GENETIC EFFICIENCY IN LOBLOLLY PINE SEED ORCHARDS

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Abstract.--Using seven allozyme loci as genetic markers, gene frequencies in the bulked seed crop of two seed orchards were compared to those expected on the basis of maximum genetic efficiency. Significant deviations from expected values were found for allelic frequencies in embryos as well as in both the ovule and pollen pools which produced the embryos. While contamination from pollen sources in surrounding stands (estimated at 28%) may be important in causing deviations from expectation in the pollen pool, self-fertilization does not appear to be a problem since the proportion of selfed progeny was estimated to be very low (< 1.5%) in these orchards. When seeds were separated by size there was a substantial loss of genetic variability in the ovule pool within a size class. Several other factors which might be responsible for decreased genetic efficiency are considered. Implications of these findings for seed orchard management are discussed.

Additional keywords: Pinus taeda, allozyme variation, seed orchards, self-fertilization, pollen contamination

The purpose of seed orchards is to produce mass quantities of genetically improved seed for reforestation. The efficiency of seed orchards is defined as the degree to which seed crops approach maximal production as well as reflect the genetic superiority and variability present among orchard clones. Factors influencing the efficiency of seed production have received considerable attention in the literature, and techniques for increasing productivity through fertilization regimes, irrigation, insect control, and other cultural treatments have been discussed (see reviews in Faulkner 1975). Genetic efficiency, on the other hand, is difficult to measure, and relatively few studies of factors influencing genetic efficiency have been reported (e.g., Adams and Joly 1980a, Bergmann 1968, Eriksson et al. 1973, Muller-Starck 1978, Squillace 1977).

To achieve maximum genetic efficiency in wind-pollinated seed orchards, the following conditions would need to be met (Woessner and Franklin 1973):

1. Orchard ramets must be more or less completely isolated from surrounding unselected trees.

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2. Natural self-fertilization must occur at insignificant rates.

3. Ramet pollen flight and female flower receptivity must coincide.

4. Ramets must be equally productive of ovules and pollen.

5. Crosses among clones must be equally compatible.

One recent investigation suggests that contamination from background pollen sources could be a serious problem in wind-pollinated seed orchards. Based on monoterpene markers, Squillace and Long (1981) estimated that as much as 80% of the seed in a small slash pine orchard was the result of fertilizations by non-orchard pollen. Selfing, however, may have only a minor effect on genetic efficiency. In a loblolly pine study, the proportion of selfs in the progeny of five seed orchard clones was estimated with the aid of allozyme markers to average only 1.2% (Adams and Joly 1980a). Clonal variation in male and female flower productivity and phenology is well known to orchard managers and has been documented quantitatively in a number of cases (Bergmann 1968, Eriksson et al. 1973, Jonsson et al. 1976). In addition, lack of complete cross-compatibility among clones is suggested by the variable success of two-parent crosses in breeding programs (Woessner and Franklin 1973).

While it is clear that all the conditions above are violated at least to some extent in wind-pollinated seed orchards, it is unclear how these violations ultimately affect genetic efficiency of seed crops. Given a large number of single locus genetic markers, the genetic efficiency of seed orchards can be evaluated (Adams and Joly 1980a). We illustrate the technique in this paper by using seven allozyme loci to analyze genetic efficiency in two loblolly pine seed orchards. We also use allozymes to explore the genetic consequences of seed sizing in seed orchard crops.

MATERIALS AND METHODS

This study was conducted in two loblolly pine seed orchards owned by Champion International Corporation, Newberry, South Carolina. One orchard contains clones selected for high specific gravity wood (HSG); the other, clones selected for low specific gravity wood (LSG). The orchards are each approximately 2 hectares (5 acres) in size, and are separated from each other by a 100-m-wide strip containing a Virginia pine (*Pinus virginiana* Mill.) seed orchard. This three-orchard complex is surrounded by a 122-m-wide isolation strip comprising a cleared area, a slash pine (*Pinus elliottii* Engelm.) plantation, and a mixed loblolly-hardwood stand from which all flowering age loblolly pines are periodically removed.

At the time of seed sampling in 1976, orchard grafts were an average of 16 years old and were in full pollen and seed production. A total of 202 ramets of 23 clones were present in the LSG orchard and 183 ramets of 25 clones in the HSG orchard. Numerous roguings had resulted in considerable imbalance of clonal representation in both orchards, with the number of ramets per clone ranging from 1-25.

Beginning in 1975, the seed crops from the two orchards were combined and processed in bulk. Random samples of the bulked seed crops of 1976 and 1978 were obtained by sampling a large number of seeds from each of several storage bins and mixing them together. However, in 1978, the seeds were separated into small (23,000/lb), medium (18,000/lb), and large (13,000/lb) size classes prior to storage. For this year, a random sample of seed was obtained for each size class.

A large number of seeds from the 1976 sample (493) and from each of the 3 size classes in 1978 (221-245) were assayed electrophoretically using the techniques described by Adams and Joly (1980b). The megagametophyte (1N) and embryo (2N) tissues of each seed were analyzed separately and the genotype of each tissue determined at seven allozyme loci: GDH, LAP-1, PGI-2, GOT2, 6PGD, PGM-1, PGM-2. Details of the banding patterns of these allozymes, and analyses of their Mendelian genetics are also found in Adams and Joly (1980b).

Based on the diploid genotypes of the embryos, allelic frequencies at all seven loci were calculated for each of the four seed samples. Since the megagametophyte has the same haploid genotype as the female gamete, the parental origin of the genes in each embryo could be determined. Therefore, allelic frequencies among the female gametes (ovule pool) and among the male gametes (pollen pool) forming the embryos in each seed sample were also calculated.

Utilizing chi-square goodness-of-fit tests, the estimated allelic frequencies in the 1976 seed crop were then compared to those expected on the basis of random mating among the orchard clones. Expected frequencies were first determined separately for each orchard on the basis of the genotypes of the orchard clones. These were known from a previous study (Adams and Joly 1980b). Frequencies expected in the bulked seed crop were then calculated by weighting the LSG frequencies by 0.68, and HSG frequencies by 0.32, the average of each orchard's relative seed production over the five year period from 1970 to 1974. The range of the relative proportion of seed produced by the HSG orchard was relatively small over the five years (0.26 to 0.38); thus, it is unlikely that the weights used are very different from the true relative production in 1976. The calculated frequencies for the bulked seed crop are those expected when all the conditions necessary for maximal genetic efficiency (i.e., full genetic efficiency) are met, including the assumption that all clones produce equal numbers of ovules and pollen grains. However, the large discrepancy in the number of ramets representing each clone in the two orchards likely leads to considerable imbalance in the genetic contribution of each clone to the seed crop. To test the degree to which correction for clonal imbalance in ramet numbers might account for observed frequencies in the 1976 seed crop, expected frequencies were also calculated by first weighting each clonal genotype by the number of ramets with that genotype present during pollination in 1975.

For the 1978 seed crop, it was of interest to determine the degree to which the three seed size classes differed in genetic composition. To test the variation in allelic frequencies over size classes, chi-square tests of heterogeneity were conducted for each of the seven loci.

RESULTS AND DISCUSSION

Significant deviations of observed allelic frequencies in embryos from those expected under full genetic efficiency were found at five of the seven loci investigated in the 1976 seed crop sample (Table 1). Furthermore, significant deviations from expectation occurred in both the ovule and pollen pools, indicating that violation of the conditions for full genetic efficiency involve both male and female gametes. These deviations are not surprising, since the expectations given in Table 1 were formulated on the assumption of an equal number of ramets per clone. Correcting expected frequencies for the actual differences in ramet numbers, however, does little to improve the fit of observed allelic frequencies (Table 2). While only three loci showed significant deviations from expectation in embryos, a total of four loci still showed significant deviations in the ovule pool, and two loci were significant in the pollen pool.

When one considers the wide range in mean flower production per ramet that has been observed among orchard clones (Jonsson et al. 1976, Eriksson et al. 1973, Bergmann 1968), it is understandable why correction for number of ramets alone may not adequately account for seed production differences among clones. For example, in a study of variation in flowering among 15 clones in an 8 year-old loblolly pine orchard, the average number of cones produced per ramet ranged from 1 to 268 among clones, and the average male flower score (i.e., a subjective scale of 0 to 5, where 0 was no flowers and 5, very heavy flower production) ranged from 0.2 to 4.2 (Bergmann 1968). In order to adequately assess the influence of differential flower production among clones on seed orchard efficiency, detailed flowering data would be required. Unfortunately, no such data were recorded for these orchards during the 1975 pollination season.

To explain observed allelic frequencies in the ovule pool, information on variation among clones in relative production of sound seeds would be needed, and data on flowering variation by itself may not be enough. In an earlier study in loblolly pine, strong clonal variation was observed in the total number of seeds per cone; also, variation among clones was found in percent of empty seeds (Bergmann 1968). Factors which may account for differences among clones in sound seed production include intrinsic differences in the number of ovules produced per cone, differences in the proportion of ovules fertilized (which may be related to timing of flowering or incompatibility) and variation in the proportion of fertilized ovules which mature (which may be related to levels of self-fertilization).

In addition to variation in pollen production among clones, deviations from expected allelic frequencies in the pollen pool may result from a high proportion of fertilizations due to selfing and/or due to pollen from non-orchard sources, as well as variations among clones in time of pollen shedding and cross-compatibility. Differences among clones in time of pollen shedding or cross-compatibility are likely to have an effect on allele frequencies only when an allele is unique to a particularly early or late flowering, or extremely incompatible, clone. Thus, the impact of these two factors on overall allelic frequencies is probably not great. However, it is evident that pollen contamination from non-orchard sources is responsible for at least some fertilizations in the 1976 seed crop, based on the presence of allele 6PGD-7 in

the pollen pool sample (Tables 1 and 2). This allele was not found among the orchard clones, but was found in wild loblolly trees sampled within approximately 400 m of the orchards. Using four allozyme markers which were present in the natural stand sample, but could not be produced by the clones in either orchard, the proportion of seed fertilized by non-orchard sources was estimated. The mean for the four markers was 0.28 + 0.06. In addition, an estimate of pollen contamination between orchards was obtained with an allozyme marker that was present among the clones in the HSG orchard, but was found in neither the LSG orchard nor the surrounding stands. This estimate was 0.10 + 0.02. Thus, pollen contamination from non-orchard sources may be a major reason why allelic frequencies in the pollen pool deviate from expectation.

A high proportion of fertilizations due to selfing, on the other hand, does not appear to be an important factor contributing to deviations from expected frequencies in the pollen pool. To expand an earlier study in these orchards based on seed from five clones in a single year (Adams and Joly 1980a), we estimated the proportion of selfs in the progeny of a much larger sample of clones (7 to 24) in each of three years. Our estimates were never greater than 0.013 for any one year (including 1976) and the pooled estimate (weighted mean) for the 3 years was 0.004 + 0.008, in close agreement with the results of the earlier study.

Even though these orchards are apparently far from full genetic efficiency, and their clones probably vary considerably in the relative proportions of male and female gametes they produced, the 1976 bulked seed crop apparently still possesses much genetic diversity. All alleles present among the orchard clones, including seven alleles expected to occur at frequencies of less than 5% (Tables 1 and 2), were found in the ovule pool sample, and the same seven alleles plus an additional non-orchard allele were found in the pollen pool. Thus, based on this very limited sample of loci, no measurable decrease was detected in the level of genetic variation in the seed crop relative to that present among the orchard clones.

1978 Seed Crop

Allelic frequencies in embryos were quite variable among the three seed size classes and were significantly heterogeneous at 5 of the 7 loci investigated (Table 3). For example, PGM1-2 varied in frequency from 0.006 in small seed to 0.149 in large seed. An examination of frequencies in the ovule and pollen pools, however, revealed that the heterogeneity among seed size classes was primarily due to variation in the ovule pool (Table 4), as would be expected if clones differ in the average size of seeds they produce. While five of the loci showed significant heterogeneity of allelic frequencies in the ovule pool, only one (PGM1) was significantly heterogeneous ($P < 0.05$) in the pollen pool.

These results indicate that clonal variation in seed size can result in substantial differentiation in the genetic and clonal composition of different seed size classes. Furthermore, there is strong evidence that genetic variability is reduced within seed size classes due to the lack of seeds from individual clones. In five cases (i.e., alleles LAP2-3, PGI2-1, 6PGD-1, PGM1-2, PGM2-1), an allele found in the ovule pool of one size class was not detected in one or more of the other size classes (Table 4). These alleles

are unique to one or a few clones; thus, their absence in a sample indicates that seeds from the clones they mark are at very low frequency, or are not present at all in that seed size class. LAP2-3, for example, is unique to clone 3-40 in the HSG orchard. This allele was found at a frequency of 0.130 in the ovule pool of the small size class seed, 0.037 in medium seed and was not detected in large seed, indicating that seeds from clone 3-40 are small, and are probably completely absent in the large size class. PGI2-1, and PGM2-1 also are unique to individual clones, and 6PGD-1 is unique to two clones. PGM1-2 is found in four clones, and the absence of this allele in the ovule pool sample of small seeds illustrates the significant reduction in clonal representation (at least in the female contribution) that might occur within seedlots as a result of seed sizing.

In addition to the reduction in genetic diversity within seed size classes due to loss of seeds from individual clones, further reduction may occur due to large differences among the remaining clones in relative seed production. For example, since one-half the progeny of clone 3-40 carry the marker LAP2-3, which occurred at an estimated frequency of 0.103 in the ovule pool of the small seed, 20.6% (i.e., 2 x 10.3%) of the small seed can be estimated to have come from ramets of this clone. Similarly, only four clones (3-13, 5-13, 3-16 and 7-45), all heterozygous for allele PGMI-2, were responsible for 46% of the large seed. Because pollen pools were relatively homogeneous over seed sizes, imbalances among clones in total genetic contribution to each size class were not as great as would be indicated from ovule pool frequencies alone. Nevertheless, based on allelic frequencies in embryos (Table 3), 11.2% of the genes in small seeds can be estimated to come from clone 3-40, and 29.8% of the genes in large seed can be estimated to come from the four clones which carried PGM1-2. Thus, some clones appear to be substantially over-represented in the large and small seed sizes, even when contributions of genes through the pollen pool are taken into account.

CONCLUSIONS

Although pollen contamination can lead to increased variability in the seed crop, it may be the single most important factor in reduction of genetic efficiency. Substantial decrease in genetic gains can result from pollen contamination (Squillace 1981). In the two seed orchards studied, it is apparent that the 122-m isolation strip composed of a continuous stand of trees was not effective in preventing undesirable levels of pollen contamination in the 1976 seed crop. Based on the data of Wang, Perry et al. (1960), a cleared isolation strip may be more effective in reducing pollen contamination. Various other methods have been proposed for reducing pollen contamination, including removing orchards from the species area, increasing the size of the orchard or the isolation strip, cooling the orchard to delay flowering relative to flowering in outside stands (Silen and Keane 1969, Fashler and Devitt 1980), and the use of supplemental mass pollination (Woessner and Franklin 1973).

While contamination is important, by itself it does not explain all the deviations in allelic frequencies from those expected. Clonal differences in phenology and numbers of male and female flowers also must be responsible for some of these deviations. However, the relative importance of phenological factors may vary from year to year depending on whether flowering time is condensed or extended. Similarly, the relative importance of clonal deviation in

numbers of flowers may depend on whether it is a generally good or poor flowering year.

Supplemental mass pollination (SMP) is one of the more promising methods suggested as a means to improve genetic efficiency in wind-pollinated seed orchards. SMP could help reduce pollen contamination to an acceptable level. In addition, SMP could increase full seed yield by ensuring that each female flower has sufficient cross pollen applied at the appropriate time to allow maximum seed production per cone. Imbalances in clonal representations which occur in the seed crop due to clonal variation in numbers of male and female flowers and floral phenology could be reduced by appropriately adjusting the pollen mix. Besides the increase in genetic efficiency, selection intensity could be increased in the choice of pollen parents to be included in the mix, and seed crops could be genetically tailored for specific uses (Franklin, 1971). Although the technology may not yet be available to accomplish SMP with dependable results and at a relatively low cost, further research, possibly using allozyme markers (Bridgewater and Trew 1981), may yield effective and practical methods.

Seed sizing could result in a significant loss of variation if seed is culled by size, or if seed from only one seed size is planted in an area. Thus, it is important that seedlings of different seed sizes be mixed for outplanting. These conclusions are in agreement with those of Silen and Osterhaus (1979) who found that in Douglas-fir [Pseudotsuga menziesii (Mirb.) Franco], seed size can vary substantially among wind pollinated families.

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Table 1.--Observed allelic frequencies in the 1976 bulk seed crop, expected frequencies assuming full genetic efficiency, and chi-square values for goodness-of-fit.

Allozyme Locus	Allele	Expected Frequency	Observe: Ovule Pool Frequency	N	χ^2 (df)	Pollen Pool Frequency	N	χ^2 (df)	Embryos Frequency	N	χ^2 (df)
GDR	1	0.900	0.929	492	4.60(1)*	0.909	492	0.44(1)	0.919	984	3.95(1)*
	2	0.100	0.071			0.091			0.081		
LAP2	1	0.509	0.481	483	4.12(2)	0.468	483	4.25(2)	0.475	966	7.89(2)*
	2	0.485	0.507			0.522			0.514		
	3	0.006	0.012			0.010			0.011		
PGI2	2	0.927	0.925	489	3.78(2)	0.956	489	11.62(2)**	0.940	978	2.46(2)
	3	0.053	0.065			0.022			0.044		
	4	0.020	0.010			0.022			0.016		
GOT2	1	0.178	0.103	486	18.68(1)**	0.130	485	7.64(1)**	0.115	971	26.34(1)**
	3	0.822	0.897			0.870			0.885		
6PGD	1	0.029	0.049	493	12.24(4)*	0.043	493	39.57(4)**[1]	0.046	986	19.71(4)**1
	2	0.423	0.414			0.349			0.381		
	3	0.035	0.018			0.077			0.048		
	4	0.039	0.049			0.022			0.035		
	5	0.473	0.470			0.505			0.488		
	7	0	0			0.004			0.002		
PGM1	1	0.959	0.925	492	14.47(1)**	0.945	492	2.45(1)	0.935	984	14.42(1)**
	2	0.041	0.075			0.055			0.065		
PGM2	1	0.015	0.020	492	0.83(1)	0.010	492	0.83(1)	0.015	984	0.00(1)
	2	0.985	0.980			0.990			0.985		

[1] Alleles 5 and 7 were bulked prior to calculating the x2.

*Significant at 0.05 probability level

**Significant at 0-01 probability level

Table 2.--Expected allelic frequencies in the 1976 seed crop when clonal representation is weighted by ramet numbers but full genetic efficiency is otherwise assumed, and chi-square values for goodness-of-fit of observed (shown in Table 1) to expected frequencies.

Allozyme		Expected	X2 (do		
Locus	Allele	Frequency	Ovule Pool	Pollen Pool	Embryos
GDH	1	0.912	1.77(1)	0.06(1)	0.60(1)
	2	0.088			
LAP2	1	0.449	2.02(2)	0.81(2)	2.66(2)
	2	0.539			
	3	0.012			
PGI2	2	0.889	7.43(2)*	26.30(2)**	26.25(2)**
	3	0.087			
	4	0.024			
GOT2	1	0.135	4.49(1)*	0.07(1)	3.33(1)
	3	0.865			
6PGD	1	0.015	49.79(4)**	49.13(4)**1	66.68(4)**1
	2	0.403			
	3	0.039			
	4	0.032			
	5	0.511			
	7	0			
PGM1	1	0.952	7.85(1)**	0.53(1)	6.22(1)*
	2	0.048			
PGM2	1	0.020	0.00(1)	2.51(1)	1.26(1)
	2	0.980			

'Alleles 5 and 7 were bulked prior to calculating the x2.

*Significant at 0.05 probability level.

**Significant at 0.01 probability level.

Table 3.--Comparison of allelic frequencies in embryos among three seed size classes (small, medium, large) in the 1978 bulk seed crop.

Allozyme		Small		Medium		Large		Heterogeneity X2 (do
Locus	Allele	Frequency	N	Frequency	N	Frequency	N	
GDH	1	0.888	490	0.890	438	0.898	462	0.29(2)
	2	0.112		0.110		0.102		
LAP2	1	0.401	484	0.454	436	0.454	460	22.00(4)**
	2	0.543		0.521		0.539		
	3	0.056		0.025		0.007		
PGI2	2	0.941	490	0.903	434	0.892	460	18.00(4)**
	3	0.029		0.069		0.091		
	4	0.03]		0.028		0.017		
GOT2	1	0.118	484	0.133	436	0.128	454	0.55(2)
	3	0.882		0.867		0.872		
6PGD	1	0.002	490	0	440	0.008	466	19.30(10)*
	2	0.396		0.431		0.363		
	3	0.018		0.039		0.039		
	4	0.024		0.041		0.024		
	5	0.556		0.480		0.560		
	7	0.004		0.009		0.006		
PGM1	1	0.994	490	0.946	442	0.851	442	77.36(2)**
	2	0.006		0.054		0.149		
PGM2	1	0.035	490	0.020	442	0.003	364	10.49(2)**
	2	0.965		0.980		0.997		

*Significant at 0.05 probability level.

**Significant at 0.01 probability level.

Table 4.--Comparison of allelic frequencies in the ovule pool among three seed size classes (small, medium, large) in the 1978 bulk seed crop.

Allozyme		Small		Medium		Large		Heterogeneity
Locus	Allele	Frequency	N	Frequency	N	Frequency	N	X2 (df)
GDH	1	0.927	245	0.890	219	0.897	234	2.03(2)
	2	0.073		0.110		0.103		
LAP2	1	0.309	243	0.477	218	0.472	233	39.99(4)**
	2	0.588		0.486		0.528		
	3	0.103		0.037		0		
PGI2	11	0	245	0.037	217	0.121	231	55.31(6)**
	2	0.930		0.848		0.754		
	3	0.037		0.097		0.121		
	4	0.033		0.018		0.004		
GOT2	1	0.127	245	0.142	219	0.103	232	1.70(2)
	3	0.873		0.858		0.897		
6PGD	1	0	245	0	221	0.004	233	23.45(8)**
	2	0.376		0.443		0.309		
	3	0.004		0.014		0.009		
	4	0.004		0.050		0.026		
	5	0.616		0.493		0.652		
PGM1	1	1.00	245	0.950	221	0.769	221	81.87(2)**
	2	0		0.050		0.231		
PGM2	1	0.053	245	0.018	221	0	182	12.38(2)**
	2	0.947		0.982		1.00		

[1] PGI2-1 is detectable in megagametophytes only.

*Significant at 0.05 probability level.

**Significant at 0.01 probability level.