

modified approach, the best families are selected on the basis of single traits. It employs unequal weighting in favor of families that are superior for several traits and considers the correlation between traits for the selected families. For growth and anthracnose resistance, which is already partially reflected in the growth trait, the modified approach might easily be applied.

These findings will be of most use to breeders attempting to improve black walnut for both timber and nut production. Breeders strictly interested in timber production may find it more appropriate to emphasize growth and wood quality traits. However, because of the direct impact of anthracnose on both tree growth and nut quality and yield, multicrop breeders can hardly afford to pass up opportunities to improve disease resistance. We believe that the potential for making gains in both traits is attractive, and suggest that rating of anthracnose incidence be considered in genetic improvement programs for black walnut. Our procedures are simple to use, and ratings obtained in only one or two epidemic years should be sufficient for effective selection of resistant families or individuals.

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Literature Cited

BERRY, F. H.: Etiology and control of walnut anthracnose. Maryland Agric. Exp. Stn. Bull. A-113, 22p. (1960). — BERRY, F. H.: Control of walnut anthracnose with fungicides in a black walnut plantation. Plant Dis. Rep. 61: 378–379 (1977). — BEY, C. F. and KUNG, F. H.: Multiple-trait selection in black walnut. Proc. Joint IUFRO Meeting S.02.04. 1–3: 117–128, Stockholm (1974). — BEY, F., A. S. MICKELSON, and M. GERARDO.: Black walnut seedling seed orchard development—a case history. Northeast Area Nurserymen's Conf. Proc., p. 48–56 (1972). — BLACK, W. M., and D. NEELY.: Effects of temperature, free moisture, and relative humidity on the occurrence of walnut anthracnose. Phytopathology 68: 1054–1056 (1978). — FUNK, D. T., R. C. SCHLESINGER, and F. PONDER, JR.: Autumn-olive as a nurse plant for black walnut. Bot. Gaz. 140 (suppl.): S110–S114 (1979). — KUNG, F. H., and C. F. BEY.: Heritability construction for provenance and family selection. Lake States For. Tree Improv. Conf. Proc. 13 (1977): 136–146 (1979). — SHORTLE, W. S., A. L. SHIGO, P. BERRY, and J. ABUSAMRA.: Electrical resistance in tree cambium zone: relationship to rates of growth and wound closure. For. Sci. 23: 326–329 (1979).

Estimation of Outcrossing in Ponderosa Pine, *Pinus ponderosa* Laws., from Patterns of Segregation of Protein polymorphisms and from Frequencies of Albino Seedlings

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Summary

Six protein polymorphisms and one seedling character, albinism, were used to estimate the level of outcrossing in ponderosa pine, and to investigate several other aspects of the mating system. The six protein polymorphisms give a homogeneous set of estimates for outcrossing with a weighted average of 0.96, and this estimate is in good agreement with estimates based upon seedling albinism and with a previous estimate based upon one protein polymorphism. Row by columns contingency tests of progeny arrays from different seed trees detected no tree-to-tree heterogeneity in the mating system. For two of the six protein polymorphisms, estimates of allelic frequencies in the effective pollen pool differed significantly from the frequencies of adult trees in the population.

Key words: Outcrossing, ponderosa pine, protein polymorphism, albinism.

Zusammenfassung

Sechs Proteinpolymorphismen sowie ein Sämlingsmerkmal, (Albinismus), wurden benutzt, um den Grad der Fremdung innerhalb der Baumart *Pinus ponderosa* abzuschätzen und um einige andere Aspekte des Kreuzungsschemas zu untersuchen. Die sechs Proteinpolymorphismen erge-

ben in Bezug auf die Fremdung eine homogene Reihe von Schätzungen mit einem gewichteten Durchschnitt von 0,96. Sie stimmt mit den Schätzungen überein, die sich auf den Sämlingsalbinismus und einen Proteinpolymorphismus beziehen. Kontingenztests hinsichtlich der Nachkommenschaftsanordnungen aus verschiedenen Samenbäumen zeigten in dem Kreuzungssystem keine Heterogenität zwischen den Einzelbäumen. Für zwei der sechs Proteinpolymorphismen unterschieden sich die Schätzwerte hinsichtlich der Allelfrequenz in den Pollen-pools wesentlich von den Frequenzen der Elternbäume in der Population.

Introduction

The mating system is an important factor influencing several aspects of the genetic architecture of a population, most particularly the proportions of genotypes, the distribution of genetic variability, and the degree to which the genome is organized. Populations in predominantly outbred species are expected to have levels of heterozygosity approximating the expectations of the binomial square law, while populations in predominantly inbreeding populations are expected to exhibit a reduction in heterozygosity proportional to the degree of selfing. The mating system also influences genetic correlations among loci within a population. While outbred populations will exhibit independence between loosely linked and unlinked loci, in-

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breeding may induce genetic correlations across loci, even when the loci are loosely linked or on separate chromosomes. The mating system also influences the degree of differentiation of populations. Because outbreeding populations generally have a greater potential for gene flow, populations within an outbreeding species, all else being equal, will form a more homogeneous set, whereas populations within an inbreeding species will be relatively more differentiated. Thus, the degree of inbreeding profoundly affects the expected level of heterozygosity, associations between loci, and the degree of differentiation among populations.

Recently, it has been demonstrated for several species of plants that rates of outcrossing are not species-specific constants but rather vary both spatially and temporally. Long term studies of a population of barley, *Hordeum vulgare*, constructed from crosses among 22 inbred strains indicated that the level of outcrossing increased by an order of magnitude in approximately 20 generations (KAHLER *et al.* 1975). Rates of outcrossing in the annual *Collinsia sparsiflora* vary from 1% to more than 50% across populations (ALLARD and KAHLER 1971), and rates of outcrossing vary across populations from .38 to .89 in *Clarkia exilis* and from .08 to .83 in *Clarkia trembloriensis* (VASEK and HARDING 1976). Similarly, populations of *Plectritis congesta* range from 48% to 89% outcrossed (CAREY and GANDERS 1980) and populations of *Elymus canadensis* are 0% to 25% outcrossed (SANDERS and HAMRICK 1980). Outcrossing in balsam fir, *Abies balsamea*, varies in an elevational transect from 97% at lower elevation to 78% at the upper extreme of the distribution (D. B. NEALE, pers. comm.). These examples of heterogeneity in the mating systems of different populations warn us against the fallacy of assuming a single population to be representative of a species.

Estimates of rates of outcrossing may be generated with several different techniques (see JAIN 1979, for a recent summary). The frequencies of grossly abnormal seedlings (FRANKLIN 1968; SORENSON 1973) and the frequencies of single-gene morphological characters (ALLARD and WORKMAN 1963; IMAM and ALLARD 1965) have been used most commonly to quantitatively estimate rates of outcrossing, but protein polymorphisms detected by electrophoresis may also be used to study mating systems (CLEGG, 1980). Trees heterozygous for rare alleles will produce rare homozygotes by selfing; the frequency of specific genotypes in the progeny of heterozygous mothers has been used to estimate the rate of selfing in Norway spruce and Scots pine (MÜLLER 1976, 1977) and also in loblolly pine (ADAMS and JOLY 1980). Finally, protein genotypes from seedlings collected as seed from natural populations have been subjected to maximum likelihood analyses to estimate the rate of outcrossing as well as to infer the genotype of the maternal parent and to estimate the frequencies of alleles in the effective pollen pool (BROWN and ALLARD 1970; CLEGG *et al.* 1978). This method has been used to estimate the rate of outcrossing in *Eucalyptus obliqua* (BROWN *et al.* 1975), *Pinus ponderosa* (MITTON *et al.* 1977), and *Abies balsamea* (NEALE 1978). Furthermore, this technique may be used to determine whether frequencies in the pollen pool are representative of the whole population and to test whether the pollen successful in fertilizing seeds is homogeneous across maternal parents (BROWN *et al.* 1975, NEALE 1978).

In this study we investigated the rate of outcrossing in one population of ponderosa pine, *Pinus ponderosa* var.

scopulorum, using both protein genotypes and the frequency of albino seedlings in open-pollinated families. Six protein polymorphisms were used to study the mating system in a natural population. We collected seeds from trees of known protein genotypes and compared the observed genotypes with those inferred by the maximum likelihood estimation procedure. We have tested progeny arrays to determine whether the pollen successful in fertilizing seeds was homogeneous across maternal parents (BROWN *et al.* 1975, NEALE 1978). Finally, we have compared the estimate of outcrossing obtained from protein polymorphisms with the estimate obtained from the observed frequency of albino seedlings.

Materials and Methods

The trees utilized in this study are all from a site 1 km west of Boulder, Colorado, at an elevation of 1740 m. This stand of trees has received extensive investigation of growth rate, intensity of cone production, and microdifferentiation of protein polymorphisms (MITTON *et al.* 1977, 1979; LINHART *et al.* 1979, 1981b, 1981c). A total of 31 families were utilized, and 10 to 22 of these families were sampled for each polymorphism to generate the genotypic arrays needed for the estimation of the rate of outcrossing.

Enzyme polymorphisms were identified from seedling and adult needle tissue by the method of MITTON *et al.* (1977, 1979). The enzyme polymorphisms utilized for this study include peroxidase (Per-2), fluorescent esterase (FE), phosphohexose isomerase (PHI), glutamate dehydrogenase (GDH), phosphoglucosmutase (PGM-2), and shikimate dehydrogenase (SHDH). Simple patterns of Mendelian inheritance for Per-2, FE, PHI, GDH and PGM-2 are described in MITTON *et al.* (1979). Inheritance of patterns of SHDH are described in LINHART *et al.* (1981a). All polymorphisms except GDH have 3 or more alleles; GDH has 2 alleles. For the analyses presented here, however, alleles have been pooled to two alleles per polymorphism. Detailed allelic frequencies for this population are presented and analyzed in LINHART *et al.* (1981a, 1981c). The alleles lost by pooling ranged in frequency from .01 to .08. In each case, the pooling was done to maximize the efficiency of the maximum likelihood procedure.

The frequency of alleles in the pollen pool and the level of outcrossing (t) were estimated by the method of BROWN and ALLARD (1970) which had been modified slightly by CLEGG *et al.* (1978). From an initial estimate of pollen pool frequencies and the rate of outcrossing, this method infers the maternal genotype for each progeny array, and given this distribution of parental genotypes, it then estimates jointly the pollen frequencies and rate of outcrossing with a maximum likelihood procedure. The new estimates of pollen gene frequencies and outcrossing are once again used to infer maternal genotypes and this process is repeated until the maternal genotypes, and pollen frequencies, and outcrossing rates do not change with further iteration. This method of estimation is inefficient and it may not generate estimates of pollen frequencies and outcrossing if all three maternal genotypes are not present in the sample. Three of the enzyme polymorphisms (Per, PHI, PGM-2) fell into this category, and for these polymorphisms we utilized a direct estimation procedure (ALLARD and WORKMAN 1963; IMAM and ALLARD 1965). The estimates of frequencies in the pollen are those obtained

from the progeny array, and the rate of outcrossing (t) and its standard error (SE) are

$$t = H/p, SE = [(1/p)^2 \left(\frac{H(1-H)}{N_1} \right) + \left(\frac{H}{p^2} \right)^2 \left(\frac{p(1-p)}{N_2} \right)]^{1/2}$$

where p is the allelic frequency of the allele in the least common homozygote, H is the proportion of heterozygotes in the progeny of the most common homozygote, N₁ is the total number of progeny, and N₂ is the number of progeny sampled from the most common type of homozygote.

Homogeneity in the mating system was tested in two ways. First, we used the method of RAO (1973) to test whether the estimates of outcrossing were heterogeneous across loci. Then we looked at data from each locus, to determine whether there was variation in the mating system from individual to individual. This test, conducted with row by column contingency test (SOKAL and ROHLF 1963), compares the numbers of heterozygous offspring among homozygous mothers (BROWN, MATHESON, and ELDRIDGE 1975; NEALE 1978). This test may detect either variation in the pollen pool (pollen with different allelic frequencies arriving at different trees) and/or variation among trees for outcrossing rate.

Table 1. — Progeny genotypes for shikimate dehydrogenase and inferred maternal genotypes.

Progeny Genotype			Family [Id. No.]	Maternal Genotype	
22	23	33		Inferred	Observed
4	8	2	43	23	23
11	5	1	180	23	23
14	5	0	175	22	22
15	1	0	49	22	22
0	7	7	21	33	33
12	4	0	81	22	22
7	7	4	171	23	23
6	7	1	214	23	23
9	2	0	57	22	22
0	11	3	90	33	33
7	8	0	13	22	23
4	7	5	4	23	23

Table 2. — Allelic frequencies in the zygotic pool and the pollen pool, and estimates of rates of outcrossing (t) for 6 protein polymorphisms. X² tests the differences in allelic frequencies between the zygotic and pollen pool.

Protein	# families	# progeny	Pollen		Zygotic		X ²	t _d ± S E		t _{ml} ± S E	
			f(c)	± S E	f(c)	± S E					
Per-2	22	407	.869	.017	.729	.021	25.4***	1.038	.142	-	-
FE	25	446	.624	.018	.611	.023	0.2	.702	.101	1.004	.039
PHI	24	423	.947	.011	.977	.007	5.3*	1.113	.321	-	-
GDH	22	85	.682	.022	.700	.022	0.3	.945	.127	.923	.043
PGM-2	23	413	.917	.014	.920	.012	0.0	1.157	.262	-	-
SHDH	<u>12</u>	<u>83</u>	.721	.034	-	-	-	.601	.157	.893	.064
	128	2,257									

Note: S E = standard error, * = p < .05, *** = p < .001, f (c) is the frequency of the most common allele, and t_d and t_{ml} are estimates of outcrossing obtained by the direct and the maximum likelihood methods. Dashes indicate that estimates were not obtained.

Albino seedlings are found at this site and among seedlings raised from seed in the greenhouse. We have assumed that all observed albinos are expressions of a single recessive allele at a single locus (SORENSEN 1973), but we have no breeding data to substantiate this assumption. It is clear, however, that the albinism is not a dominant trait.

Results

The progeny arrays for each locus may be summarized as in Table 1, which presents the data for the SHDH polymorphism. Analyses of this array and other arrays are presented in Table 2. The rates of outcrossing obtained by the direct method vary from .601 to 1.157 and form a homogeneous set (P = .10) with a weighted mean of .841. If the direct estimates of t are replaced by maximum likelihood estimates whenever possible (Table 2), the estimates again form a homogeneous set (P < .50) with a weighted mean of .960. The later estimate is in good agreement with our previous estimate of outcrossing (.956) which was based solely upon the Per-2 locus (MITTON *et al.* 1977).

The genotypes of progeny and the inferred maternal genotypes are presented in Table 1 as a demonstration of the inference of the maternal genotypes (BROWN and ALLARD 1970, CLEGG *et al.* 1978). In most of the studies in which this technique has been employed, it was not possible to directly observe the genotype of the maternal parent. At this site we can compare the known maternal genotypes with those inferred from the progeny arrays. For example, shikimate dehydrogenase (Table 1) has one family (13) with a discrepancy between the observed and inferred maternal genotype: the inferred genotype is homozygous, but the observed genotype is heterozygous. Within the progeny, there were 7 fast homozygotes (22), and 8 heterozygotes (23). With the gene frequencies observed in the pollen pool, two slow homozygotes (33) were expected in this number of progeny from a heterozygous parent. This array is clearly not impossible for a heterozygous parent, but it is more likely to come from a homozygous parent. Similarly, there are 2 discrepancies for the PER locus, 2 for the FE locus and 1 for PGM-2. In each case, a maternal parent known to be heterozygous was inferred to be a homozygote. There is perfect agreement for all families for the PHI and GDH loci. From these data, which have an average of 18 progeny per family, the inference of the maternal genotype was correct

Table 3. — Summary of Row by Columns G-tests of progeny arrays of homozygous females for analysis of heterogeneity in the mating system

Enzyme	families	progeny	df	G	Probability
Per-2	15	279	14	17.7	p > .10
FE	11	225	10	13.9	p > .10
PHI	22	391	21	14.6	p > .50
GDH	11	189	10	12.3	p > .25
PGM-2	20	365	19	16.7	p > .50
SHDH	3	43	2	2.3	p > .10

95% of its time. More accurate inference of maternal genotype could be achieved with a larger sample size.

The progeny genotypes were treated with a contingency chi-square test to determine if there was heterogeneity in the outcrossing rate among trees or in the pollen received by the maternal parents. The progeny genotypes of known homozygous mothers were placed in a 2 × m contingency table, where m is the number of homozygous mothers sharing the same genotype. Wherever sample sizes were sufficient, χ^2 values and degrees of freedom were summed across different homozygous genotypes. Results of these analyses are summarized in Table 3. None of the tests revealed significant heterogeneity in the proportion of heterozygous offspring of homozygous mothers. Thus, there is no evidence of either differential outcrossing rate or patchiness in the pollen cloud for this site.

Chi-square tests were performed on allelic frequencies in the mature trees and in the effective pollen pool to determine whether the effective pollen pool was a random sample of the adult trees (Table 2). For two of the six protein loci, there were significant differences between these samples. Although the differences are statistically significant, they appear to be slight.

We were able to estimate the rate of outcrossing from the occurrence of albino seedlings with both seedlings germinated in the greenhouse and seedlings found at our field site. In the greenhouse, we found 10 albinos in 1,651 seedlings from 71 families. If the frequency of albinism is multiplied by four (this assumes that albinos are produced only by selfing of rare heterozygous trees) an estimate of 2.42% selfing is obtained. At our field site, 31 of 5116 seedlings were albino, producing a second estimate of 2.42% selfing. Thus, the rate of outcrossing is estimated to be 97.6 percent.

The albino seedlings may be used yet another way to estimate the rate of outcrossing. The method above assumes that all albino seedlings are produced by selfing of trees heterozygous for the allele; in reality, they can also be produced by outcrosses between heterozygous trees. We can allow for this possibility if we can estimate the frequency of the albino allele in the total population. From seedlings raised in the greenhouse, we infer that 8 of 71 trees are heterozygous for the albino allele. Thus, we estimate that the frequency of the albino allele at our field site is .056. If the trees heterozygous for the albino allele were 100% outcrossed, we would expect to find $.5 \times .056 = .028$ of the seedlings to be albino. Of 225 seedlings raised in the greenhouse from 8 heterozygous trees, 10, or .044 are albino. The frequency of selfing in the trees heterozygous for the albino allele may be estimated with the use of the following equation:

$$aa_{\text{obs}} = (t) (a_m) (a_p) + (1-t) (a_m)^2$$

where aa_{obs} is the observed frequency of selfing albinos, a_m and a_p are the frequency of the albino gene in maternal and paternal parents, and t is the proportion of seeds that are outcrossed. The maternal frequency is .5, for we are only analyzing the data from heterozygous mothers, and a_p is .056, the estimate of this allele in the entire population. These estimates from greenhouse seedlings, when utilized in the formula above, provide an estimate of outcrossing of 92.8%. At the field site, 31 of 901 seedlings were albino, and these data yield an estimate of outcrossing of 97.3 percent.

Heterozygous maternal trees vary from 1.6 to 9.7 percent for the frequency of albinos in their progeny. The two trees with the highest frequencies of albinos were adjacent to one another, suggesting that their elevated frequencies of albinos are due to pollen exchange rather than an enhancement of selfing.

Discussion

Conifer seeds rarely travel more than 200m, and although pollen is capable of moving immense distances, normal or average distances for effective pollen dispersal are modest (LEVIN and KERSTER 1974). For example, MÜLLER (1976) used a leucine aminopeptidase polymorphism in *Pinus sylvestris* to study the distance of effective pollen dispersal. Seeds collected from trees close to and distant from a single tree with an unusual genotype were analyzed for their genotypes. The frequency of the unusual pollen decreased with distance from the source, approaching zero beyond 80 meters. Müller concluded that trees were more likely to accept pollen from very close pollen sources than to accept pollen as if sampling the population randomly. This conclusion is in good agreement with theoretical expectations (GLEAVES 1974), and it is also consistent with our data on the frequency of albino seedlings in the progeny of two adjacent heterozygous mothers. These adjacent trees have elevated frequencies of albinos, for in addition to the albinos produced by selfing, a higher proportion of albinos is produced by outcrossing with trees carrying the recessive allele. Thus, there must be some patchiness in the pollen cloud. Yet this patchiness must be slight, for tests aimed at detecting heterogeneity in the mating systems (Table 3) reveal no significant heterogeneity.

Although there is no significant degree of heterogeneity in the pollen pool, there is significant heterogeneity in the microgeographic distribution of the adult trees within this 2 ha site (LINHART *et al.* 1980b, 1980c). Patches or clusters of trees, probably reflecting familial structure, are present in this population. The presence of these clusters is probably attributable to historical accident and restricted movement of seed. Photographs taken at this site almost a century ago reveal that the density of trees was lower. Seeds dropped by the few trees present decades ago probably did not travel very far, so the clusters of trees evident at the site today probably represent related individuals.

Estimates of rates of outcrossing are obtained here by very different methods, but the agreement among the methods is good. Each method has some implicit assumptions and despite the probability that the assumptions are not perfectly correct, the results are effectively equivalent. The use of protein polymorphisms assumes that there is not differential survival between pollination and germination. The phenomenon of simple polyembryony, with each seed initially containing several genetically het-

erogeneous embryos, provides an opportunity for such selection. The estimates from the maximum likelihood procedure (CLEGG, KAHLER, and ALLARD 1978) for FE, GDH, and SHDH and the estimates from direct estimation (ALLARD and WORKMAN 1963) for Per-2, PHI, and PGM-2 form a homogeneous set with a weighted mean of .960 (Table 2). The maximum likelihood analysis is preferable in that it makes more complete use of the data and has smaller standard errors, but if all maternal genotypes are not available, the direct estimation procedure must be used. Simple observation of rare recessive phenotypes also provides an estimate of outcrossing, but in this method, it is assumed that all recessive phenotypes are produced by selfing. As the frequency of the recessive allele becomes more and more common, this assumption becomes increasingly inappropriate, and the estimate of selfing becomes artificially inflated. Our observations of albino seedlings in the greenhouse and in the field revealed identical estimates of outcrossing rates of .976. The method of estimation of outcrossing presented last in this study represents an improvement over the simple tallying of recessive phenotypes. This method, in utilizing an estimate of frequencies in the pollen pool, attempts to apportion matings into selfs and outcrosses, and thus achieves a more accurate estimate of outcrossing. This method provided estimates of 93 and 97% outcrossing. Although the estimates of outcrossing obtained with protein polymorphisms and visible mutations are quite similar, we expect that the use of protein polymorphisms will predominate in future studies of mating systems, for protein polymorphisms are common in forest trees (HAMRICK, LINHART, MITTON 1979), but visible mutations may not always be available.

A previous estimate of outcrossings in ponderosa pine, based on only the PER locus at a site at an elevation of 2100 m, was .956. The estimates obtained for this site, at the lower edge of the distribution of ponderosa pine in the Front Range, bracket that earlier estimate. Thus, there is no evidence of variation in the mating system between these sites.

Our results indicate that ponderosa pine in these populations is more highly outcrossed than several other forest trees (SORENSEN 1973, WRIGHT 1976). This is particularly noteworthy for the cones were collected by hand or with the aid of a pole pruner, and thus were collected from the lower part of the crown. In *Pinus resinosa*, seeds from the lower portion of the crown are self-pollinated more often than seeds from the upper portion of the crown (FOWLER, 1965). This result is probably due to the predominance of female cones high in the crown and male cones lower in the crown, and because this distribution of cones is common in pines, the results of FOWLER (1965) may be general for pines. The high levels of outcrossing observed here may be due to a combination of factors. First of all, the trees at this site are not large or tall, so that differences commonly observed between upper and lower crown may not be well developed. In addition, these trees are in a very open stand (approximately 100 mature trees per hectare), and the open space between trees allows free movement and mixing of pollen. Finally, this region is famous for its strong and turbulent winds, which may thoroughly mix the pollen cloud.

Highly outcrossed species are expected to have high levels of heterozygosity and little between-population differentiation relative to predominantly self-pollinated species. The observations available to date for ponderosa pine

are consistent with these observations. The average heterozygosity of ponderosa pine protein loci is approximately 12% (O'MALLEY, ALLENDORF, and BLAKE 1979) and this value is high relative to species with other mating systems (HAMRICK, LINHART, and MITTON 1979). Although stands of ponderosa pine are highly variable, only about 12% of the variation of protein polymorphisms is associated with differentiation between populations (O'MALLEY, ALLENDORF and BLAKE 1979).

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Literature Cited

- ADAMS, W. T. and R. J. JOLY: Allozyme studies in loblolly pine seed orchards: Clonal variation and frequency of progeny due to self-fertilization. *Silvae Genetica*, 29: 1-4 (1980). — ALLARD, R. W. and A. L. KAHLER: Allozyme polymorphisms in plant population: Stadler Genetics Symposia 3: 9-24 (1971). — ALLARD, R. W. and P. L. WORKMAN: Population studies in predominantly self-pollinated species. IV Seasonal fluctuations in estimated values of genetic parameters in lima bean populations. *Evolution* 17: 470-480 (1963). — BROWN, A. H. D., A. C. MATHESON, and K. G. ELDRIDGE: Estimation of the mating system of *Eucalyptus obliqua* L'HÉRÉT. by using allozyme polymorphisms. *Aust. J. Bot.* 23: 931-949 (1975). — CAREY, K. and F. R. GANDERS: Heterozygote advantage at the fruit wing locus in *Plectritis congesta* (Valerianaceae). *Evolution* 34: 601-607 (1980). — CLEGG, M. T., A. L. KAHLER, and R. W. ALLARD: Estimation of life cycle components of selection in an experimental plant population. *Genetics* 89: 765-792 (1978). — CLEGG, M. T.: Measuring plant mating systems. *Bioscience* 30: 814-818 (1980). — FOWLER, D. P.: Effects of inbreeding in red pine, *Pinus resinosa* AIT. III. Factors affecting natural selfing. *Silvae Genetica* 14: 36-46 (1965). — GLEAVES, J. T.: Gene flow mediated by wind-borne pollen. *Heredity* 31: 355-366 (1973). — HAMRICK, J. L., Y. B. LINHART, and J. B. MITTON: Relationships between life history characteristics and electrophoretically detectable genetic variation in plants: *Ann. Rev. Ecol. Syst.* 10: 173-200 (1979). — IMAM, A. G. and R. W. ALLARD: Population studies in predominantly self-pollinated species. VI. Genetic variability between and within natural populations of wild oats from differing habitats in California *Genetics* 51: 49-62 (1965). — JAIN, S. K.: Estimation of outcrossing rates: Some Alternative procedures. *Crop Sci.* 19: 23-26 (1979). — KAHLER, A. L., M. T. CLEGG, and R. W. ALLARD: Evolutionary changes in the mating system of an experimental population of barley (*Hordeum vulgare* L.) *Proc. Nat. Acad. Sci. U. S. A.* 72: 943-946 (1975). — LINHART, Y. B., J. B. MITTON, D. M. BOWMAN, K. B. STURGEON, and J. L. HAMRICK: Genetic aspects of fertility differentials in ponderosa pine. *Genet. Res. Camb.* 33: 237-242 (1979). — LINHART, Y. B., M. L. DAVIS, and J. B. MITTON: Genetic control of shikimate dehydrogenase in ponderosa pine. *Biochem. Genet.*, in press (1981a). — LINHART, Y. B., J. B. MITTON, K. B. STURGEON, and M. L. DAVIS: An analysis of genetic architecture in populations of ponderosa pine. In M. T. CONKLE, (ed.) *Isozymes of North American Forest Trees and Forest Insects*. In press (1981b). — LINHART, Y. B., J. B. MITTON, K. B. STURGEON, and M. L. DAVIS: Genetic variation in space and time in a population of ponderosa pine. *Heredity*, in press (1981c). — MITTON, J. B., Y. B. LINHART, J. L. HAMRICK, and J. S. BECKMAN: Observations on the genetic structure and mating system of ponderosa pine in the Colorado Front Range. *Theor. Appl. Genet.* 57: 5-13 (1977). — MITTON, J. B., Y. B. LINHART, K. B. STURGEON and J. L. HAMRICK: Allozyme polymorphisms detected in mature needle tissue of ponderosa pine. *J. of Hered.* 70: 86-89 (1979). — MÜLLER, G.: Short note: Cross fertilization in a conifer stand inferred from enzyme gene-markers in seeds. *Silvae Genetica* 26: 223-226 (1977). — NEALE, D. B.: Allozyme studies in balsam fir. Masters thesis. University of New Hampshire (1978). — O'MALLEY, D. M., F. W. ALLENDORF, and G. M. BLAKE: Inheritance of isozyme variation and heterozygosity in *Pinus ponderosa* *Biochem. Genet.* 17: 233-250 (1979). — RAO, C. R.: *Linear statistical inference and its application*. 2nd ed. John Wiley and Sons, New York (1973). — SANDERS, T. B. and J. L. HAMRICK: Variation in the breeding system of *Elymus canadensis* (1980). — SOKAL, R. R. and F. J. ROHLF: *Biometry* (1963). — SORENSEN, F. C.: Frequency of seedlings from natural self-fertilization in coastal Douglas-fir. *Silvae Genetica* 22: 20-24 (1973). — VASEK, F. C. and J. HARDING: Outcrossing in natural populations. V. Analysis of outcrossing, inbreeding, and selection in *Clarkia exilis* and *Clarkia trembloriensis*. *Evolution* 80: 403-411 (1976). — WRIGHT, J. W.: *Introduction to forest genetics*. Academic Press, New York. 463 pp. (1976).