Estimation of the Outcrossing Rate of Douglas-Fir
[Pseudotsuga Menziesii (Mirb.) Franco] using Allozyme Polymorphisms

By Y. A. El-Kassaby, F. C. Yeh and O. Sziklai

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Abstract

The haploid megagametophyte and corresponding diploid embryo tissues from 36 seed trees from a natural stand of coastal Douglas-fir [Pseudotsuga menziesii (Mirb.) Franco] were assayed for electrophoretically demonstrable variation in proteins at four polymorphic loci (glucose-6-phosphate dehydrogenase, isocitrate dehydrogenase, phosphoglucomutase, and 6-phosphogluconic dehydrogenase). The probability of detecting genotypic arrays of progeny from seed trees of known genotypes was used as an estimator of outcrossing rate (t). The level of outcrossing (t = 0.9) yielded an average inbreeding coefficient (F) of 0.05 for these loci. It is not known whether this level of inbreeding is common in coastal Douglas-fir. However, if such inbreeding levels in progenies from are common, some of the improvement in performance seen pollinated controls may result from the break up of the family structure of the stands.

Key words: Allozyme, Inbreeding, Outcrossing Rate, Pseudotsuga menziesii

Zusammenfassung

Der haploide Megagametophyt und das entsprechende diploide Embryogewebe von 36 Samenbäumen aus einem natürlichen Bestand von Pseudotsuga menziesii Mirb. Franco wurden auf elektrophoretisch sichtbare Proteinvarianzen an 4 polymorphen Loci (Glukose-6-Phosphat-Dehydrogenase, Isocitrat-Dehydrogenase, Phosphoglucomutase, und 6-Phosphogluco-Dehydrogenase) untersucht. Es wurde die Wahrscheinlichkeit, eine genotypische Aufstellung der Nachkommenschaft der Samenbäume von bekannten Genotypen zu entdecken, benutzt, um die Fremdungsraten zu bestimmen. Das Fremdungs niveau (t = 0.9) ergab erwartungsgemäß durchschnittlichen Inzucht coefficients of 0.05 für diese Loci. Es ist nicht bekannt, ob dieses Inzucht niveau bei Douglas-fir ist. Jedoch, falls ein solches Inzucht niveau mög lich sein sollte, mag ein Teil der Verbreitung im Erscheinungsbild von Nachkommenschaften aus Samenplanta gen daraus resultieren, daß die Familienstruktur der Bestände durchbrochen worden ist.

Introduction

Forest trees are predominantly outbreeding (Stern and Rocchi, 1974), but the relative rate of outbreeding and inbreeding is natural to selection (Kahler et al., 1975). Knowledge of a species' breeding system is an important prerequisite for understanding the genetic structure of that species.

Electrophoretically detectable genetic variations (allozymes) are useful markers for studying mating systems in conifers in that the co-dominant nature of these allozymes enables identification of both phenotypes and genotypes without progeny testing. Furthermore, populations are often polymorphic for many enzyme loci, thus providing the investigator with a large set of gene markers. These advantages have resulted in an increased use of enzyme loci for the acquisition of quantitative measures of mating system parameters. Estimates obtained using enzyme markers are now available for several species: Eucalyptus obliqua (Brown et al., 1975); Picea abies (Muller, 1976 and Lundqvist, 1979); Pinus ponderosa (Mitton et al., 1977); Pinus sylvestris (Rudin et al., 1977 and Krzakowa, 1980); and Eucalyptus delegatensis (Morgan and Brown, 1980).

In this paper we present a method for estimating outcrossing rate (t) based on the probability of detecting the genotypic array of progenies from seed trees of known genotypes, and a quantitative measure of t for a natural stand of coastal Douglas-fir [Pseudotsuga menziesii (Mirb.) Franco].

Materials and Methods

Data Collection

Data were collected from individual haploid megagametophytes and their corresponding diploid embryos. In the fall of 1978, cones were collected from the upper one-third of the crown from each of 36 trees growing in a 40-year-old, open-grown stand of Douglas-fir. Ten seeds from each of 36 trees were utilized for this study.

Four polymorphic loci were studied. Inheritance at these loci has been previously determined as part of a population genetics study of Douglas-fir (El-Kassaby et al. in preparation). The loci studied were glucose-6-phosphate dehydrogenase (G6P), isocitrate dehydrogenase (IDH), phosphoglucomutase (PGM) and 6-phosphogluconic dehydrogenase (6PG-1). These loci were selected because of the clarity of the zymograms from the embryo tissue. The electrophoretic procedures, staining recipes, and enzyme nomenclature used have been reported elsewhere (Yeh and O'Malley, 1980).

Data Interpretation

Determination of the pollen genotype depends upon the maternal genotype. Once the genotype of the megagametophyte of a particular seed has been scored, it is a relatively simple process to determine the pollen contribution to the embryo of the same seed. Two mutually exclusive possibilities exist, assuming that there are i alleles (A1, A2, ..., and An) in an enzyme system:

1. The maternal genotype is homozygous (i.e., A1A1). Then the maternal contribution is A1 in all cases. The possible embryo genotypes are A1A1, A1A2, ..., or A2A2. Embryos having an A1A1 constitution exhibit a banding pattern described by a single band having the same relative migration distance as the corresponding megagametophyte. For the heterozygous embryos the banding pattern is a two-banded configuration for a monomorphic enzyme (i.e., PGM) or a three-banded configura-

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tion in the case of dimeric enzymes (i.e., G6P, IDH, and 6PG-I) (ES-KASSARY et al., in preparation).

2. The maternal genotype is heterozygous (i.e., A, A). The possible embryo genotypes vary according to the contribution of the maternal and pollen parents:
   a) When the maternal contribution is A, the embryo genotypes are A, A, A, A, . . . or A, A.
   b) When the maternal contribution is A, the embryo genotypes are A, A, A, A, . . . or A, A.

**Statistical Analyses**

Allele frequencies for the four loci studied were computed from the genotypic frequency of 360 embryos. Probabilities for obtaining progeny of a particular genotype were used to estimate the outcrossing rate (t).

Estimation procedures for the outcrossing rate (t) varied according to the maternal and progeny genotypes. The following formulae were used to estimate t:

1. Homozygous progeny from homozygous (A, A) maternal trees:
   \[
p(A, A) = \frac{t p_{AA} + (1 - t)}{p_{AA} + 1}
   \]
   \[t = \frac{p(A, A) - 1}{p_{AA} - 1}
   \]

2. Heterozygous progeny from homozygous (A, A) maternal trees:
   \[
p(A, A) = \frac{t p_{AA} + (1 - t)}{p_{AA} + 1}
   \]
   \[t = \frac{p(A, A) - 1}{p_{AA} - 1}
   \]

3. Homozygous progeny from heterozygous maternal tree of given A allele:
   \[
p(A, A) = \frac{t p_{AA} + 1 - t}{p_{AA} + 1}
   \]
   \[t = \frac{2(p(A, A)) - 1}{2p_{AA} - 1}
   \]

4. Heterozygous progeny from heterozygous maternal tree of given A allele:
   \[
p(A, A) = \frac{t p_{AA} + 1 - t}{p_{AA} + 1}
   \]
   \[t = \frac{2(p(A, A)) - 1}{2p_{AA} - 1}
   \]

where \( t \) = outcrossing rate.

\( p_{AA} = \) frequency of A allele from the progeny information.

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\( p(A, A) = \) frequency of A, A progeny from the total progeny of the A, A parent trees.

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For each locus, outcrossing rates were estimated for each condition and a weighted t was computed according to the number of trees within each condition.

**Results and Discussion**

The allelic frequencies of each locus as determined from haploid megagametophytes and from the observed genotypic frequencies in the embryos, together with tests for conformity to Hardy-Weinberg equilibrium are presented in Table 1. Both IDH and PGM exhibited significant departures from equilibrium genotypic frequencies in the embryos due to excess of homozygotes. However, the disproportionate chi-square values for the IDH locus were obtained mainly in the rare homozygous IDH (90/90) “expected” class, a situation where the chi-square statistics are unreliable (KIMURA and CROW, 1970).

<table>
<thead>
<tr>
<th>Locus</th>
<th>Allele</th>
<th>Maternal</th>
<th>Embryos</th>
</tr>
</thead>
<tbody>
<tr>
<td>G6P</td>
<td>80</td>
<td>0.014</td>
<td>0.013</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>0.014</td>
<td>0.009</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0.012</td>
<td>0.047</td>
</tr>
<tr>
<td></td>
<td>HWE X²</td>
<td>2.50</td>
<td>2.63</td>
</tr>
<tr>
<td>IDH</td>
<td>65</td>
<td>0.014</td>
<td>0.009</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>0.028</td>
<td>0.044</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0.022</td>
<td>0.074</td>
</tr>
<tr>
<td></td>
<td>122</td>
<td>0.009</td>
<td>0.073</td>
</tr>
<tr>
<td></td>
<td>HWE X²</td>
<td>0.77</td>
<td>89.83*</td>
</tr>
<tr>
<td>PGM</td>
<td>94</td>
<td>0.150</td>
<td>0.139</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0.753</td>
<td>0.760</td>
</tr>
<tr>
<td></td>
<td>105</td>
<td>0.097</td>
<td>0.101</td>
</tr>
<tr>
<td></td>
<td>HWE X²</td>
<td>1.76</td>
<td>31.76*</td>
</tr>
<tr>
<td>6PG-1</td>
<td>85</td>
<td>0.014</td>
<td>0.023</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0.958</td>
<td>0.956</td>
</tr>
<tr>
<td></td>
<td>107</td>
<td>0.028</td>
<td>0.021</td>
</tr>
<tr>
<td></td>
<td>HWE X²</td>
<td>0.09</td>
<td>5.05</td>
</tr>
</tbody>
</table>

* \( p = 0.005 \)

Estimates of outcrossing rate are presented in Table 2.

<table>
<thead>
<tr>
<th>Locus</th>
<th>t</th>
</tr>
</thead>
<tbody>
<tr>
<td>G6P</td>
<td>0.84</td>
</tr>
<tr>
<td>IDH</td>
<td>1.02</td>
</tr>
<tr>
<td>PGM</td>
<td>0.62</td>
</tr>
<tr>
<td>6PG-1</td>
<td>1.12</td>
</tr>
<tr>
<td>Average (( \bar{t} ))</td>
<td>0.90</td>
</tr>
</tbody>
</table>

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this stand could be increased by about three percent by eliminating natural inbreeding.

Knowledge of a species' breeding system is of a practical importance because of its genetic consequences. The mixed mating system of many Pinaceae species (Sullivan, 1974) and particularly the opportunity for spatial and temporal variations in the system (Brown et al., 1975, Moran and Brown, 1980, and Yeh and Layton, 1979), raises problems for provenance and progeny testing using open polinated seeds. If one provenance sample is less vigorous than another this may reflect useful adaptive variation or merely an ephemeral difference in the proportion of inbreds in the seed crop. Similarly, an unwarranted assumption of random mating may cause over estimation of additive genetic variance (Namkung, 1960).

Finally, to effectively use knowledge of a species' breeding system it is also necessary to understand the mechanisms through which the breeding system is maintained. Investigation of reproductive biology, phenology, and polination ecology are therefore an implicit component of breeding system studies.

**Literature Cited**


**Performance Level – Standardized Score for Progeny Test Performance**

By A. V. Hatcher, F. E. Bridgewater and R. J. Weir


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**Summary**

Performance levels are standardized scores for families in progeny tests of the North Carolina State University-Industry Tree Improvement Cooperative. These scores are one method of combining data from genetic tests with unbalanced experimental designs and unbalanced mating designs. Mean performance levels across tests are used to rank specific crosses (full-sib and clonal half-sib) values to determine their relative worth in tree improvement programs. With appropriate transformation, one may determine what proportion of a normally distributed population individual full-sib or half-sib average performance levels outrank.

Key words: Progeny testing, standard scores, performance level

**Zusammenfassung**


**Introduction**

A major reason for progeny testing is to rank parents based on the average performance of their offspring. Progeny tests of forest trees are replicated in time and space