings and inferred parents (β for seedlings in the northwest direction at Benshell was just slightly higher than in the northeast direction). Although microhabitat selection is probably operative at the seedling stage in these stands, there is no evidence from this study that it significantly alters the patterns of dispersal that occur as a result of seed fall.

A gene frequency analysis of pollen and seedling pools was undertaken to determine if any obvious patterns in genetic structure of shelterwood stands could be detected by dividing the total pollen and seedling pools into discrete groups. Chi-square tests showed that there was significant ($P < .05$) heterogeneity among pollen pools in both shelterwoods. Non-random mating (selfing or positive assortative) among shelterwood leave trees could lead to heterogeneity in allele frequencies among the pollen pools of maternal parents of different single locus genotypes. Non-random mating can be caused by: (1) mating within subdivided populations, (2) cross incompatabilities, (3) phenological differences among leave trees, or (4) chance differences in outcross pollen pools of individual trees. The outcrossing rates for the 6 leave trees used in this analysis were also estimated (see chapter II). Mean estimated outcrossing rates among leave trees were high ($\hat{t}_{m_i}$ = .975 Benshell, $\hat{t}_{m_i}$ = .965 Cutmore), although estimates ranged from approximately .90-1.00. Given these high estimates of outcrossing and the fact that selfing should effect pollen pool allele frequencies at individual loci equally, selfing alone cannot satisfactorily explain pollen pool heterogeneity at some loci and not at others. The comparison of single locus ($\hat{t}_s$) and multilocus ($\hat{t}_m$) estimates of outcrossing (see chapter II) indicates that population subdivision may be present among trees in uncut stands, but there was no evidence for population subdivision among leave trees in shelterwoods. In the pollen substructure analysis it was shown that a small number (5-6) of leave trees contribute a large proportion of the pollen to pollen pools of individual leave trees. If there are chance differences in allele

frequencies among the groups of large pollen contributors of individual leave tree pollen pools, then this may explain the observed allele frequency heterogeneity at some loci but not all loci. Assortative mating, cross incompatabilities, and flowering phenology differences among trees may be factors which in part determine the subsets of pollen parents of individual leave trees.

The seedling subpopulations sampled within the shelterwoods showed very little genetic differentiation. Given the high outcrossing rates (see chapter II) in these stands, large seed and pollen dispersal distances, and the apparent environmental uniformity in these stands, the lack of differentiation in seedling subpopulations is not unexpected. However, differentiation may not manifest itself until seedlings reach older age classes. This would be true especially if microhabitat selection is operative after the 3-5 year old seedling stage sampled in this study. It is also possible that the loci assayed in this study and/or genes marked by them are selectively neutral or are not differentially selected during the regeneration phase of these shelterwoods.

The estimates of effective population size (N_e) were similar for both shelterwoods, although the neighborhood area (NA) for Cutmore (the less dense stand) was twice as large. Effective population sizes were equivalent due to larger dispersal variances at the Cutmore stand than at Benshell. It then might be assumed that individual trees at the less dense stand (Cutmore) would contribute equivalent proportions of offspring (seedlings or pollen) to offspring pools as would individual trees at Benshell. Inspection of Figures III.5 and III.7 illustrate that there are differences between stands in the proportions

of offspring contributed by individual trees, depending on the distance from the offspring pool. Individual parent trees 60-140 m from pollen pools at Cutmore contribute proportionately more pollen than do parent trees at these distances at Benshell (Figure III.5). For seedlings, individual parent trees contribute proportionately more offspring at 0-40 m at Cutmore than at Benshell (Figure III.7). These results suggest that as density decreases in stands, leave trees close to seedling pools contribute more seedlings. If the spacing of leave trees is uniform in shelterwoods, there will always be fewer leave trees at short distances from offspring pools than there will be trees at greater distances. Population substructure would then be more likely to develop as a result of seed dispersal patterns than due to pollen dispersal patterns.

Estimates of effective population size in forest trees have relied almost exclusively on estimates of pollen or seed dispersal variance from isolated point source trees. Wright (1952) estimated variance of pollen dispersal for a variety of trees, including an isolated Douglas-fir. He estimated the effective number of males (N_m) to be about 25 trees. Assuming 74 breeding trees per hectare in old growth Douglas-fir, as Campbell (1979) has done, Wright's estimate of neighborhood area would be approximately 0.33 ha. Campbell (1979) used the data of Wright (1952) and Isaac (1930) to estimate effective number of males (N_m) and females (N_f) to be 147 and 1169, respectively, in old growth stands. Campbell estimated neighborhood area to be 2 ha for males and 15 ha for females. Campbell's estimate of male neighborhood area is comparable to the neighborhood areas estimated for the shelterwood stands (Table III.5), but the estimate based on

seed dispersal is much larger. This can be attributed to the larger estimate of seed dispersal variance of Isaac (1930).

Effective population size has been studied under different plant densities in forest trees. Wang et al. (1960) estimated N_e to be at least 365 trees in Pinus elliottii, given a density of 120 trees/ha. Neighborhood area was approximately 3.04 ha. Bannister (1964) has estimated effective population size in Pinus radiata for various densities, however, these estimates were based on Wang et al.'s pollen dispersal variance estimates. The estimates of Wang et al. (1960) and Bannister (1964) make the assumption that effective population size (N_e) is density-dependent, whereas neighborhood area (NA) is density-independent in wind-pollinated plants (Levin and Kerster 1974). This assumption is equivalent to assuming that variance of seed and pollen dispersal are not effected by plant density. The results of this study suggest that variance of seed and pollen dispersal does change with plant density, thereby rendering both effective population size and neighborhood area density-independent.

The large reduction in density of parent trees, due to the shelterwood harvest, does not appear to have significantly impacted the genetic structure of the regeneration of these stands. Inbreeding in regeneration was nonsignificant (see chapter I) and the amount of outcrossing was no less than in uncut stands (see chapter II). There was little evidence for population subdivision of seedling regeneration, although there was some indication that the genetic composition of pollen pools among leave trees were different. The lack of family substructure in regenerated stands is in part due to moderate seed and pollen dispersal distances within stands. The effective population

size also appears to be independent of parent tree density. However, some of the above conditions may not prevail in stands of very low parent tree densities. More research will be needed to determine at what parent tree densities the population structure of regenerated stands is impacted.

Table III.1. Total numbers of individuals sampled among the leave trees, pollen pool, seed crop, and seedlings of two shelterwood stands for genetic structure analyses.

Stand	Sampled population	Tissue		
		Bud	Seed	Total
Benshell	Leave trees	13	36	49
	Pollen pool	--	547	547
	Seed crop	--	65	65
	Seedlings (systematic sample)	240	--	240
	Seedlings (leave tree sample)	300	--	300
Cutmore	Leave trees	5	43	48
	Pollen pool	--	574	574
	Seed crop	--	42	42
	Seedlings (systematic sample)	240	--	240
	Seedlings (leave tree sample)	300	--	300

Table III.2. Conditional probabilities of maternal genotype producing progeny genotype assuming no selfing and two alleles at a locus, where p and q are the frequencies of the A_1 and A_2 alleles in the outcross pollen pool.

Maternal genotype	Progeny genotype		
	A_1A_1	A_1A_2	A_2A_2
A_1A_1	p	q	0
A_1A_2	(1/2) p	1/2	(1/2) q
A_2A_2	0	p	q

Table III.3. Estimates of mean squared pollen dispersal distance ($\hat{\beta}$) (standard errors in parentheses) by quadrant, based on distances between most likely parents (inferred by joint likelihood of parentage and distance) and all pollen gametes sampled from six leave trees in each shelterwood stand.

		Benshell				Cutmore			
		Selfs included		Selfs excluded		Selfs included		Selfs excluded	
Quadrant	Orientation	n[a]	$\hat{\beta}$	n[a]	$\hat{\beta}$	n[a]	$\hat{\beta}$	n[a]	$\hat{\beta}$
1	NW	67	2570.34 (314.02) m	61	2823.17 (361.70) m	126	4315.38 (384.44) m	112	4854.80 (458.74) m
2	SW	132	2517.83 (219.15) m	120	2769.59 (252.83) m	77	4338.79 (494.45) m	69	4841.84 (582.89) m
3	SE	93	4900.17 (508.12) m	84	5425.17 (591.93) m	37	9115.54 (1498.59) m	33	10220.45 (1779.15) m
4	NE	107	3800.11 (367.37) m	97	4191.85 (425.62) m	197	8962.90 (638.58) m	175	10089.67 (762.71) m

[a]Number of pollen gametes.

Table III.4. Estimates of the mean squared distance ($\hat{\beta}$) (standard errors in parentheses) between seedlings and their most likely parents (inferred from joint likelihood of parentage and distance) for the Benshell and Cutmore shelterwoods.

		Benshell		Cutmore	
Quadrant	Orientation	n[a]	$\hat{\beta}$	n[a]	$\hat{\beta}$
1	NW	74	2480.03 (288.30)	59	2448.75 (318.80)
2	SW	54	1839.58 (250.34)	68	2715.72 (329.33)
3	SE	54	1356.51 (184.60)	48	2608.52 (376.51)
4	NE	48	2451.79 (353.88)	57	3708.86 (491.25)

[a]Number of seedlings.

Table III.5. Estimates of effective population ($\hat{N}_e$) and neighborhood area ($\hat{N}A$) in two shelterwood stands.

Stand	$\hat{\sigma}_s^2(m^2)$	$\hat{\sigma}_p^2(m^2)$	$\hat{d}$ (tree/m^2)	$\hat{t}_m$	$\hat{N}_f$	$\hat{N}_m$	$\hat{N}_e$	$\hat{N}A$ (ha)
Benshell	635.36	1036.00	.003705	.959	29	47	38	1.01
Cutmore	1367.84	2071.79	.001482	.945	25	37	31	2.01

Definition of Variables

$\hat{\sigma}_s^2$ = Variance of seed dispersal distance

σ_p^2 = Variance of pollen dispersal distance

$\hat{d}$ = Parent tree density

$\hat{t}_m$ = Multilocus outcrossing rate

$\hat{N}_f$ = Effective number of females

$\hat{N}_m$ = Effective number of males

$\hat{N}_e$ = Total effective population size

$\hat{N}A$ = Neighborhood area

Table III.6. Estimated allele frequencies in the pollen pools of six leave trees in the Benshell shelterwood.

Locus	Allele	Pollen pool					
		BS08	BS24	BS26	BS39	BS57	BS65
PGM	1	.103	.213	.094	.095	.089	.092
	2	.865	.787	.833	.821	.871	.867
	3	.032	--[b]	.073	.084	.040	.041
	N[a]	126	47	96	95	101	98
PGI2	2	.992	.957	1.000	1.000	.980	.990
	3	.008	.043	--	--	.020	.010
	N	126	47	96	95	101	98
LAP1	2	.437	.425	.448	.474	.455	.490
	5	.270	.298	.240	.232	.248	.265
	7	.294	.277	.313	.295	.297	.245
	N	126	47	96	95	101	98
LAP2	2	.016	.021	.010	.074	--	.071
	3	.976	.979	.979	.926	.960	.929
	5	.008	--	.010	--	.040	--
	N	126	47	96	95	101	98
GOT1	1	.992	.979	.990	1.000	.990	1.000
	2	.008	.021	.010	--	.0.0	--
	N	126	47	96	95	101	98
GOT2	1	.079	.021	.010	.053	.063	.093
	2	.905	.979	.938	.937	.884	.887
	3	.016	--	.052	.011	.053	.021
	N	126	47	96	95	95	97
GOT3	1	.032	.064	.056	.063	.010	.052
	2	.960	.936	.944	.926	.980	.938
	3	.008	--	--	--	--	.010
	5	--	--	--	.001	.010	--
	N	126	47	90	95	99	97
G-6PD	1	.960	.957	.969	1.000	.970	.949
	3	--	--	--	--	--	.020
	4	.040	.043	.031	--	.030	.031
	N	125	46	96	94	101	98
GLYD	1	.008	--	--	--	--	--
	2	.690	.596	.574	.642	.594	.680
	3	.302	.404	.426	.358	.406	.320
	N	126	47	47	95	101	97

Table III.6 (continued)

Locus	Allele	Pollen pool BS08	BS24	BS26	BS39	BS57	BS65
CAT	1	.310	.319	.500	.316	.248	.296
	2	.690	.681	.500	.684	.752	.704
	N	126	47	96	95	101	98
GDH	1	1.000	1.000	1.000	.989	1.000	1.000
	2	--	--	--	.011	--	--
	N	126	47	96	95	101	98
6PGD	1	.016	--	.021	.021	.010	.020
	2	.960	.957	.917	.937	.891	.959
	3	.024	.043	.063	.042	.099	.020
	N	126	47	96	95	101	98
IDH	1	.175	.225	.156	.200	.228	.184
	2	.810	.745	.813	.726	.752	.796
	3	.008	--	.010	.032	.020	.010
	4	--	--	.021	.032	--	--
	5	.008	--	--	.011	--	.010
	N	126	47	96	95	101	98
DIA	1	.310	.234	.208	.179	.330	.245
	3	.690	.766	.792	.811	.670	.755
	4	--	--	--	.011	--	--
	N	126	47	96	95	100	98

[a]Number of pollen gametes sampled.

[b]Allele was not detected in pollen pool sample.

Table III.7. Estimated allele frequencies in the pollen pools of six leave trees in the Cutmore shelterwood.

Locus	Allele	Pollen pool					
		CS08	CS31	CS41	CS43	CS70	CS80
PGM	1	.100	.144	.129	.135	.140	.088
	2	.867	.822	.819	.757	.813	.796
	3	.033	.033	.052	.108	.047	.115
	N[a]	90	90	116	111	107	113
PGI2	2	1.000	1.000	.983	.973	.990	1.000
	3	--[b]	--	.017	.027	.010	--
	N	90	90	117	111	107	114
LAP1	2	.511	.389	.487	.468	.505	.461
	5	.211	.311	.162	.216	.196	.209
	7	.278	.300	.350	.315	.299	.330
	N	90	90	117	111	107	115
LAP2	2	--	--	.026	.027	.009	.061
	3	.989	.978	.974	.973	.991	.930
	5	.011	.022	--	--	--	.009
	N	90	90	117	111	107	115
GOT1	1	1.000	.978	1.000	1.000	1.000	1.000
	2	--	.022	--	--	--	--
	N	90	90	117	111	107	115
GOT2	1	.129	.089	.086	.090	.167	.044
	2	.871	.911	.905	.883	.792	.945
	3	--	--	.009	.027	.042	.011
	N	70	90	116	111	96	91
GOT3	1	.014	.033	.034	.054	.051	.061
	2	.972	.967	.966	.946	.949	.929
	3	.014	--	--	--	--	--
	5	--	--	--	--	--	.010
	N	71	90	117	111	98	90
G6PD	1	.988	.944	.932	.982	.939	1.000
	3	--	.044	.017	--	.010	--
	4	.012	.011	.051	.018	.051	--
	N	84	90	117	111	98	109
GLYD	1	--	--	--	.010	--	.010
	2	.694	.667	.523	.695	.636	.505
	3	.306	.333	.477	.295	.364	.476
	4	--	--	--	--	--	.010
	N	72	90	107	105	99	105

Table III.7 (continued)

Locus	Allele	Pollen pool CS08	CS31	CS41	CS43	CS70	CS80
CAT	1	.447	.367	.282	.243	.178	.281
	2	.553	.633	.718	.757	.822	.719
	N	85	90	117	111	107	114
GDH	1	.989	.989	1.000	1.000	1.000	1.000
	2	--	.011	--	--	--	--
	4	.011	--	--	--	--	--
	N	90	90	117	111	107	110
6PGD	1	--	--	.009	.027	.019	.026
	2	.944	.944	.821	.937	.944	.939
	3	.056	.056	.171	.036	.037	.035
	N	90	90	117	111	107	114
IDH	1	.167	.178	.231	.198	.140	.254
	2	.800	.756	.761	.757	.841	.693
	3	--	.011	.009	.009	.009	.018
	4	.022	.011	--	.009	--	.026
	5	.011	.044	--	.027	.009	.009
	N	90	90	117	111	107	114
DIA	1	.272	.272	.293	.198	.275	.248
	3	.716	.716	.698	.802	.725	.752
	4	.012	.012	.009	--	--	--
	N	81	90	116	111	102	109

[a]Number of pollen gametes sampled.

[b]Allele was not detected at specific pollen pool sample.

Table III.8. Allele frequency contingency chi-square analysis for 14 loci among the pollen pools of 6 leave trees in the Benshell shelterwood.

Locus	No. of alleles	Chi-square	D.F.	P
PGM1	3	13.225	10	.211
PGI2	2	7.325	5	.197
LAP1	3	2.334	10	.993
LAP2	4	31.849	15	.006
GOT1	2	2.951	5	.707
GOT2	3	16.069	10	.097
GOT3	4	11.770	15	.696
G6PD	3	13.313	10	.206
GLYD	3	7.423	10	.685
CAT	2	16.530	5	.005
GDH	2	4.925	5	.425
6PGD	3	10.576	10	.391
IDH	5	19.516	20	.488
DIA	3	13.996	10	.173
(Totals)		171.802	140	.034

Table III.9. Allele frequency contingency chi-square analysis for 14 loci among the pollen pools of 6 leave trees in the Cutmore shelterwood.

Locus	No. of alleles	Chi-square	D.F.	P
PGM1	3	13.672	10	.188
PGI2	2	7.077	5	.214
LAP1	3	8.823	10	.548
LAP2	4	22.211	15	.102
GOT1	2	12.127	5	.033
GOT2	3	17.839	10	.057
GOT3	4	15.369	15	.425
G6PD	4	27.372	15	.025
GLYD	4	23.045	15	.083
CAT	2	20.329	5	.001
GDH	3	12.149	10	.275
6PGD	3	31.449	10	< .001
IDH	5	23.232	20	.277
DIA	3	7.078	10	.718
(Totals)		241.773	155	< .001

Table III.10. Estimated genotype frequencies for 14 allozyme loci assayed from bud tissues of 50 seedlings in each of 6 subpopulations in the Benshell shelterwood.

Locus	Genotype	Seedling Subpopulation					
		BS08	BS24	BS26	BS39	BS57	BS65
PGM1	11	--[a]	--	--	.06	.04	--
	12	.28	.18	.20	.21	.20	.16
	22	.60	.68	.68	.67	.62	.74
	13	.04	.02	--	--	.02	.02
	23	.04	.12	.12	.06	.10	.08
	33	.04	--	--	--	.02	--
PGM2	13	.02	--	--	--	--	--
	23	.16	.10	.16	.10	.10	.04
	33	.76	.86	.82	.88	.90	.92
	24	--	.02	--	--	--	--
	34	.06	.02	.02	.02	--	.04
PGI2	22	.98	.92	.98	1.000	.92	1.000
	23	.02	.08	.02	--	.04	--
	24	--	--	--	--	.04	--
LAP1	22	.42	.20	.30	.29	.36	.34
	25	.18	.22	.26	.31	.22	.14
	55	.12	.10	.02	.08	.02	.10
	27	.10	.30	.30	.22	.24	.24
	57	.10	.06	.10	.06	.02	.14
	77	.08	.10	.02	.04	.14	.04
	88	--	.02	--	--	--	--
LAP2	22	.04	.02	.02	.02	--	--
	23	.02	.06	.04	.10	.02	.06
	33	.90	.88	.88	.82	.94	.92
	25	--	--	.02	--	.02	--
	35	.04	.04	.04	.06	.02	.02
GDH	11	.98	1.000	.96	.98	.98	1.000
	12	.02	--	.04	.02	.02	--
GOT2	11	--	.02	--	--	--	--
	12	.18	.16	.16	.10	.12	.12
	22	.78	.66	.80	.86	.88	.82
	13	--	.02	--	.02	--	--
	23	.04	.14	.04	--	--	.06
	33	--	--	--	.02	--	--
GOT4	11	1.000	1.000	1.000	1.000	1.000	1.000

Table III.10 (continued)

Locus	Genotype	Seedling Subpopulation					
		BS 08	BS 24	BS 26	BS 39	BS 57	BS 65
G6PD	11	.84	.90	.88	.90	.96	.94
	13	--	.02	.06	.04	--	--
	14	.16	.08	.06	.06	.04	.06
SDH	11	.06	.02	.04	--	--	.02
	12	.24	.16	.16	.12	.14	.26
	22	.26	.46	.52	.78	.54	.44
	13	.06	.04	.10	--	.06	--
	23	.26	.22	.10	.10	.14	.20
	33	.12	.08	.08	--	.12	.08
	24	--	.02	--	--	--	--
6PGD	12	.04	.02	.02	.12	.04	.06
	22	.92	.92	.86	.78	.78	.88
	13	.02	--	--	--	--	--
	23	.02	.06	.12	.10	.16	.06
	33	--	--	--	--	.02	--
IDH	11	--	--	.02	.02	.04	.02
	12	.34	.30	.22	.29	.24	.36
	22	.64	.70	.66	.63	.66	.54
	23	--	--	.04	.02	--	
	24	.02	--	.04	.04	.02	.04
	15	--	--	--	--	.02	--
	25	--	--	.02	--	.02	.04
DIA	11	.10	.02	.06	.06	.06	.04
	13	.28	.44	.50	.30	.34	.44
	33	.62	.54	.44	.64	.60	.52
MDH3	22	.70	.68	.60	.80	.68	.74
	23	.30	.30	.34	.14	.24	.24
	33	--	.02	.06	.06	.06	.02
	26					.02	

[a]Genotype was not observed.

Table III.11. Estimated genotype frequencies for 14 allozyme loci assayed from bud tissues of 50 seedlings in each of 6 subpopulations in the Cutmore shelterwood.

Locus	Genotype	Seedling Subpopulation					
		CS08	CS31	CS41	CS43	CS70	CS80
PGM1	11	.04	--[a]	--	.02	.02	--
	12	.20	.14	.18	.18	.20	.24
	22	.72	.66	.66	.58	.59	.64
	13	--	.02	.02	--	.04	.06
	23	.04	.18	.12	.22	.14	.06
	33	--	--	.02	--	--	--
PGM2	23	.08	.12	.06	.02	--	.08
	33	.90	.82	.88	.92	.92	.88
	34	.02	.06	.06	.06	.04	.04
	13	--	--	--	--	.02	--
	35	--	--	--	--	.02	--
PGI2	22	1.000	.94	.98	.96	.94	.94
	23	--	.04	.02	.02	.04	.02
	24	--	.02	--	.02	.02	.04
LAP1	22	.24	.24	.34	.20	.27	.30
	25	.28	.26	.22	.22	.27	.20
	55	.16	.04	.06	.08	.04	.08
	27	.18	.32	.10	.28	.24	.26
	57	.08	.06	.16	.08	.10	.08
	77	.06	.08	.12	.14	.08	.08
LAP2	22	--	.02	--	--	--	--
	23	.04	.02	.04	.06	.04	--
	33	.94	.90	.94	.92	.92	.98
	34	--	--	--	.02	.02	--
	25	--	.02	--	--	--	--
	35	.02	.04	.02	.02	.02	.02
GDH	11	1.000	1.000	1.000	1.000	1.000	1.000
GOT2	11	--	.02	.06	--	.02	.04
	12	.16	.26	.22	.14	.27	.08
	22	.76	.70	.66	.86	.69	.84
	23	.08	.02	.06	--	.02	.04
GOT4	11	.98	1.000	1.000	1.000	1.000	1.000
	12	.02	--	--	--	--	--

Table III.11 (continued)

Locus	Genotype	Seedling Subpopulation					
		CS08	CS31	CS41	CS43	CS70	CS80
G6PD	11	1.000	.84	.82	.88	.88	.90
	13	--	.02	--	--	.02	.04
	14	--	.10	.16	.08	.10	.06
	15	--	.04	.02	.02	--	--
	44	--	--	--	.01	--	--
SDH	11	--	.06	--	.02	.02	.06
	12	.14	.20	.20	.24	.14	.18
	22	.50	.46	.34	.40	.53	.46
	13	.06	.04	.06	.06	.04	--
	23	.18	.22	.34	.22	.25	.22
	33	.12	.02	.06	.06	.02	.04
	24	--	--	--	--	--	.04
6PGD	12	.08	.04	--	.06	.04	--
	22	.76	.94	.94	.76	.88	.90
	13	--	--	--	.02	--	--
	23	.16	.02	.06	.16	.08	.08
	33	--	--	--	--	--	.02
IDH	11	.04	.02	.04	.02	.02	.02
	12	.36	.40	.28	.18	.43	.20
	22	.52	.56	.64	.68	.51	.74
	13	--	--	--	.02	--	--
	23	--	--	--	.02	--	--
	14	--	--	--	.02	--	--
	24	.04	.02	.02	.04	.02	.08
	15	.02	--	--	.02	.02	--
	25	--	--	.02	--	--	--
DIA	11	.04	.02	.10	.12	.10	.08
	13	.62	.36	.32	.42	.37	.26
	23	--	--	--	--	--	.02
	33	.34	.62	.58	.46	.53	.64
MDH3	22	.72	.72	.72	.72	.69	.48
	23	.22	.18	.22	.22	.25	.42
	33	--	.10	.06	.04	.04	.10
	27	.02	--	--	--	--	--
	36	.02	--	--	--	--	--
	66	.02	--	--	--	--	--
	26	--	--	--	.02	.02	--

[a]Genotype was not observed.

Table III.12. Allele frequency contingency chi-square analysis for 13 loci among 6 seedling subpopulations in the Benshell shelterwood.

Locus	No. of alleles	Chi-square	D.F.	P
PGM1	3	8.437	10	.586
PGM2	4	13.514	15	.562
PGI2	3	18.581	10	.045
LAP1	4	20.783	15	.143
LAP2	3	5.214	10	.876
GDH	2	3.425	5	.634
GOT2	3	15.759	10	.106
G6PD	3	14.183	10	.164
SDH	4	20.784	15	.143
6PGD	3	16.134	10	.095
IDH	5	17.503	20	.620
DIA	2	2.964	5	.705
MDH3	3	9.561	10	.479
(Totals)		166.842	145	.103

Table III.13. Allele frequency contingency chi-square analysis for 13 loci among 6 seedling subpopulations in the Cutmore shelterwood.

Locus	No. of alleles	Chi-square	D.F.	P
PGM1	3	10.750	10	.377
PGM2	5	19.710	20	.476
PGI2	3	5.946	10	.819
LAP1	3	9.582	10	.477
LAP2	4	10.710	15	.772
GOT2	3	15.816	10	.105
GOT4	2	4.988	5	.417
G6PD	4	18.840	15	.221
SDH	4	20.586	15	.150
6PGD	3	17.987	10	.055
IDH	5	22.172	20	.331
DIA	3	14.308	10	.159
MDH3	4	27.082	15	.028
(Totals)		198.476	165	.038

Table III.14. Summary of F-statistics for 13 loci among 6 seedling sub-populations in the Benshell shelterwood.

Locus	F(IS)	F(IT)	F(ST)
PGM1	.038	.046	.008
PGM2	-.049	-.040	.009
PGI2	-.031	-.014	.016
LAP1	.101	.109	.009
LAP2	.256	.261	.006
GDH	-.014	-.008	.006
GOT2	-.003	.010	.013
G6PD	-.052	-.042	.010
SDH	.183	.194	.013
6PGD	-.005	.009	.014
IDH	-.089	-.084	.004
DIA	-.033	-.028	.005
MDH3	.054	.062	.008
Mean	.051	.060	.009

Table III.15. Summary of F-statistics for 13 loci among 6 seedling sub-populations in the Cutmore shelterwood.

Locus	F(IS)	F(IT)	F(ST)
PGM1	-.017	-.010	.007
PGM2	-.050	-.043	.007
PGI2	-.021	-.015	.006
LAP1	.089	.095	.007
LAP2	.106	.112	.007
GOT2	.045	.060	.016
GOT4	-.010	-.002	.008
G6PD	-.004	.010	.014
SDH	.057	.064	.008
6PGD	-.007	.013	.019
IDH	-.048	-.036	.012
DIA	-.004	.011	.015
MDH2	.147	.165	.021
Mean	.036	.047	.011

Table III.16. Estimated percentages and cumulative percentages of pollen in pollen pools of Benshell leave trees from parents at increasing distances from leave trees.[a]

	Pollen pool													
	BS08		BS24		BS26		BS39		BS57		BS65		Mean	
Distance (m)	%	Cum %	%	Cum %	%	Cum %	%	Cum %	%	Cum %	%	Cum %	%	Cum %
0 (self)	8.0(1)[b]	8.0(1)	2.2(1)	2.2(1)	10.0(1)	10.0(1)	6.3(1)	6.3(1)	10.5(1)	10.5(1)	1.0(1)	1.0(1)	6.3(1)	6.3(1)
.01-20.02	18.4(2)	26.4(3)	15.2(3)	17.4(4)	10.0(2)	20.0(3)	9.5(3)	15.8(4)	13.7(3)	24.2(4)	4.1(1)	5.1(2)	11.8(2.3)	18.1(3.3)
20.13-40.23	14.4(2)	40.8(5)	2.2(1)	19.6(5)	7.8(2)	27.8(5)	20.0(4)	35.8(8)	1.0(1)	25.2(5)	36.0(7)	41.2(9)	13.6(2.8)	31.7(6.1)
40.24-60.35	6.4(1)	47.2(6)	17.4(2)	37.0(7)	33.3(6)	61.1(11)	18.9(5)	54.7(13)	24.2(4)	49.4(9)	16.5(5)	57.7(14)	19.5(3.8)	51.2(9.9)
60.36-80.47	15.2(6)	62.4(12)	15.2(4)	52.2(11)	10.0(2)	71.1(13)	16.6(6)	66.3(19)	2.1(3)	51.5(11)	4.1(4)	61.8(18)	10.5(4.2)	61.7(14.1)
80.48-100.58	8.0(5)	70.4(17)	21.7(5)	73.9(16)	6.7(4)	77.8(17)	5.3(3)	71.6(22)	3.1(3)	54.6(14)	3.1(2)	64.9(20)	8.0(3.7)	69.7(17.8)
100.59-120.70	4.0(3)	74.4(20)			1.1(1)	78.9(18)	1.0(1)	72.6(23)	4.2(4)	58.8(18)	3.1(2)	68.1(22)	2.2(1.8)	71.9(19.6)
120.71-140.82										58.8(18)	3.1(2)	71.1(24)	0.5(0.3)	72.4(19.9)
140.83-160.9									5.3(2)	64.1(20)	1.0(1)	72.2(25)	1.1(0.5)	73.5(20.4)
161+									4.2(3)	68.4(23)			0.7(0.5)	74.2(20.9)
Immigrant[c]	25.6(?)[d]	100.0	26.1(?)[d]	100.0	21.1(?)[d]	100.0	27.4(?)[d]	100.	31.6(?)[d]	100.0	27.8(?)[d]	100.0	26.6(?)[d]	100.0

[a]Based on distance between maximum likelihood pollen parents and pollen in pollen pool samples of six leave trees.

[b]Number of most likely paternal parents contributing percentage of pollen.

[c]Immigrant pollen was that percentage of the pollen pool which did not have a paternal parent among the leave trees in the study area.

[d]Number of parents contributing immigrant pollen was unknown.

Table III.17. Estimated percentages and cumulative percentages of pollen in pollen pools of Cutmore leave trees from parents at increasing distances from leave trees.[a]

	Pollen pool													
	CS08		CS31		CS41		CS43		CS70		CS80		Mean	
Distance (m)	%	Cum %	%	Cum %	%	Cum %	%	Cum %	%	Cum %	%	Cum %	%	Cum %
0 (self)	14.7(1)[b]	14.7(1)	1.1(1)	1.1(1)	5.7(1)	5.7(1)	3.8(1)	3.8(1)	18.7(1)	18.7(1)	11.0(1)	11.0(1)	9.2(1)	9.2(1)
.01-20.12	7.3(2)	22.0(3)	--	1.1(1)	--	5.7(1)	--	3.8(1)	12.1(2)	30.8(3)	8.8(1)	19.8(2)	4.7(0.8)	13.9(1.8)
20.13-40.23	7.3(2)	29.3(5)	12.2(2)	13.3(3)	13.2(4)	18.9(5)	27.4(6)	31.2(7)	6.6(3)	37.4(6)	7.7(1)	27.5(3)	12.4(3.0)	26.3(4.8)
40.24-60.35	18.8(4)	41.1(9)	4.4(2)	17.7(5)	16.0(3)	34.9(8)	19.8(3)	51.0(10)	8.8(2)	42.2(8)	11.0(4)	38.5(7)	13.1(3.0)	39.4(7.8)
60.36-80.47	14.7(4)	55.8(13)	21.1(4)	38.8(9)	21.7(6)	56.6(14)	2.8(3)	53.8(13)	9.9(1)	56.1(9)	16.5(6)	55.0(13)	14.5(4.0)	53.9(11.8)
80.48-100.58	10.3(4)	66.1(17)	22.2(5)	61.0(14)	14.2(4)	70.8(18)	1.9(1)	55.7(14)	3.3(1)	59.4(10)	5.5(2)	60.5(15)	9.6(2.8)	63.5(14.6)
100.59-120.70	7.3(2)	73.4(19)	4.4(2)	65.4(16)	6.6(3)	77.4(21)	11.3(5)	67.0(19)	1.1(1)	60.5(11)	--	60.5(15)	5.1(2.2)	68.6(16.8)
120.71-140.82	2.9(2)	76.3(21)	8.9(4)	7443(20)	0.9(1)	78.3(22)	9.4(3)	76.4(22)	8.8(2)	69.3(13)	5.5(2)	66.0(17)	6.1(2.3)	74.7(19.1)
140.83-160.9			5.5(2)	79.8(22)	3.8(2)	82.1(24)	0.9(1)	77.3(23)	5.5(2)	74.8(15)	2.2(2)	68.2(19)	3.0(1.5)	77.7(20.6)
161+					0.9(1)	83.0(25)	4.7(4)	82.1(27)	8.8(5)	85.5(20)	4.4(1)	72.5(20)	3.1(1.8)	80.8(22.4)
Immigration[c]	23.5(?)[d]	100.0	20.0(?)[d]	100.0	17.0(?)[d]	100.0	17.9(?)[d]	100.0	16.5(?)[d]	100.0	27.5(?)[d]	100.0	20.4(?)[d]	100

[a]Based on distance between maximum likelihood pollen parents and pollen in pollen pool samples of six leave trees.

[b]Number of most likely paternal parents contributing percentage of pollen.

[c]Immigrant pollen was that percentage of the pollen pool which did not have a paternal parent among the leave trees in the study area.

[d]Number of parents contributing immigrant pollen was unknown.

Table III.18. Estimated percentages and cumulative percentages of seedlings within four subpopulations in the Benshell shelterwood contributed by maternal parents at increasing distances from seedling subpopulations.

	Subpopulation									
	BS08		BS26		BS39		BS65		Mean	
Distance (m)	%	Cum %	%	Cum %	%	Cum %	%	Cum %	%	Cum %
0	14 (1)[b]	14 (1)	6 (1)	6 (1)	4 (1)	4 (1)	4 (1)	4 (1)	7 (1.0)	7 (1.0)
.01-20.12	18 (3)	32 (4)	34 (5)	40 (6)	14 (3)	18 (4)	14 (1)	18 (2)	20 (3.0)	27 (4.0)
20.13-40.23	24 (4)	56 (9)	22 (5)	62 (11)	29 (6)	47 (10)	20 (5)	38 (7)	24 (5.0)	51 (9.0)
40.24-60.35	16 (2)	72 (10)	28 (6)	90 (17)	25 (7)	72 (17)	46 (11)	84 (18)	29 (6.5)	80 (15.5)
60.36-80.47	12 (3)	84 (13)	8 (4)	98 (21)	22 (5)	94 (22)	12 (5)	96 (23)	13 (4.3)	93 (19.8)
80.48-100.58	14 (5)	98 (18)	--	98 (21)	6 (3)	100 (25)	4 (2)	100 (25)	6 (2.5)	99 (22.3)
100.59-120.70	2 (1)	100 (19)	2 (1)	100 (22)					1 (0.5)	100 (22.8)

[a]Based on distance between maximum likelihood maternal parent and seedlings in subpopulations.

[b]Number of most likely maternal parents contributing to percentage of total seedlings in subpopulation.

Table III.19. Estimated percentages and cumulative percentages of seedlings within four subpopulations in the Cutmore shelterwood contributed by maternal parents at increasing distances from seedling subpopulations.[a]

	Subpopulation									
	CS08		CS31		CS41		CS43		Mean	
Distance (m)	%	Cum %	%	Cum %	%	Cum %	%	Cum %	%	Cum %
0	14 (1)[b]	14 (1)	18 (1)	18 (1)	14 (1)	14 (1)	4 (1)	4 (1)	12 (1.0)	12 (1.0)
.01-20.12	34 (3)	48 (4)	--	18 (1)	--	14 (1)	--	4 (1)	9 (0.7)	21 (1.7)
20.13-40.23	10 (1)	58 (5)	52 (5)	70 (6)	54 (6)	68 (7)	54 (5)	58 (6)	43 (4.3)	64 (6.0)
40.24-60.35	14 (4)	72 (9)	2 (1)	72 (7)	14 (3)	82 (10)	12 (3)	70 (9)	10 (2.8)	74 (8.8)
60.36-80.47	18 (5)	90 (14)	20 (5)	92 (12)	16 (5)	98 (15)	14 (2)	84 (11)	17 (4.3)	91 (13.1)
80.48-100.58	10 (4)	100 (18)	6 (3)	98 (15)	2 (1)	100 (16)	2 (1)	86 (12)	5 (2.3)	96 (15.4)
100.59-120.70			--	98 (15)			12 (6)	98 (18)	3 (1.5)	99 (16.9)
120.71-140.82			2 (1)	100 (16)						
Immigrant[c]							2 (1)	100 (19)	0.5 (0.3)	100 (17.2)

[a]Based on distance between maximum likelihood maternal parent and seedlings in subpopulations.

[b]Number of most likely maternal parents contributing to percentage of total seedlings in subpopulation.

[c]Immigrant seedlings were that percentage of seedlings that could not have had any of the shelterwood leave trees as its maternal parent.

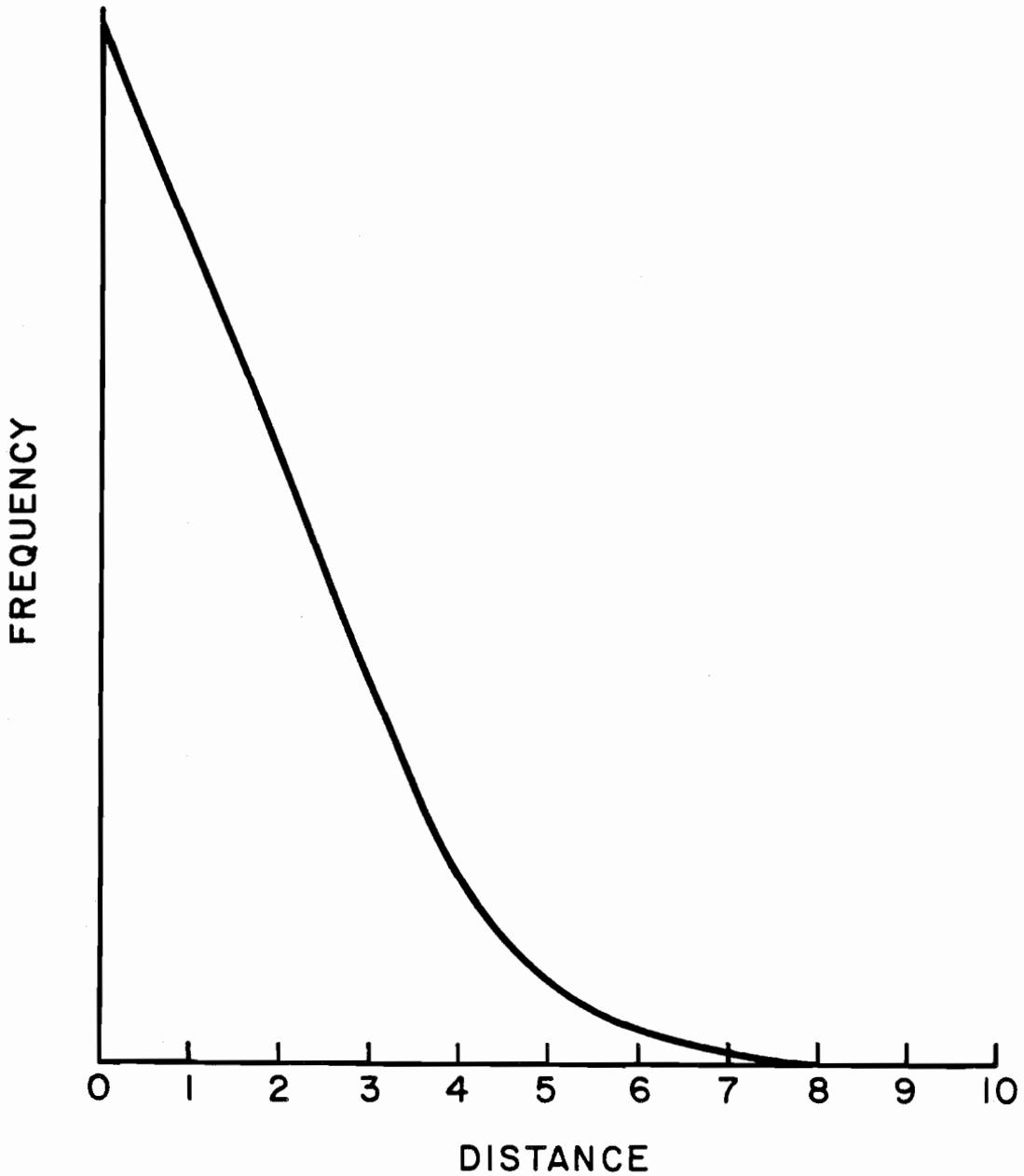

Figure III.1. Shape of the negative exponential curve with a mean squared dispersal distance (β) of 10 units.

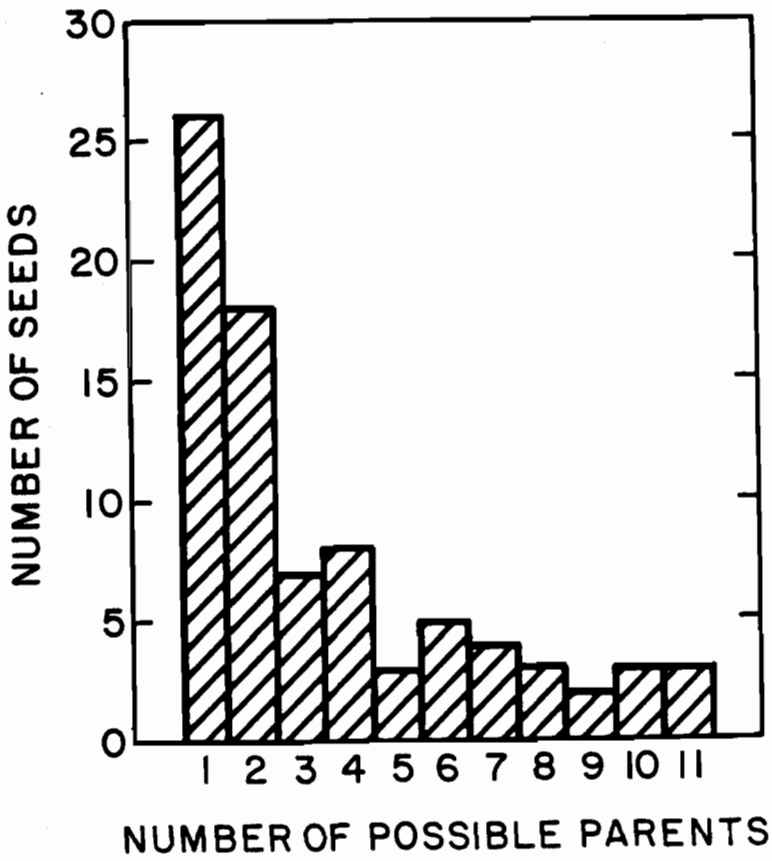

Figure III.2. Histogram of the number of possible parents for individual seeds inferred from likelihood of parentage model for the Benshell and Cutmore shelterwoods combined.

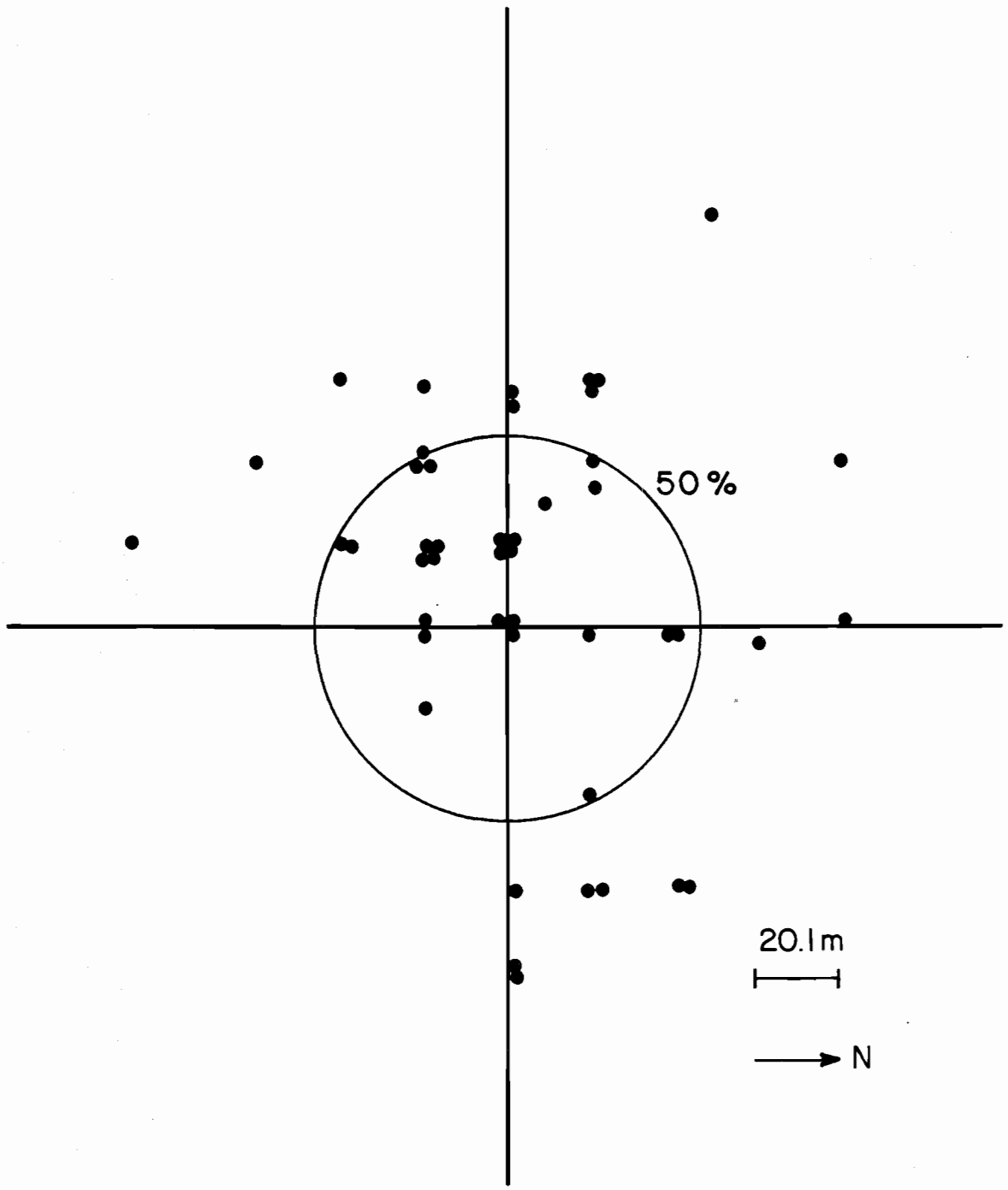

Figure III.3. Composite plot of seed locations relative to their inferred most likely parent in the Benshell shelterwood. Concentric circle delineates the estimated area within which 50 percent of seeds would be dispersed.

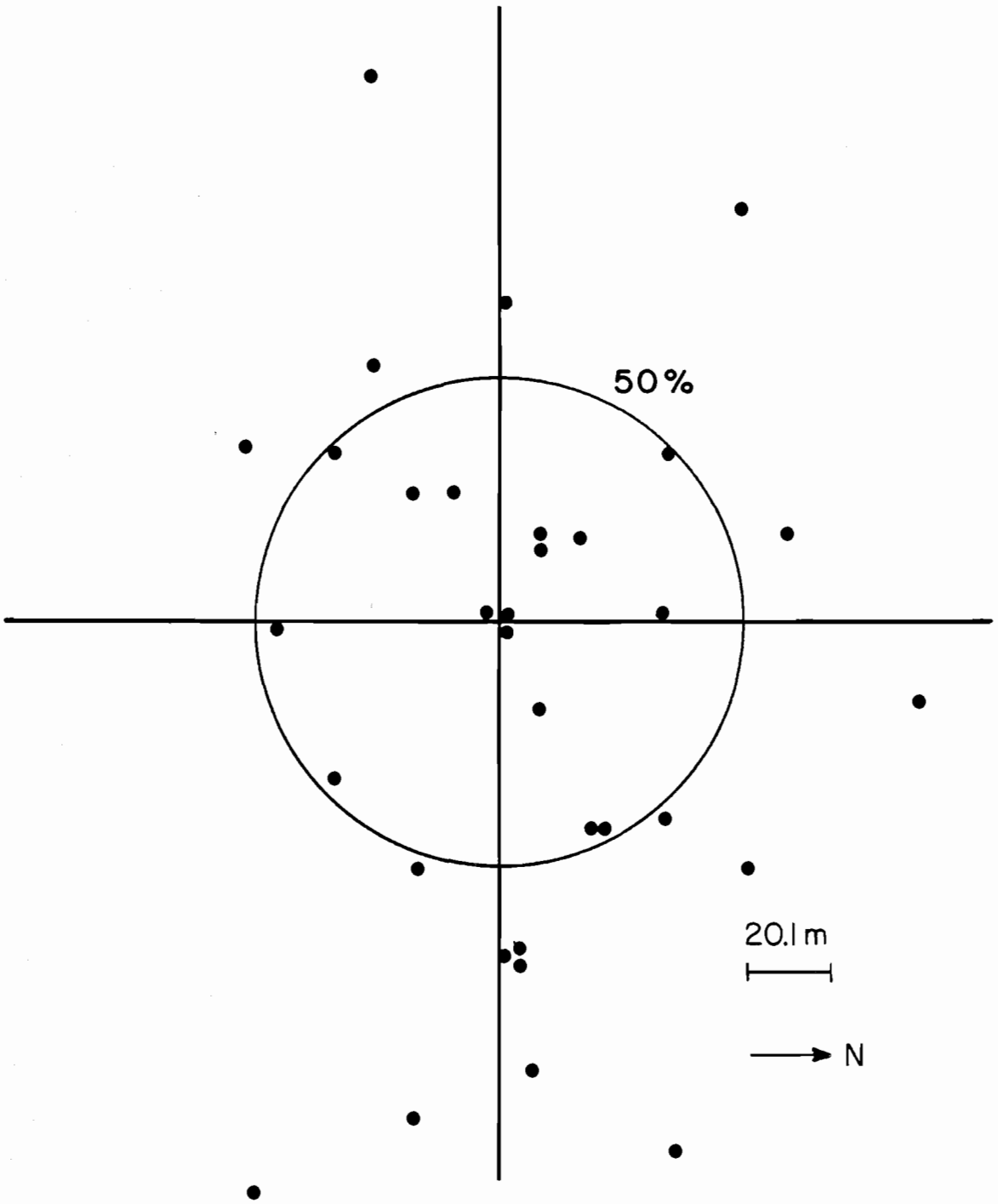

Figure III.4. Composite plot of seed locations relative to their inferred most likely parent in the Cutmore shelterwood. Concentric circle delineates the estimated area within which 50 percent of seeds would be dispersed.

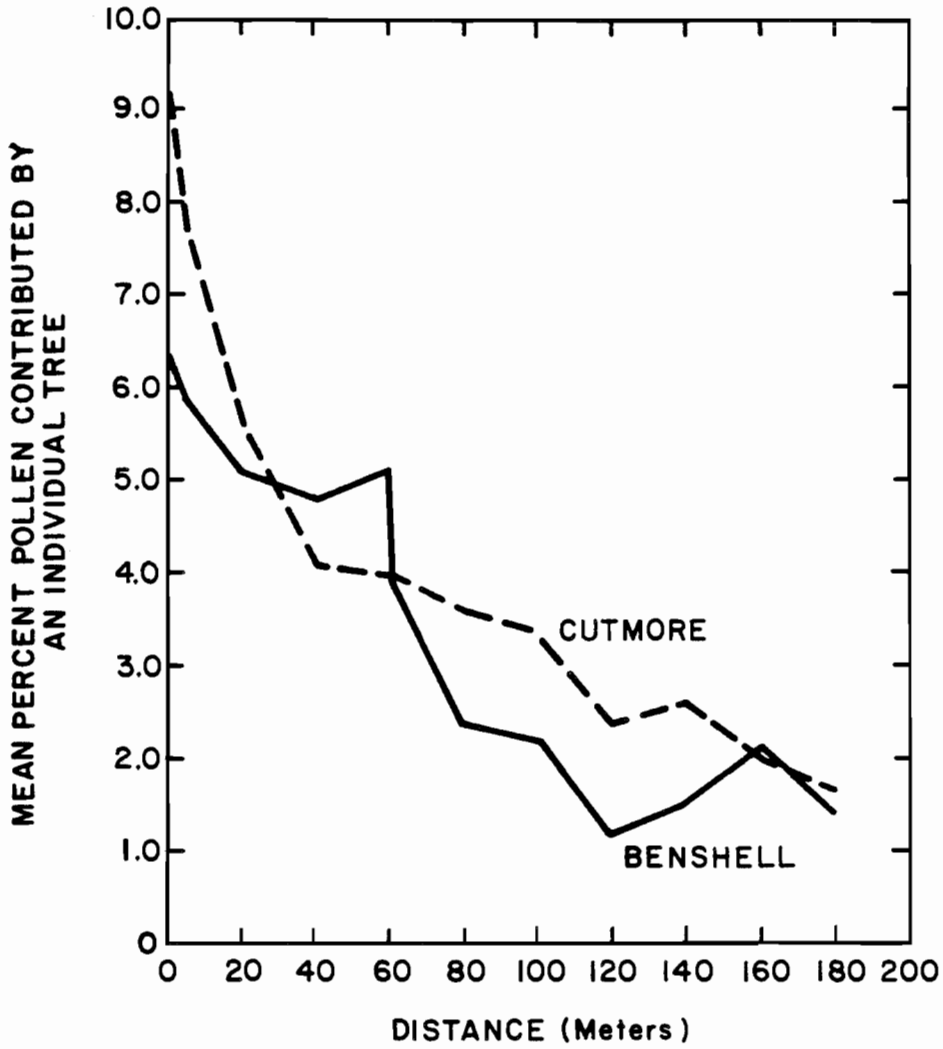

Figure III.5. Estimated mean percent of pollen contributed to pollen pools of shelterwood leave trees by individual pollen parents at varying distances from the point where the pollen pool was sampled.

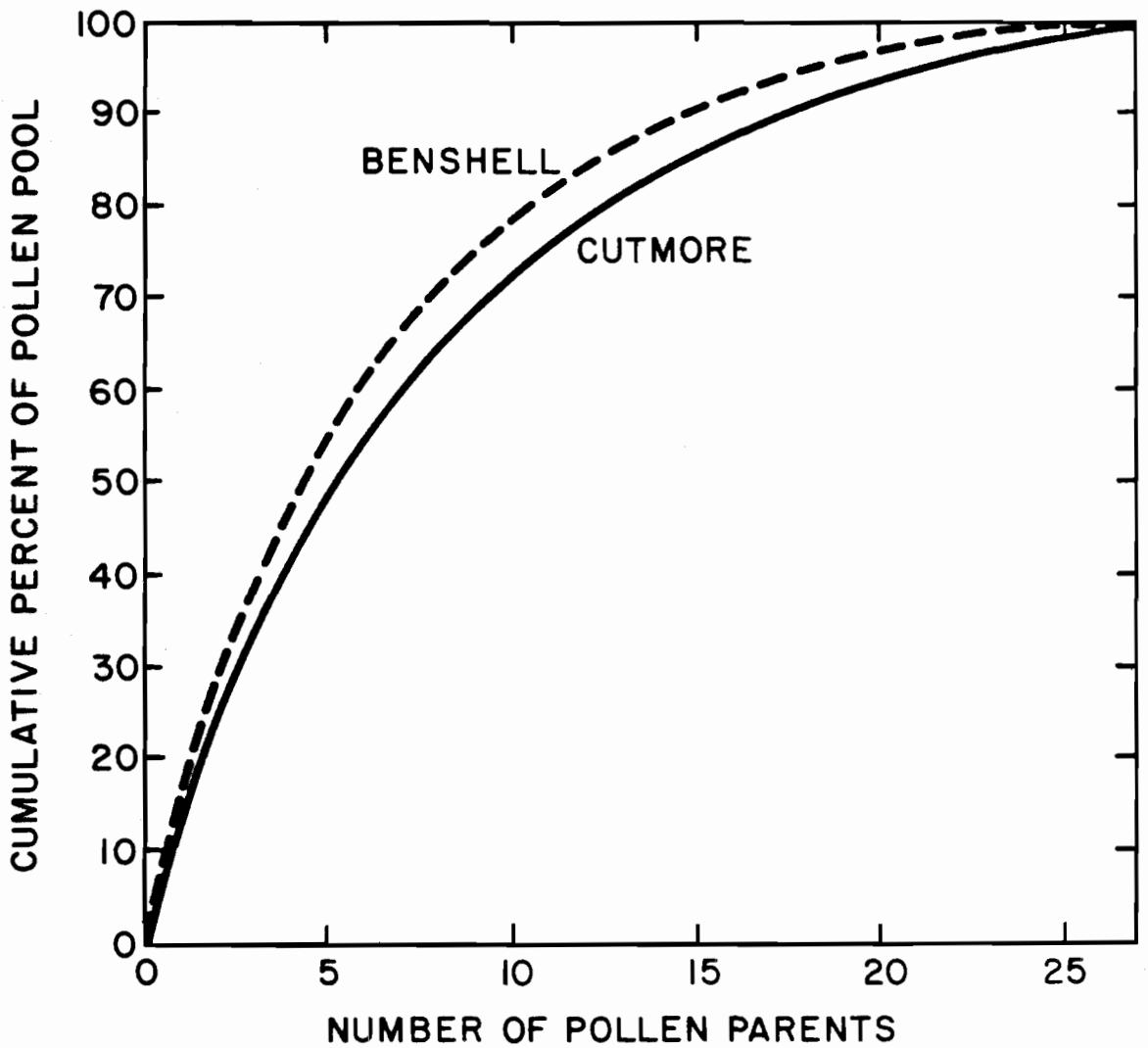

Figure III.6. Cumulative frequency plot of percentages of pollen gametes contributed to pollen pools by individual pollen parents.

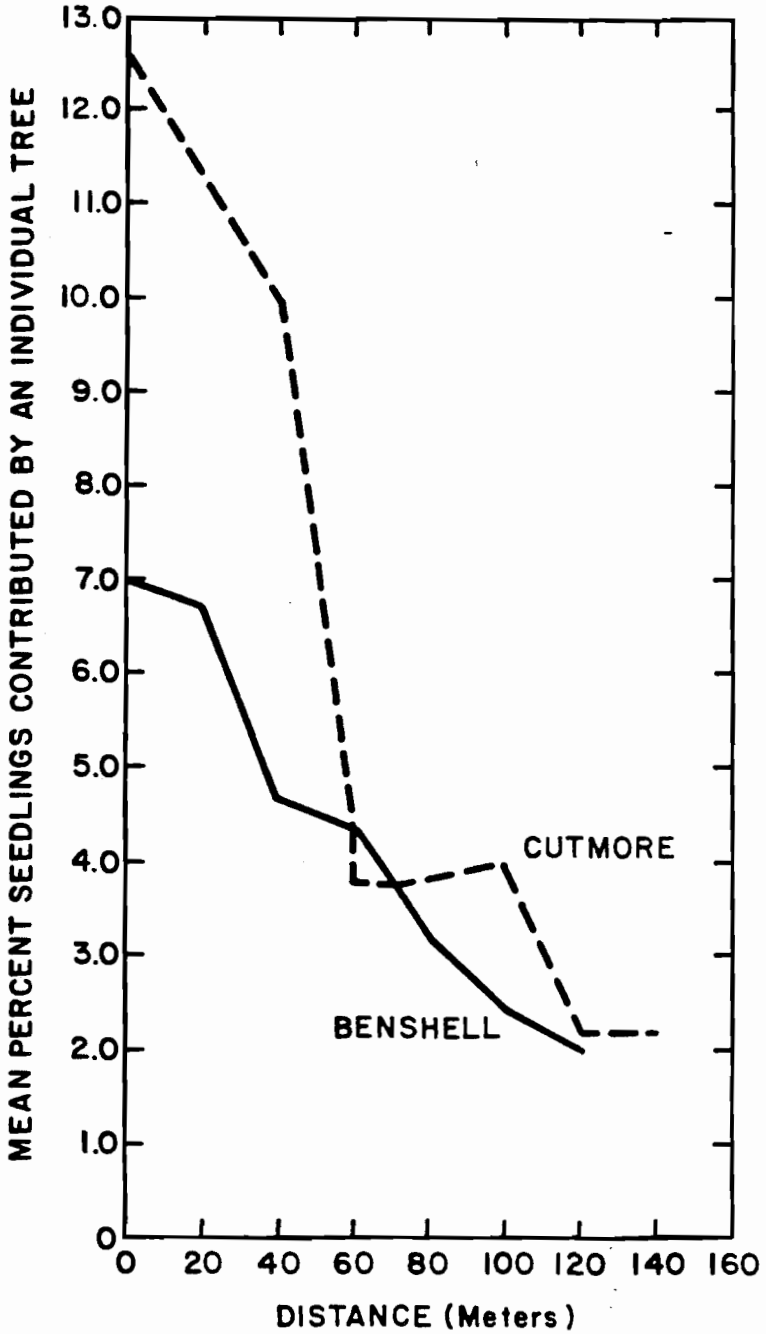

Figure III.7. Estimated mean percentages of seedlings contributed to seedling pools by individual maternal parents at varying distances from the seedling pools.

SUMMARY AND CONCLUSIONS

The goal of this disseration was to contribute to the knowledge of population genetic structure in Douglas-fir and to evaluate the potential impact of the shelterwood regeneration system on genetic structure. The study had three principal objectives: (1) assess the genetic implications of the shelterwood regeneration system; (2) quantify the mating system in uncut stands and shelterwood stands; and (3) develop methods to estimate effective seed and pollen dispersal distance within shelterwoods and explore the mechanisms for development of family structure in shelterwood regenerated stands.

This study was supported by the Fundamental Forestry Intensified Research program. This program was initiated primarily to improve forest regeneration methods in southwest Oregon. Genetic improvement of planting stock can be employed to directly increase regeneration success. However, an understanding of the basic evolutionary biology of important tree species is necessary for maximizing the efficiency of domestication programs involving tree improvement. Several components of the evolutionary biology of Douglas-fir have been explored in this study: (1) genic variability in the life cycle, (2) mating system, (3) family structure in regenerated populations, (4) gene flow within populations, and (5) effective population size. In addition, the results have several potential applications for forest management in this region.

In chapter I, levels of genic diversity at four life cycle stages (uncut stand, shelterwood leave trees, seed crop, seedling regeneration) were estimated in each of two Douglas-fir shelterwoods.

There were no significant differences among life cycle stages for 3 measures of genic diversity (mean number of alleles per locus, percent polymorphic loci, and mean expected heterozygosity). Genic variability in the shelterwoods was also only slightly less than that estimated from the breeding unit within which the shelterwoods were contained. This supports the general observation that the within population component of total genetic variability in Douglas-fir is very high. Genotype frequencies were not significantly different than Hardy-Weinberg proportions at all life cycle stages. The seedling regeneration stage at the Benshell shelterwood had a slight excess of homozygotes, suggesting that some inbreeding may have occurred in the regeneration of this stand.

In chapter II, the outcrossing rate in uncut stands and in shelterwoods was estimated using single and multilocus estimation techniques. There was significant heterogeneity among single locus population estimates ($\hat{t}_s$) within stands. Multilocus population estimates ($\hat{t}_m$) indicated that there were no significant differences in outcrossing between uncut natural stands and shelterwoods, although a trend of decreased outcrossing with decreasing plant density was suggested by the results. Comparison between single locus and multilocus population estimates in both uncut and shelterwood stands suggested that in addition to selfing, matings in uncut stands may include crosses among related individuals, possibly as a result of family structure in these stands. There was no evidence for related matings in shelterwoods, other than selfs, possibly because family structure was eliminated by the shelterwood harvest. The outcrossing rate was also estimated for 6 individual leave trees in each of the

two shelterwoods ($\hat{t}_{m_i}$). The mean of single tree estimates was very close to the population estimate for each stand, although there was significant heterogeneity in outcrossing estimates among leave trees at one shelterwood (Benshell).

Several analyses were performed in chapter III in order to investigate genetic structure in the offspring of shelterwoods. There was no evidence for population substructure among 6 groups of 50 seedlings each in either of the shelterwood stands. However, there was some evidence for heterogeneity in allele frequencies among the pollen pools of 6 shelterwood leave trees. The mean effective seed and pollen dispersal distance was estimated to be approximately 63 m and 70 m in these stands, respectively. A minimum estimate of the effective population size in these stands was determined to be approximately 31-38 trees. Given the estimated seed and pollen dispersal distances and the density of leave trees in these shelterwoods, there does not appear to be much of a potential for the development of family substructure in the regeneration of these stands, as a result of restricted gene flow alone. However, there was some evidence that if plant density was much less than that of these shelterwoods, family structure might develop, principally as a result of restricted seed dispersal.

This study was conducted in only two shelterwood stands within a single breeding zone in southwest Oregon. Nevertheless, some preliminary conclusions can be drawn. There is no evidence from this study that there is a significant loss of genetic variability in regenerated stands, after a single rotation of shelterwood harvesting. This implies that shelterwood harvesting and regeneration of

Douglas-fir could be used without appreciable loss in productivity due to narrowing of the genetic base or change in genetic composition of populations. It may also be possible to use the shelterwood system for maintaining in situ genetic reserves. The mating system analysis indicates that inbreeding in shelterwood trees is no greater, or possibly less, than in uncut stands, therefore it should be possible to collect open-pollinated seed from seed trees in thinned stands without appreciable inbreeding in seed progenies. The family structure analyses would indicate that comparison plus-tree selection would be appropriate for selecting plus trees in shelterwood regenerated stands, due to the lack of family substructure in these stands. Finally, the estimates of effective seed and pollen dispersal and population size could be useful for designing in situ gene conservation areas.

This investigation has provided further insight into the population genetic structure of Douglas-fir in southwest Oregon. The generalized population structure seems to be one of large within population variability, high outcrossing and fecundity, moderate seed and pollen dispersal distances and effective population size, and close adaptation to environmental parameters. This combination of population genetic and life history characteristics leads to a population structure of continuously overlapping, moderately sized groups of randomly mating individuals which are closely adapted to environmental variables across the continuum.

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APPENDICES

APPENDIX A.

LIST OF RAW DATA FILES AND COMPUTER PROGRAMS

All raw allozyme data and several computer programs used in this study are available upon request from the Forest Science Data Bank, Department of Forest Science, Oregon State University, Corvallis, Oregon 97331. Supporting documentation is provided for all data sets and computer programs by the Data Bank. Listed below are the data files and computer programs with a brief description of each.

DATA SETS:

1. RF00401 Allozyme genotypes (15 loci) for grid sample seedlings from each of two shelterwoods. Four seedlings were sampled at each of 60 grid points for a total of 240 seedlings per shelterwood.
2. RF00402 Allozyme genotypes (15 loci) for 6 subpopulations of 50 seedlings each in each of two shelterwood stands (300 seedlings per shelterwood).
3. RF00403 Allozyme genotypes (15 loci) for trees in uncut stands adjacent to shelterwoods. Allozymes assayed from bud tissues.
4. RF00404 Allozyme genotypes (14 loci) of ovules (megagametophytes) and pollen gametes for progenies from all shelterwood leave trees and seed trees in uncut stands.
5. RF00405 Multilocus allozyme genotypes for all shelterwood leave trees, assayed from either seeds and/or bud tissues, and allozyme genotypes for seed trees in uncut stands assayed from seed tissues.

COMPUTER PROGRAMS

1. RF004NC Program written in Fortran V which summarizes seed progeny data for input into single locus mating system estimation program (RF004SL).
2. RF004SL Program written in Fortran V for single locus mating system estimation (see Appendix C).
3. RF004ML Program written in Fortran V for multilocus population or individual tree mating system estimation (see Appendix C).

APPENDIX B.

CONTINGENCY CHI-SQUARE ANALYSES FOR SIX PAIRS OF LIFE CYCLE STAGES IN EACH OF TWO SHELTERWOOD STANDS.

Table	Life cycle stage pair	Shelterwood
1	Uncut: leave tree	Benshell
2	Uncut: leave tree	Cutmore
3	Leave tree: megagametophyte, seed trap	Benshell
4	Leave tree: megagametophyte, seed trap	Cutmore
5	Leave tree: pollen, seed trap	Benshell
6	Leave tree: pollen, seed trap	Cutmore
7	Leave tree: embryo, seed trap	Benshell
8	Leave tree: embryo, seed trap	Cutmore
9	Embryo, seed trap: regeneration	Benshell
10	Embryo, seed trap: regeneration	Cutmore
11	Leave tree: regeneration	Benshell
12	Leave tree: regeneration	Cutmore

Table B.1. Allele frequency contingency chi-square analysis for 10 loci among 2 life cycle stages (uncut and leave tree) in the Benshell population.

Locus	No. of alleles	Chi-square	D.F.	P
PGM1	3	2.501	2	.286
PGI2	4	3.113	3	.374
LAP1	3	.959	2	.619
LAP2	4	5.876	3	.117
GDH	2	1.026	1	.311
GOT2	3	2.593	2	.273
G6PD	3	1.911	2	.384
6PGD	3	.361	2	.834
IDH	3	4.069	2	.130
DIA	2	.057	1	.811
(Totals)		22.465	20	.315

Table B.2. Allele frequency contingency chi-square analysis for 9 loci among 2 life cycle stages (uncut and leave tree) in the Cutmore population.

Locus	No. of alleles	Chi-square	D.F.	P
PGM1	3	3.465	2	.176
PGI2	4	1.573	3	.665
LAP1	3	.819	2	.663
LAP2	4	2.548	3	.466
GOT2	3	.068	2	.966
G6PD	4	.216	3	.974
6PGD	3	1.667	2	.434
IDH	4	5.575	3	.134
DIA	2	.034	1	.853
(Totals)		15.964	21	.771

Table B.3. Allele frequency contingency chi-square analysis for 13 loci among 2 life cycle stages (leave tree and megagametophytes in seed traps) in the Benshell population.

Locus	No. of alleles	Chi-square	D.F.	P
PGM1	3	4.146	2	.125
PGI2	3	1.561	2	.458
LAP1	7	3.986	6	.690
LAP2	3	6.821	2	.033
GOT1	1	0.000	0	1.000
GOT3	6	4.043	5	.543
G6PD	3	2.925	2	.231
GLYD	4	2.933	3	.402
CATA	2	1.456	1	.227
GDH	2	.951	1	.329
6PGD	3	2.380	2	.304
IDH	3	1.511	2	.469
DIA	2	1.421	1	.233
(Totals)		34.044	29	.237

Table B.4. Allele frequency contingency chi-square analysis for 14 loci among 2 life cycle stages (leave trees and megagametophytes in seed traps) in the Cutmore population.

Locus	No. of alleles	Chi-square	D.F.	P
PGM1	3	.256	2	.879
PGI2	4	1.412	3	.702
LAP1	7	9.815	6	.132
LAP2	4	2.303	3	.511
GOT1	1	0.000	0	1.000
GOT2	3	1.709	2	.425
GOT3	4	2.816	3	.420
G6PD	4	3.496	3	.321
GLYD	2	.005	1	.943
CATA	2	.339	1	.560
GDH	1	0.000	0	1.000
6PGD	3	3.217	2	.200
IDH	4	2.413	3	.491
DIA	2	.158	1	.691
(Totals)		27.939	30	.573

Table B.5. Allele frequency contingency chi-square analysis for 13 loci among 2 life cycle stages (leave trees and pollen gametes inferred from embryos in seed traps) in the Benshell population.

Locus	No. of alleles	Chi-square	D.F.	P
PGM1	3	1.601	2	.449
PGI2	2	.979	1	.322
LAP1	3	2.428	2	.296
LAP2	3	4.624	2	.099
GOT1	2	1.036	1	.308
GOT3	5	3.840	4	.428
G6PD	3	3.889	2	.143
GLYD	3	1.232	2	.540
CATA	2	.030	1	.862
GDH	2	.979	1	.322
6PGD	3	1.706	2	.426
IDH	3	2.290	2	.318
DIA	2	1.608	1	.204
(Totals)		26.242	23	.289

Table B.6. Allele frequency contingency chi-square analysis for 14 loci among 2 life cycle stages (leave trees and pollen gametes inferred from embryos in seed traps) in the Cutmore population.

Locus	No. of alleles	Chi-square	D.F.	P
PGM1	3	2.353	2	.308
PGI2	3	1.462	2	.481
LAP1	3	.079	2	.961
LAP2	3	6.156	2	.046
GOT1	1	0.000	0	1.000
GOT2	3	2.175	2	.337
GOT3	2	.092	1	.761
G6PD	3	2.740	2	.254
GLYD	2	3.475	1	.062
CATA	2	.001	1	.969
GDH	1	0.000	0	1.000
6PGD	3	.121	2	.941
IDH	5	4.523	4	.339
DIA	2	.056	1	.813
(Totals)		23.233	22	.388

Table B.7. Allele frequency contingency chi-square analysis for 13 loci among 2 life cycle stages (leave trees and embryos in seed traps) in the Benshell population.

Locus	No. of alleles	Chi-square	D.F.	P
PGM1	3	3.390	2	.183
PGI2	2	1.898	1	.168
LAP1	3	.431	2	.805
LAP2	3	9.316	2	.009
GDH	2	1.926	1	.165
GOT1	2	.532	1	.465
GOT3	6	2.599	5	.761
G6PD	3	3.249	2	.197
GLYD	4	2.436	3	.486
CATA	2	.632	1	.426
6PGD	3	2.046	2	.359
IDH	5	1.638	4	.801
DIA	2	2.036	1	.153
(Totals)		32.130	27	.227

Table B.8. Allele frequency contingency chi-square analysis for 12 loci among 2 life cycle stages (leave trees and embryos in seed traps) in the Cutmore population.

Locus	No. of alleles	Chi-square	D.F.	P
PGM1	3	.306	2	.858
PGI2	3	1.521	2	.467
LAP1	3	1.675	2	.432
LAP2	4	4.698	3	.195
GOT2	3	3.522	2	.171
GOT3	3	.829	2	.660
G6PD	4	5.179	3	.159
GLYD	2	1.118	1	.290
CATA	2	.046	1	.829
6PGD	3	.464	2	.793
IDH	5	5.035	4	.283
DIA	2	.014	1	.904
(Totals)		24.406	25	.496

Table B.9. Allele frequency contingency chi-square analysis for 9 loci among 2 life cycle stages (embryos in seed traps and regeneration) in the Benshell population.

Locus	No. of alleles	Chi-square	D.F.	P
PGM1	3	.879	2	.644
PGI2	3	3.289	2	.193
LAP1	3	4.117	2	.127
LAP2	3	1.523	2	.467
GDH	2	3.289	1	.069
G6PD	3	5.585	2	.061
6PGD	3	1.710	2	.425
IDH	4	4.043	3	.256
DIA	3	2.444	2	.294
(Totals)		26.880	18	.081

Table B.10. Allele frequency contingency chi-square analysis for 9 loci among 2 life cycle stages (embryos in seed traps and regeneration) in the Cutmore population.

Locus	No. of alleles	Chi-square	D.F.	P
PGM1	3	2.188	2	.334
PGI2	3	1.217	2	.544
LAP1	3	.733	2	.693
LAP2	3	1.088	2	.580
GOT2	2	.396	1	.529
G6PD	3	3.233	2	.198
6PGD	3	2.096	2	.350
IDH	4	1.471	3	.689
DIA	2	.830	1	.362
(Totals)		13.254	17	.719

Table B.11. Allele frequency contingency chi-square analysis for 10 loci among 2 life cycle stages (leave tree and regeneration) in the Benshell population.

Locus	No. of alleles	Chi-square	D.F.	P
PGM1	3	.555	2	.757
PGI2	4	3.384	3	.336
LAP1	3	2.724	2	.256
LAP2	4	18.825	3	.000
GDH	2	.656	1	.418
GOT2	3	4.894	2	.086
G6PD	3	.123	2	.940
6PGD	3	2.555	2	.278
IDH	3	2.128	2	.344
DIA	3	2.014	2	.365
(Totals)		37.857	21	.013

Table B.12. Allele frequency contingency chi-square analysis for 9 loci among 2 life cycle stages (leave tree and regeneration) in the Cutmore population.

Locus	No. of alleles	Chi-square	D.F.	P
PGM1	3	3.562	2	.168
PGI2	4	.491	3	.920
LAP1	3	.087	2	.957
LAP2	4	2.599	3	.457
GOT2	3	2.624	2	.269
G6PD	4	5.211	3	.156
6PGD	3	2.442	2	.294
IDH	4	.894	3	.826
DIA	2	.301	1	.583
(Totals)		18.212	21	.635

APPENDIX C.

DESCRIPTION OF SINGLE AND MULTILOCUS OUTCROSSING (t) ESTIMATORS FOR THE MIXED MATING MODEL AND COMPUTER PROGRAMS FOR CALCULATING ESTIMATES

The purpose of this appendix is to describe the mating system estimation procedures used in this study and the computer programs that were developed for calculating estimates. Computer programs and test data sets can be obtained from the Forest Science Data Bank, Department of Forest Science, Oregon State University, Corvallis, OR 97331 (see Appendix A for listing of programs). Three separate outcrossing estimators will be described: 1) single locus population, 2) multilocus population, and 3) multilocus individual tree.

1. Single locus outcrossing (t_s) estimation

A locus must be polymorphic in order to estimate t_s (the proportion of viable progeny resulting from outcrossing) for a given population. Shaw and Allard (1982) presented a single locus estimation procedure based on a diallelic mixed mating model. Application of this procedure requires that all loci be diallelic. Therefore, if more than two alleles are observed at a locus, alleles must be bulked to form two allelic classes. In this situation, Shaw and Allard could estimate two parameters; t_s and p (the frequency of the A_1 allele or allelic class in the outcross pollen pool). t_s and p were estimated for all possible combinations of allelic classes (i.e., 3 alleles, 3 combinations) and a mean estimate of t_s across combinations was calculated for each locus. This procedure is rather cumbersome because of

the need for bulking alleles and computing multiple estimates of t_s for loci with more than two alleles. Shaw (1980) also observed that multiple estimates of t_s for a locus were extremely variable. In this study, the diallelic model of Shaw and Allard (1982) was applied for diallelic loci only. A triallelic model was then developed for loci with three alleles. For the two loci (GOT3 and IDH) where four or more alleles were detected in pollen gametes, a triallelic model was also used by preserving the two most common alleles and bulking the remaining alleles into a third synthetic class.

The single locus triallelic model is an extension of the diallelic model. With a triallelic model there are six possible parental genotypic classes, and conditional expectations for each of the three alleles (A_1, A_2, A_3) in pollen gametes of the progeny of each parental genotype are given in Table C.1. Three parameters, t_s, p, and q (frequency of the A_2 allele in the outcross pollen pool) can be estimated simultaneously by the method of maximum likelihood. The general form of the likelihood equation is,

$$L(\emptyset) = \frac{N!}{R_{11}!\ldots R_{kl}!} \prod_{i=1}^{\pi} \prod_{j=1} (M_{ij})^{R_{ij}} ,$$

where the $\emptyset$'s are the parameters to be estimated (t_s, p, and q), M_{ij} is the expected proportion of pollen gametes carrying the jth allele in progeny of the ith maternal genotype, R_{ij} is the observed number of the ijth parent-gamete combination (see Table C.2), and N is the sum of R_{ij}. Deleting the combinational term and using the

notation of Tables C.1 and C.2 the expanded form of the likelihood equation becomes:

$$L(\emptyset) = [(1 - t_s) + t_s p]^{R_{11}} \quad [t_s q]^{R_{12}} \quad [t_s r]^{R_{13}}$$

$$[1/2(1 - t_s) + t_s p]^{R_{21}} \quad [1/2(1 - t_s) + t_s q]^{R_{22}} \quad [t_s r]^{R_{23}}$$

$$[t_s p]^{R_{31}} \quad [(1 - t_s) + t_s q]^{R_{32}} \quad [t_s r]^{R_{33}}$$

$$[1/2(1 - t_s)]^{R_{41}} \quad [t_s q]^{R_{42}} \quad [1/2(1 - t_s) + t_s r]^{R_{43}}$$

$$[t_s p]^{R_{51}} \quad [1/2(1 - t_s) + t_s q]^{R_{52}} \quad [1/2(1 - t_s) + t_s r]^{R_{53}}$$

$$[t_s p]^{R_{61}} \quad [t_s q]^{R_{62}} \quad [(1 - t_s) + t_s r]^{R_{63}}$$

The joint maximum likelihood estimates of the parameters t_s, p, and q are the estimates which maximize the likelihood equation. The likelihood equation is maximized by setting the first derivative of the log-likelihood function, with respect to each parameter, to zero and then solving the set of simultaneous equations numerically by using Fisher's method of scoring (Rao 1973). The first derivative of the log-likelihood function with respect to each parameter is known as the "score" of that parameter. The negative expectation of the second derivative of the log-likelihood function with respect to each parameter is known as the "information" for that parameter. The vector of scores of all parameters divided by the matrix of information is the difference vector. With each iteration of the scoring method, the difference values are added to the parameter estimates. The maximum likelihood parameter estimates are those values of $\hat{t}_s$, $\hat{p}$, and $\hat{q}$ at the iteration where the difference values approach zero. Large

sample approximations of the variances and covariances of parameters are those corresponding terms in the inverse of the information matrix.

Two computer programs were developed for single locus mating system estimation. The program RF004NC (see Appendix A) tabulates the observed number of R_{ij}'s for each parental genotypic class (M_{ij}) from the megagametophyte-embryo pairs of parent trees in the sample population. Details concerning the formating of allozyme data, bulking of alleles, etc., are discussed in the documentation of RF004NC. The output from RF004NC is equivalent to the R_{ij} matrix (Table C.2) for each locus. For a triallelic locus, the summarized data is in the form:

$$R_{11}, R_{12}, N_{1.}, R_{21}, R_{22}, N_{2.}, \ldots, R_{61}, R_{62}, N_{6.} ,$$

where $N_{i.} = R_{i1} + R_{i2} + R_{i3}$. For a diallelic locus the summarized data is in the form:

$$R_{11}, N_{1.}, R_{21}, N_{2.}, R_{31}, N_{3.} ,$$

where $N_{i.} = R_{i1} + R_{i2}$.

The program RF004SL (see Appendix A) uses the summarized data from RF004NC to estimate the parameters t_s and p for the diallelic model and t_s, p, and q for the triallelic case. Details concerning the input format for RF004SL are discussed in the program documentation. RF004SL solves the joint maximum likelihood function numerically, as was discussed above. Output from RF004SL includes the estimates of t_s, p, q, and/or r($r = 1-p-q$) for each locus, variance-covariance matrix of these estimates, and a likelihood ratio test of the null hypothesis, $t_s = 1.0$ (Rao 1973).

2. Multilocus Population Outcrossing (t_m) Estimation

The multilocus population outcrossing estimation procedure takes advantage of information at multiple loci to estimate the mating system parameter, t_m. All matings are classified 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, then the progeny with that pollen gamete is classified as a definite 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. Matings of this type are classified as ambiguous.

The multilocus population estimator used in this study is similar to that of Green et al. (1980). The maximum likelihood equation for the estimation of t_m is given as,

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

where R_i is the number of detectable outcross pollen gametes observed among the N_i progeny sampled from the ith maternal parent, and G_i is the conditional probability of detecting an outcross pollen gamete in progeny of the ith maternal parent, given that an outcross has

occurred. The G_i (detection probability) was estimated for each maternal parent in the population as

$$\hat{G}_i = 1 - \prod_{j=1}^{k} \hat{f}_{ij} \quad ,$$

where $\hat{f}_{ij}$ is the estimated frequency in the outcross pollen pool of alleles carried at the jth locus in the ith maternal parent and k is the number of loci. The estimator for $\hat{G}_i$ assumes that alleles at the k loci are associated independently in the outcross pollen pool.

Values for three variables (N_i, R_i, and G_i) are needed for each of the i maternal parents in order to solve the likelihood equation. The computer program RF004ML determines R_i and estimates G_i for each maternal parent with N_i progeny, before t_m can be estimated. Three types of data are required as input in RF004ML: (1) multilocus (k loci) allozyme genotypes of all maternal parents in the population sample; (2) estimates of outcross pollen pool allele frequencies and respective variances for as many as 3 alleles per locus; and (3) multilocus pollen gamete genotypes for all the N_i progeny sampled from each maternal parent. The outcross pollen pool allele frequencies are estimated by using the single locus mating system program, RF004SL. RF004SL uses either a diallelic or triallelic model (see section C.1), therefore no more than three alleles at a locus can be used to estimate the G_i's and t_m. If there are more than three alleles at a locus, the two most common alleles are preserved and all remaining alleles are bulked into a composite allelic class.

For predominantly outcrossing species, estimation is more efficient if more loci are assayed, at the cost of fewer individuals

sampled, for a fixed number of electrophoretic runs (Shaw and Brown 1982). The exact number of loci, maternal parents, and progenies per parent to optimize efficiency of estimation is highly dependent upon allele frequencies and the outcrossing rate, but without evidence from simulation, it was determined during the course of the present study that 8-12 loci and 200-300 progenies should be assayed for a highly outcrossed species such as Douglas-fir in order to derive estimates of t_m with standard errors of less than .05.

The maximum likelihood equation is solved numerically by RF004ML using Fisher's method of scoring (Rao 1973). Unlike the single locus estimator, only one parameter, t_m, is estimated. The first derivative of the log-likelihood function, with respect to t_m (the score of t_m), is set equal to zero and solved numerically. The score (S_t) and information (I_t) of t_m are given as follows (Green et al. 1980):

$$S_t = \sum_i (N_i - R_i)G_i / (1 - G_i t_m) - \sum_i R_i / t_m,$$

$$I_t = \sum_i N_i G_i^2 / (1 - G_i t_m) + \sum_i \frac{N_i G_i}{t_m} .$$

The score divided by the information is called the difference ($S_t/I_t = D_t$) and is added to the estimate of t_m with each iteration until the value of D_t approaches zero. The large sample approximation of the variance of $\hat{t}_m$ ($VAR_{\hat{t}_m}$) is the inverse of the information of t_m (i.e., $1/I_t$) (Green et al. 1980). This estimate of the variance of $\hat{t}_m$ assumes that the G_i are known and constant (Green et al. 1980). Since this assumption is normally violated (as in this

study), $VAR\hat{t}_m$ must be considered a lower bound estimate. The output from RF004ML includes the R_i, N_i and G_i for all i maternal parents in the sample, the estimate of t_m, $VAR\hat{t}_m$, and the chi-square value for the likelihood ratio test of the $H_o: t_m = 1.0$.

3. Multilocus Outcrossing (t_{m_i}) Estimation for Individual Trees

A multilocus estimation procedure was developed to estimate outcrossing for individual trees ($\hat{t}_{m_i}$) in order to investigate within stand heterogeneity in outcrossing. The maximum likelihood estimator of Green et al. (1980) can be modified for single trees:

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

where R_i is the number of detectable outcross pollen gametes among the N_i progeny sampled from the ith maternal parent and G_i is the detection probability for the ith maternal parent. The likelihood equation can be solved directly; the maximum likelihood estimator of t_{m_i} is,

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

where $\hat{r}_i = R_i/N_i$, the observed proportion of detectable outcrosses. A large sample approximation of the variance of $\hat{t}_{m_i}$ is given as:

$$VAR\ \hat{t}_{m_i} = \left[\frac{\partial \hat{t}_{m_i}}{\partial \hat{r}_i}\right]^2 VAR\ \hat{r}_i + \left[\frac{\partial \hat{t}_{m_i}}{\partial \hat{G}_i}\right]^2 VAR\ \hat{G}_i + 2\ \frac{\partial \hat{t}_{m_i}}{\partial \hat{r}_i}\ \frac{\partial \hat{t}_{m_i}}{\partial \hat{G}_i}\ COV(\hat{r}_i, \hat{G}_i)$$

Expressions for the VAR $\hat{r}_i$ and VAR $\hat{G}_i$ can easily be derived, but an expression for COV($\hat{r}_i$, $\hat{G}_i$) is extremely complicated. The G_i are estimated from frequency of alleles in the outcross pollen pool as was shown for the estimation of t_m. Estimates of outcross pollen pool allele frequencies were obtained through the single locus outcrossing estimation procedure by the joint estimation of t_s, p, and q, where p and q were the estimated frequencies of the A_1 and A_2 alleles in the outcross pollen pool. Therefore, in order to estimate p and q, and in turn G_i with the single locus procedure, information on a large sample of maternal parents must be combined. If it is then desired to estimate t_{m_i} for one of the maternl parents from the population sample used to estimate the G_i, then the G_i and r_i for that maternal parent are not statistically independent. 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 data set used to estimate G_i. In this situation, the covariance of $\hat{G}_i$ and $\hat{r}_i$ would be expected to be small, relative to the total variance of $\hat{t}_{m_i}$. However, the uncertainty about the magnitude of COV($\hat{r}_i$, $\hat{G}_i$) can be eliminated if $\hat{G}_i$ and $\hat{r}_i$ are made independent. This can be done simply by removing the observations on the ith maternal parent from the data set when outcross pollen pool allele frequencies (p and q) are estimated by the single locus procedure. Thus, for each i maternal parent for which an estimate of t_{m_i} is desired, a separate set of outcross pollen pool frequencies must be estimated using RF004SL. Using this technique COV($\hat{r}_i$, $\hat{G}_i$) is zero and by substituting in expressions for VAR $\hat{G}_i$ and VAR $\hat{r}_i$, VAR $\hat{t}_{m_i}$ is approximated by,

$$\text{VAR } \hat{t}_{m_i} = \frac{1}{\hat{G}_i^2} \frac{\hat{r}_i(1-\hat{r}_i)}{N_i} + \frac{\hat{r}_i^2}{\hat{G}_i^4} \sum_{j=1}^{k} (\text{VAR } f_{ij})(\pi_{j=1}^{k} f_{ij}^2)$$

Single tree outcrossing estimates ($\hat{t}_{m_i}$) can be obtained by specifying a subroutine in RF004ML. Different values for outcross pollen pool allele frequencies must be substituted into the input data for each estimate of $\hat{t}_{m_i}$. Other details concerning the input data file and execution of RF004ML for single tree estimates are contained in the documentation to the program.

Table C.1. Conditional expectations for pollen gametes in viable seeds of maternal genotypes, based on the triallelic mixed mating model (t_s is the proportion of outcrossed pollen gametes and p, q, r are the frequencies at the A_1, A_2, and, A_3 alleles, respectively, in the outcross pollen pool).

Maternal genotype	Pollen gamete		
	A_1	A_2	A_3
A_1A_1	$(1 - t_s) + t_sp$	t_sq	t_sr
A_1A_2	$1/2(1 - t_s) + t_sp$	$1/2(1 - t_s) + t_sq$	t_sr
A_2A_2	t_sp	$(1 - t_s) + t_sq$	t_sr
A_1A_3	$1/2(1 - t_s) + t_sp$	t_sq	$1/2(1 - t_s) + t_sr$
A_2A_3	t_sp	$1/2(1 - t_s) + t_sq$	$1/2(1 - t_s) + t_sr$
A_3A_3	t_sp	t_sq	$(1 - t_s) + t_sr$

Table C.2. Matrix of R_{ij} observed pollen gametes for the ith maternal genotype class and jth pollen gamete genotype in the triallelic model.

Maternal genotype	Pollen gamete		
	A_1	A_2	A_3
A_1A_1	R_{11}	R_{12}	R_{13}
A_1A_2	R_{21}	R_{22}	R_{23}
A_2A_2	R_{31}	R_{32}	R_{33}
A_1A_3	R_{41}	R_{42}	R_{43}
A_2A_3	R_{51}	R_{52}	R_{53}
A_3A_3	R_{61}	R_{62}	R_{63}