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

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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 Proteinvariationen an 4 polymorphen Loci (Glukose-6-Phosphat-Dehydrogenase, Isocitrat-Dehydrogenase, Phosphoglukomutase, und 6-Phosphogluco-Dehydrogenase) untersucht. Es wurde die Wahrscheinlichkeit, eine genotypische Aufstellung der Nachkommenschaft der Samenbäume von bekannten Genotypen zu entdecken, benutzt, um die Fremdungsrate zu bestimmen. Das Fremdungsniveau (t = 0,9) ergab einen durchschnittlichen Inzuchtkoeffizienten von 0,05 für diese Loci. Es ist nicht bekannt, ob dieses Inzuchtniveau bei Douglasie üblich ist. Jedoch, falls ein solches Inzuchtniveau üblich sein sollte, mag ein Teil der Verbesserung im Erscheinungsbild von Nachkommenschaften aus Samenplantagen daraus resultieren, daß die Familienstruktur der Bestände durchbrochen worden ist.

Introduction

Forest trees are predominantly outbreeding (Stern and Roche, 1974), but the relative rate of outbreeding and inbreeding is subject to natural 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 Lundkvist, 1979), *Pinus ponderosa* (Mitton *et al.*, 1977); *Pinus sylvestris* (Rudin *et al.*, 1977 and Krzakowa, 1980); and *Eucalyptus delegatensis* (Moran 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 recipies, 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 allelels (A_1, A_2 . . . and A_i) in an enzyme system:

1. The maternal genotype is homozygous (i.e., A_1A_1). Then the maternal contribution is A_1 in all cases. The possible embryo genotypes are A_1A_1, A_1A_2 . . . or A_1A_i. Embryos having an A_1A_1 constitution exhibit a banding pattern described by a single band having the same relative migration distance as the corresponding megagametophytes. For the heterozygous embryos the banding pattern is a two-banded configuration for a monomeric 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-1) (El-Kassaby *et al.*, in preparation).

2. The maternal genotype is heterozygous (i.e., A_1A_2). The possible embryo genotypes vary according to the contribution of the maternal and pollen parents:
   a) When the maternal contribution is A_1, the embryo genotypes are A_1A_1, A_1A_2, . . . or A_1A_i.
   b) When the maternal contribution is A_2, the embryo genotypes are A_2A_1, A_2A_2, . . . or A_2A_i.

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_1A_1) maternal trees:

$$p(A_1A_1) = tp_{A_1} + (1 - t) \qquad t = \frac{p(A_1A_1) - 1}{p_{A_1} - 1}$$

2. Heterozygous progeny from homozygous (A_1A_1) maternal trees:

$$p(A_1A_i) = tp_{A_i} \qquad t = \frac{p(A_1A_i)}{p_{A_i}}$$

3. Homozygous progeny from heterozygous maternal tree of given A_1 allele:

$$p(A_1A_1) = tp_{A_1} + \frac{1 - t}{2} \qquad t = \frac{2\{p(A_1A_1)\} - 1}{2p_{A_1} - 1}$$

4. Heterozygous progeny from heterozygous maternal tree of given A_1 allele:

$$p(A_1A_i) = tp_{A_i} + \frac{1 - t}{2} \qquad t = \frac{2\{p(A_1A_i)\} - 1}{2p_{A_i} - 1}$$

where t = outcrossing rate.

P_{A_1} = frequency of A_1 allele from the progeny information.

P_{A_i} = frequency of A_i allele from the progeny information.

$p(A_1A_1)$ = frequency of A_1A_1 progeny from the total progeny of the A_1A_1 parent trees.

$p(A_1A_i)$ = frequency of A_1A_i progeny from the total progeny of the A_1A_i parent trees.

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 1. — Allele distribution and test for Hardy-Weinberg equilibrium ($HWE\chi^2$).

Locus	Allele	Maternal	Embryos
G6P	80	0.014	0.013
	90	0.514	0.500
	100	0.472	0.487
	$HWE\chi^2$	2.58	2.63
IDH	65	0.014	0.009
	90	0.028	0.044
	100	0.889	0.874
	122	0.069	0.073
	$HWE\chi^2$	0.77	89.83*
PGM	94	0.150	0.139
	100	0.753	0.760
	105	0.097	0.101
	$HWE\chi^2$	1.76	31.76*
6PG-1	85	0.014	0.023
	100	0.958	0.956
	107	0.028	0.021
	$HWE\chi^2$	0.09	5.05

* P = 0.005

Estimates of outcrossing rate are presented in *Table 2.* There was some inter-locus heterogeneity in the estimates of t for this stand. This heterogeneity was also observed in *Eucalyptus obligua* (Brown *et al.*, 1975), *Pinus sylvestris* (Rudin *et al.*, 1977), *Picea abies* (Lundkvist, 1979), and *Eucalyptus delegatensis* (Moran and Brown, 1980). Since t is a function of genotypic frequencies, single locus estimates of t are sensitive to evolutionary forces, in addition to the breeding system (Workman, 1969). However, the breeding system should be the only force affecting all loci equally (Lewontin and Krakaner, 1973). Consequently, t was averaged over four loci. The resultant outcrossing rate was 0.9 and the inbreeding rate, therefore, was 0.1. This is in agreement with the previously reported value of seven percent natural selfing observed in coastal Douglas-fir using a recessive mutant as a gene marker (Sorensen, 1973). If this level of inbreeding is common in coastal Douglas-fir, a portion of the improvement in performance of progenies from seed orchards over that of wind-pollinated controls may result merely from reduced inbreeding due to the breaking up of family structure of stands.

Langner (1966) reported that an increase in the coefficient of inbreeding (F) of 0.1 is accompanied by a height growth reduction of about five percent in several plant species. Therefore, our estimated value of F from the relationship $F = (1 - t)/(1 + t)$ (Brown *et al.*, 1975) suggests an expected reduction in height growth of approximately three percent. In other words, average height in

Table 2. — Estimates of the outcrossing rate.

Locus	t
G6P	0.84
IDH	1.02
PGM	0.62
6GP-1	1.12
Average ($\bar{t}$)	0.90

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 (Squillace, 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 pollinated 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 (Namkoong, 1966).

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 pollination ecology are therefore an implicit component of breeding system studies.

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Performance Level – Standardized Score for Progeny Test Performance

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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 cross (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

In den Nachkommenschaftsprüfungen des kooperativen Waldbaumzüchtungsprogramms zwischen der Universität North Carolina und der Industrie sind die Leistungsniveaus von Familien standardisierte Punktzahlen. Diese Punktzahlen sind eine Methode, um Daten von genetischen Tests von unbalancierten Versuchsplänen mit unvollständigen Kreuzungsplänen zu kombinieren. Um bestimmte Kreuzungen (Vollgeschwister) und Klone (Halbgeschwister) in eine Reihenfolge zu bringen, werden Leistungsniveaus über mehrere Tests verwendet, und auf diese Weise der relative Wert der Versuchsglieder in Züchtungsprogrammen bestimmt. Mit einer geeigneten Transformation kann man bestimmen, welcher Anteil einer normal verteilten Population über der durchschnittlichen individuellen Voll- oder Halbgeschwisterleistung liegt.

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