Mating system and multilocus associations in a natural population of *Pseudotsuga menziesii* (Mirb.) Franco

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**Summary.** Arrays of open-pollinated seeds were assayed for allozyme polymorphisms at ten loci (*Aat2, Est1, G6pd, Idh, Mdh2, Mdh3, Pgm, Sod, 6Pgd1, 6Pgd2*) to obtain estimates of the outcrossing rate and assess multilocus association in a natural population of coastal Douglas-fir, *Pseudotsuga menziesii* (Mirb.) Franco. The allele frequencies in the samples of adult trees and pollen-gamete pool were similar. Maximum-likelihood estimators of the outcrossing rate for individual loci and two multilocus models were derived using counting methods. The single-locus maximum likelihood estimates (MLEs) of the outcrossing rate were significantly heterogeneous; they varied over a more than two-fold range from 0.404 to 0.935, with an average MLE of 0.741. Both multilocus MLEs of the outcrossing rate were 0.887. The sample of trees was in random mating equilibrium when assessed on a pairwise-locus basis using Burrows’ composite measure of gametic disequilibrium, with one exception (*Mdh2 Sod*) that was attributable to a rare “gametic” class. In the sample of pollen gametes, 5 of the 45 pairwise-locus associations were nominally significant at the 0.05 level: *Idh Est1, Mdh2 Sod, Aat2 Est1, Aat2 Mdh3*, and *Est1 Mdh3*. These apparent associations were attributable in most cases to the relative excess of uncommon or rare paternal gametes of discernibly outcrossed embryos. An additional two-locus association was identified for *Mdh2 Pgm* which was marginally significant for the major partition of the contingency table that excluded paternal gametes with the rare allele *Mdh2*.

**Key words:** Douglas-fir – Mixed mating model – Outcrossing rate – Gametic disequilibrium – Counting method

**Introduction**

Mating system studies in forest trees using allozyme polymorphisms have provided knowledge pertaining to the genetic structure of continuous populations, and to the relative contributions to population differentiation of selection, family structure and gene flow (for example, as reviewed by Brown et al. 1985). A common finding among studies of the mating system in forest trees is the heterogeneity of single-locus estimates of the outcrossing rate for the same set of embryos. Violations of the assumptions on which single-locus models are based have been suggested as the causes of the apparent heterogeneity (Shaw et al. 1981; Ellstrand and Foster 1983; Brown et al. 1985; Schoen and Clegg 1986).

An estimate of the outcrossing rate based on the information obtained by genotyping each embryo for several polymorphic isozyme loci, a multilocus estimator, will be more robust than individual-locus estimates (Shaw et al. 1981). In the first step of a two-step estimation procedure of mating system parameters, the embryo and maternal genotypes are compared in order to categorize each embryo as either discernibly outcrossed or ambiguous. Each additional polymorphic locus that is scored increases the likelihood that an outcrossed embryo will be discerned. One assumption in formulating the multilocus estimator is that no associations exist between alleles at different loci; that is, it is assumed that the pollen pool is in gametic phase equilibrium. (We use the term “gametic disequilibrium” instead of “linkage disequilibrium” in order to refer to associations between independent as well as linked loci.) Since evidence of gametic disequilibrium has most often been obtained in selfing plants, either in natural populations or in field populations construct-
ed from composite crosses (Brown and Allard 1970; Allard et al. 1972; Clegg et al. 1972; Kahler et al. 1975), the prediction for outcrossing plants such as forest trees has been that any initial gametic disequilibrium will rapidly decay in the absence of strong epistatic selection. This is a prediction, however, of long-term behavior. In the reestablishment of a forest tree population after a fire, for example, there could initially be gametic disequilibrium which will slowly decay for closely-linked genes and if there is nonrandom mating. This consideration is appropriate for coastal Douglas-fir, *Pseudotsuga menziesii* [Mirb.] Franco, in British Columbia because its distribution has been associated with the recent history of fire occurrence (Schmidt 1960).

In this paper, results are reported of analyses of ten polymorphic enzyme loci assayed by electrophoresis in a population of coastal Douglas-fir naturally regenerated after fire during the year 1927. The reproductive system in gymnosperms offers an unique opportunity to study segregation, linkage, gametic disequilibrium and outcrossing from the information provided by the various components of seed. The female gametophyte (megagametophyte) is haploid and all cells within the seed coat including the egg-cell (ovule) have been derived mitotically from a single megaspore. The maternal and pollen contributions to an embryo can be unambiguously determined, in principle, by genotyping the megagametophyte and the corresponding embryo from a single seed. At the same time, the most likely genotype of the tree can be assigned by inspection of the segregation patterns among its megagameto-

**Materials and methods**

**Seed collection**

Cones were collected in 1981 by rifle-fire from a pure coastal Douglas-fir population located on Mt. Prevost near Duncan, British Columbia, at 457 m elevation, latitude 48° 52'N and longitude 123° 45'W. The stand is situated on a gentle slope extending the plot to concentric circles until 60 trees minimum distance of 45 m apart were sampled around the center. The stand is genetically different than the coastal Douglas-fir population studied by El-Kassaby et al. (1982 a, b) with the result that the inventories of isozymes analyzed in the two samples do not entirely overlap.

**Outcrossing rate**

Maximum-likelihood estimates (MLEs) of the outcrossing rate of the mixed mating model were obtained by counting methods (Smith 1957) that use the EM algorithm (Dempster et al. 1977). A single-locus estimator of the outcrossing rate, an estimator of the average outcrossing rate of a set of independent loci, and two multilocus estimators were used. It is assumed that loci segregate independently in the formation of gametes for self-fertilization. An embryo that carries a non-maternal allele at any locus is categorized as discernibly outcrossed (DOC), otherwise as ambiguous (Shaw et al. 1981). An iterative procedure is used to estimate the posterior probability of being outcrossed for each embryo in the ambiguous class. This probability is a function of the outcrossing rate, the frequency of the paternal genotype in the outcross-pollen pool, and the expected segregation ratio of the maternal tree. One multilocus estimator restricts the set of possible multilocus genotypes of the pollen gametes to those observed in the sample of embryos. We call this "restricted-gametic" estimation. In the second multilocus estimator, the expected frequency of the multilocus genotype of the paternal gamete of an outcrossed embryo is the product of the appropriate allele frequency estimates of the outcross-pollen pool (e.g., Shaw et al. 1981). We call this "product-frequency" estimation. The restricted-gametic estimator is a straightforward extension of the single-locus estimator previously published (Cheliak et al. 1983).

For a test of the hypothesis that a single-locus estimate of the outcrossing rate is equal to 1 and a test for heterogeneity of outcrossing rates for independent loci, we used the Neyman-Pearson likelihood-ratio criterion *A* (Rao 1973). For the test of heterogeneity of outcrossing rates, we calculated the likelihood-ratio test statistic:

\[-2 \log_A = -2 \left( \sum I(\hat{t}) - I(\bar{t}) \right)\]