ESTIMATING CONFIDENCE LIMITS FOR ELECTROPHORETIC MATING SYSTEM PARAMETERS IN BLACK WALNUT

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Abstract: Starch gel electrophoretic banding patterns from black walnut embryo progeny of 26 wind-pollinated trees were used to generate the mating system parameters of outcrossing rate and fixation index. Confidence intervals around these mating system parameters were estimated by the "jackknife" method of alternating serial data omission. Preliminary analyses of mating system parameters and their confidence intervals indicated inbreeding to be a relatively minor component of the species mating system.

Additional Keywords: *Juglans nigra*, outcrossing, fixation index, multi-locus.

Black walnut (*Juglans nigra* L.) is the most valuable timber species in the central hardwood region. During the last two decades the stumpage price for black walnut in Illinois has been more than five times greater than the average stumpage price for other hardwood species. The high price reflected a scarcity of black walnut trees. The scarcity has meant that the walnut timber buyer has had to buy trees that were smaller in volume and poorer in quality (Frye 1982).

The increasing scarcity of black walnut trees also means a reduction in the size of breeding populations. As the effective population size becomes smaller, the outcrossing rate also becomes smaller and the rate of inbreeding increases, theoretically resulting in inbreeding depression.

In order to test the hypothesis that the outcrossing rate has become imperfect and that the deviation from random mating is significant, (the expected value for the outcrossing rate under ideal conditions is unity), we need to set confidence intervals for the mating system parameters outcrossing rate and fixation index. The procedure we have chosen for the setting confidence intervals is called the "Jackknife Method".

Jackknifing is a data analysis method widely used for bias reduction and for setting realistic approximate confidence limits in complex situations (Miller 1974.). The method involves partitioning a data set into groups. Desired calculations are made for the complete data set, and then for each of the slightly reduced bodies of data obtained by leaving out just one of the groups. A weighted difference between the result of calculations on the portion of the sample that omits a subgroup and the result for the entire sample is called a pseudo value. There are as many pseudo values as there are subgroups. These pseudo values are then used for confidence interval calculation.

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MATERIALS AND METHODS

Nut collections were made from 26 local Jackson County, Illinois trees; mother tree identity of each nut was retained. Approximately 50 nuts from each of 26 mother trees were dehusked and stratified at 2-5 °C until used. Nuts were cracked and cotyledons and embryos removed. Extracted tissue from each nut was squashed onto a 2 x 10 mm filter paper wick for electrophoresing. General techniques of starch gel electrophoresis, gel slicing, staining, and fixing are described by Marty *et al*. (1984) and Torres *et al*. (1978).

The following 12 enzyme systems were assayed: aconitase (ACO), alcohol dehydrogenase (ADH), aspartate amino transferase (AAT), glutamic-pyruvic transaminase (GPT), 6-phosphogluconic dehydrogenase (6PG), uridine diphosphoglucose pyrophosphorylase (UGPP), phosphoglucose isomerase (PGI), shikimic dehydrogenase (SKD), fluorescent esterase (FEST), isocitric dehydrogenase (IDH), malic dehydrogenase (MDH), and phosphoglucomutase (PGM). Systems assayed were selected on the basis of minimizing cost of evaluation and presence of differential migration on starch gel in exploratory runs. Of these 12 systems, only the following six showed consistently interpretable variation patterns: ACO, AAT, ADH, FEST, PGM, and 6PG. (Both PGM and ACO were controlled by two loci: PGM1, PGM2, and AC01, ACO2, respectively.)

DATA ANALYSIS

Data were analyzed using programs of Ritland and Jain (1981). The program identifies genotypes of mother trees for each assayed enzyme system, estimates gene frequencies for the system and outcrossing rate (t) for the mother trees being evaluated; estimates are based on multi-locus maximum likelihood equations developed elsewhere (Brown and Allard 1970).

The fixation index (F) is computed from the outcrossing rate (t) as follows (Li 1976, p. 254):

$$F = (1 - t)/(1 + t)$$

Because there were eight loci used in this study, the complete data set was duplicated eight times. Data associated with a given locus were deleted from the duplicated set one at a time and an estimate of t and F recalculated. For example, with the complete set of data we had t = 0.905 and F = 0.0499, but when the data associated with the AAT1 locus were deleted in the first duplicated data set, the value of t and F became 0.894 and 0.0560, respectively (Table 1, columns 2 and 4).

In order to compute the confidence intervals by the "Jackknife" procedure, a "pseudo-value" should be calculated for each "one at a time" deletion. The pseudo-value (P.V.) for one of the n subsets is:

P.V. = n(Estimate from the complete set) - (n-1)(Estimate from data set with one subset deletion)

For example, a pseudo-value for the outcrossing rate when the AAT1 locus was deleted should be 8(0.905) - 7(0.894) = 0.982, and for the fixation index, 8(0.0499) - 7(0.0560) = 0.0074 (Table 1).

After all 8 pseudo-values were obtained, the 95% confidence interval was constructed from these pseudo values.

Table 1. Computation of confidence interval for the outcrossing rate and for the fixation index in black walnut by using the "Jackknife" procedure with electrophoretic locus data

Locus Deleted	----Outcrossing Rate-----		------Fixation Index------	
	Estimate	Pseudo-value	Estimate	Pseudo-value
None	.905		.0499	
AAT1	.894	.982	.0560	.0074
PGM1	.926	.758	.0384	.1302
PGM2	.870	1.150	.0695	-.0874
ACO1	.902	.926	.0515	.0385
ACO2	.897	.961	.0543	.0191
ADH	.911	.863	.0466	.0732
FEST	.902	.926	.0515	.0385
6PG2	.892	.996	.0571	-.0004
Mean		.945		.0269
St. Error		.040		.0222
95% Conf. Int.		.850 to 1.039		0.794 to -0.0256

Similar procedures were followed using family data. The complete data set was duplicated 26 times, but each time one and only one family was deleted from the calculation of t and F. The 95% confidence interval then was estimated from the collection of 26 pseudo-values as presented in Table 2.

RESULTS AND DISCUSSION

The mating system parameters of black walnut trees were estimated by using eight allozyme loci from seed samples of 26 wind pollinated families. Based on results in Table 1, if this experiment were repeated but with other random samples of allozyme loci, we would have 95% confidence that the outcrossing rate would be within the range of 0.850 to 1.039; and the fixation index, 0.794 to -0.0256. Because the confidence interval of the outcrossing rate includes 1.0, one may accept that these black walnut trees are completely outcrossed. Likewise, since the confidence interval for the fixation index includes zero, one may choose to believe that there is no inbreeding among black walnut.

On the other hand, looking at family parameters (Table 2), the confidence interval for the outcrossing rate would not include zero. Thus, one may also choose to believe that a mild degree of selfing might have existed in these black walnut trees.

Table 2. Computation of confidence interval for the outcrossing rate and for the fixation index in black walnut by using the "Jackknife" procedure with electrophoretic data arranged by family.

Family Deleted	-----Outcrossing Rate----- Estimate	Pseudo-value	------Fixation Index------- Estimate	Pseudo-values
None	.905		.0499	
1	.903	.955	.0510	.0231
2	.905	.905	.0499	.0507
3	.912	.730	.0460	.1468
4	.901	1.000	.0521	-.0045
5	.903	.280	.0363	.3907
6	.899	1.055	.0532	-.0322
7	.901	1.000	.0521	-.0045
8	.902	.980	.0515	.0093
9	.900	1.030	.0526	-.0184
10	.899	1.055	.0532	-.0322
11	.900	1.030	.0526	-.0184
12	.904	.930	.0504	.0369
13	.903	.955	.0510	.0231
14	.904	.930	.0504	.0369
15	.906	.880	.0493	.0645
16	.907	.855	.0488	.0782
17	.904	.930	.0504	.0369
18	.896	1.130	.0549	-.0739
19	.908	.830	.0482	.0919
20	.905	.905	.0499	.0507
21	.906	.880	.0493	.0645
22	.903	.955	.0510	.0231
23	.905	.905	.0499	.0507
24	.901	1.000	.0521	-.0045
25	.909	.805	.0477	.1057
26	.924	.430	.0395	.3099
Mean		.898		.0540
St. Err.		.036		.0196
95% Conf. Int.		.824 to .972		.0944 to .0136

The "Jackknife" procedure indicates that the mating system parameters are more sensitive to the sampling variation in enzyme system than to the family variation. For example, in Table 1, if the PGM2 loci were not studied the outcrossing rate would be at the lowest (t = 0.87) and the fixation index would be at the highest (F = 0.07). In contrast, if the PGM1 were not used the outcrossing rate would be at the highest (t = 0.93) and the fixation index would be at the lowest (F = 0.04). Thus, the exclusion of a single locus could make a difference of 0.06 in the estimation of the outcrossing rate and a difference of 0.03 in the fixation index. By comparison, the exclusion of a single family (family No. 26 versus family No. 18 in Table 2) would only change the outcrossing rate by 0.03 and the fixation index by 0.015. These two values are half of that in the previous example.

The amount of inbreeding indicated by these mating parameters is rather low in light of the present pattern of species distribution and growth. Self-pollination is effective in this species and putative high-grading has resulted in coatings among increasingly homozygous, related, slow growing and disease-prone individuals (Beineke 1972). So why was a greater rate of inbreeding not evident? Three explanations could account for these results:

1. Inbred trees resulting from pollination among related trees apparently do not reach reproductive maturity (Rink et al., in press).
2. The separation between reproductive maturation of staminate and pistillate flowers is so complete that self-pollination may be relatively rare (Gleeson 1982).
3. Sampling variation can result from collecting seed in years when weather conditions minimized overlap of maturation of flowers of the opposite sex (Masters 1974). Weather records for 1984, the year of pollination, indicated relatively cool conditions during the months of March through May, a situation not conducive to floral overlap and therefore, not conducive to inbreeding.

A follow-up study of electrophoretic banding patterns from black walnut embryos should be performed from seed collected in different years. Such a study could verify or refute the present results. It could also decrease variation in the mating system parameter estimates.

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