# POLYMORPHIC ISOENZYMES FROM MEGAGAMETHOPHYTES AND POLLEN OF LONGLEAF PINE: CHARACTERIZATION, INHERITANCE, AND USE IN ANALYSES OF GENETIC VARIATION AND GENOTYPE VERIFICATION

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Abstract.--Segregation of isoenzyme variants of 13 enzyme systems, assayed in longleaf pine (Pinus palustris Mill.) indicated control by 19 separate loci in megagametophytes and embryos of control-crossed and open-pollinated seeds. Along with megagametophyte evaluations, pollen contributions to embryos proved to be suitable for genetic evaluation and determination of genotypes. A unique multi-locus genotype was determined for each parent involved in control-crosses. Exact parentage of hybrids was determined from female and male contributions to hybrid multi-locus genotypes. Unique genotypes were determined for 62 of 68 parents evaluated. Considerable genetic variation in isoenzymal characteristics was found among 24 natural populations from the Central Gulf Coast. Allele frequencies per locus differed significantly among populations although in most cases one allele was more frequent in all populations. Numbers of polymorphic loci per population ranged from 31.6 to 57.9 percent and were correlated with latitudes at which the populations occurred ($r = 0.63$, $P<0.002$).

Additional keywords: Pinus palustris Mill., electrophoresis, number of alleles, polymorphic loci, genetic distance.

Isoenzyme analyses, by means of electrophoretic separation, are no longer a novelty in forest genetics investigations, yet the number of species and populations that have been studied is limited. Loblolly (Pinus taeda L.) is the only southern pine that has been dealt with in much detail (Adams and Jolly 1980, Conkle and Adams 1977, and Florence and Rink 1979). Conkle and Adams (1977) included some longleaf pine (Pinus palustris Mill.) seeds in *a* survey of southern pine banding patterns and concluded that similar genes were probably present in all of them. However, inheritance of isoenzymes must be established before they can be used in genetic analyses. A very limited amount of isoenzymal inheritance data has been gathered for longleaf pine (Snyder and Hamaker 1978). To be of much utility in arriving at unique genotypes for a large number of parent trees, *a* large number of polymorphic loci must be available and their inheritance must be understood. Part I of this study was designed to elucidate the inheritance of 19 loci representing 13 enzyme systems in longleaf pine when evaluations were made of both the female gametophyte and the contribution due to pollen. Although isoenzymes may be expressed in both embryo and megagametophyte tissues, the pollen

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contribution to the embryo genotype is not always apparent because of confounding of the banding patterns. The reliability of determining genotypes from pollen contributions of control-crosses was determined. Both embryo and megagametophyte tissue were used to establish unique genotypes for parents and verify the parentage of crosses.

Genetic variation has been demonstrated among seed sources of longleaf pine for metric traits such as height growth (Wells and Wakeley 1970) and disease resistance (Synder and Derr 1972). An appreciable amount of genetic variation has been found for height growth which has allowed for the differentiation of geographic zones based on growth potential. The question arises whether similar variation is present for isoenzymal characteristics and if so, what is its extent and distribution. Also, if it is present can it be used to differentiate populations. Isoenzymal analyses are currently used to estimate heterozygosities, genic diversity, and the extent of population differentiation.

Isoenzymal variation has been demonstrated for single trees of loblolly pine (Adams and Jolly 1980) and for longleaf pine (Duba, 1983). Variation has also been demonstrated for particular isoenzyme loci of loblolly pine (Florence and Rink 1979), pitch pine (Pinus rigida Mill.) (Guries and Ledig 1982), Norway spruce (Picea abies K.) (Lundkvist 1979), and Douglas-fir (Pseudotsuga menziesii (Mirb.) Franco) (Yeh and O'Malley 1980) populations, although when averaged over several loci the variation has not always shown extensive population differentiation. Some clinical trends have been indicated (Guries and Ledig 1982, Yeh and O'Malley 1980), but more isoenzymal genetic variation has been found to exist within populations than between them. Native conifers differ in the kind and amount of isoenzymal genetic variation they contain (Conkle 1980) and, in general, possess high levels as would be expected when considering their life history characteristics (Hamrick et al. 1979).

Longleaf pine is a long-lived, wind pollinated species with *a* large natural range that does not span dramatic climatic differences. Still, patterns of variation in growth characteristics have been associated with climatic factors, particularly temperature and rainfall. In connection with a study of geographic variation in growth potential of longleaf pine, various sources were utilized in Part II of this study to evaluate variation in isoenzymal characteristics. The level of genetic diversity within the species was determined and patterns of variation among populations were evaluated by analyzing protein polymorphisms that were revealed by electrophoretic separations.

## MATERIALS AND METHODS

### Part I

Control-cross seeds were utilized for the majority of these analyses, but observations from open-pollinated seeds were also included. The control-cross seeds represented 10 crossing combinations from 5 parents that were included in the U.S. Forest Service's longleaf breeding program at Gulfport, Mississippi. Open-pollinated seeds represented 63 longleaf parents from sources located in Alabama and adjoining states.

For a detailed description of the electrophoretic run conditions, consult Duba (1983). Mobility of electrophoretic bands was used to identify differing zones of activity. Segregation of band patterns in each zone was evaluated in conjunction with mobility of the zones of activity to identify separate loci and alleles (variants) at each locus. Inheritance of the enzymes was postulated based on segregations observed in band patterns of both megagametophyte and embryo tissues. Whenever a locus was determined to be heterozygous, chi-square values were calculated to evaluate the goodness-of-fit to the expected 1:1 ratio of segregation. The segregation in pollen gametophytes was compared to that from megagametophytes to evaluate the suitability of using pollen contributions to detemine genotypes.

From analyses of megagametophytes, the multi-locus genotype of the 5 parents involved in the 10 crosses and of the 63 source parents was determined. Multi-locus genotypes and segregation ratios of progeny these crosses were evaluated. Verifications of the parents responsible for particular crosses were made.

Part II

The primary center of sampling was the central gulf coast region of the natural range of longleaf pine. The entire sample consisted of 22 populations distributed through Alabama, southeast Mississippi, southwest Georgia, and the panhandle of Florida, plus 2 distant sources, 1 in central Florida and 1 in North Carolina (Figure 1). Each source was evaluated as a separate population to determine the extent and distribution of isoenzymal genetic variation.

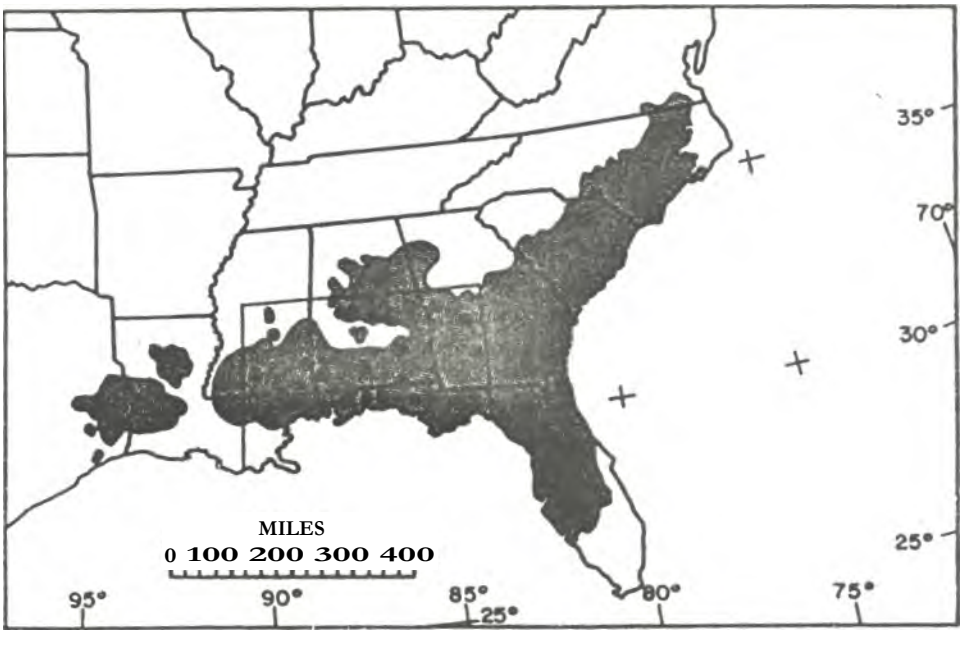


Figure 1.-- Species range of longleaf pine and relative locations of sampling points.

Direct count allele frequencies at each locus were obtained for each of the 24 populations. Within each population, genetic variation was quantified by determining the average number of alleles per locus, the proportion of polymorphic loci, and the Hardy-Weinberg expected proportion of heterozygous loci per individual. Heterogeneity chi-square values were calculated for allele frequencies among all populations to determine if frequencies were different from one population to another. Linear correlations were also computed between certain isoenzymal characteristics and latitude and longitude as well as growth potential.

## RESULTS AND DISCUSSION

### Part I

From analyses of electrophoretic banding patterns, 19 consistently staining zones of activity were observed in 13 enzyme systems. Evidence collected from segregation analyses of megagametophytes and pollen contributions to embryos of control-cross seeds (Table 1) demonstrated directly that 8 zones (ALAP, LAP-1, PGI-2, GOT-1, GOT-3, SKDH, MDH-2, AND PGD-1) were each controlled by a single locus. Evaluations from megagametophytes of open-pollinated seeds indicated control of 8 more zones (ADH, FLEST, LAP-2, PGI-1, PGM-1, PGM-2, GPD-3, and PGD-2) by a single locus each. Indirect evidence from embryos of open-pollinated seeds indicated control of the final 3 zones (GDH, GPD-1, and IDH) also by a single locus each.

Analysis of embryo bands in control-cross and open-pollinated seeds gave evidence that pollen contributions can be reliably ascertained (Table 1), although caution was required in evaluating one allelic combination at the MDH-2 locus. Pollen contributions can be utilized to verify male parents in hybrids and evaluate allele frequencies in population studies where the contributions to embryos can be consistently scored.

Table 1.--Allozyme segregation in megagametophytes and pollen produced by heterozygous parent trees

| Enzyme locus | Estimated from[a] | Allelic combination[b] X | Y | Observed number X | Total | Deviation (1) | P |
|---|---|---|---|---|---|---|---|
| ALAP | G | 1 | 2 | 18 | 39 | 0.23 | >.50 |
| | P | 1 | 2 | 12 | 24 | 0.00 | >.90 |
| LAP-1 | G | 1 | 2 | 17 | 40 | 0.90 | >.25 |
| | P | 1 | 2 | 11 | 24 | 0.17 | >.50 |
| PGI-2 | G | 1 | 2 | 20 | 44 | 0.36 | >.50 |
| | P | 1 | 2 | 15 | 24 | 1.50 | >.10 |
| | G | 1 | 3 | 56 | 115 | 0.08 | >.75 |
| | P | 1 | 3 | 42 | 84 | 0.00 | >.90 |
| GOT-1 | G | 1 | 3 | 18 | 40 | 0.40 | >.50 |
| | P | 1 | 3 | 14 | 24 | 0.67 | >.25 |
| GOT-3 | G | 1 | 2 | 22 | 40 | 0.40 | >.50 |
| | P | 1 | 2 | 15 | 24 | 1.50 | >.10 |
| SKDH | G | 1 | 2 | 51 | 89 | 1.90 | >.10 |
| | P | 1 | 2 | 20 | 42 | 0.09 | >.75 |
| MDH-2 | G | 1 | 2 | 17 | 41 | 1.19 | >.25 |
| | P | 1 | 2 | 7 | 18 | 0.89 | >.25 |
| | G | 1 | 5 | 38 | 89 | 1.90 | >.10 |
| | P | 1 | 5 | 15 | 31 | 0.03 | >.75 |
| | G | 2 | 5 | 14 | 40 | 3.60 | >.05 |
| | P | 2 | 5 | 43 | 65 | 6.78 | <.01 |
| PGD-1 | G | 1 | 4 | 16 | 41 | 1.97 | >.10 |
| | P | 1 | 4 | 11 | 21 | 0.05 | >.75 |
| | G | 1 | 5 | 80 | 120 | 13.33 | <.01 |
| | P | 1 | 5 | 40 | 62 | 5.22 | <.02 |

[a] Estimated from female gametophyte (G) or pollen (P) contributions.
[b] Allele 1 is the most frequent allele at each locus.

Segregation distortion was demonstarted in two combinations of PGD-1. Both combinations showed a deficiency for the same allele. Normal segregation was indicated for the other alleles at this locus. Therefore, caution must be exercised when using this locus for genetic evaluations. Part of this problem may have been due to band resolution between two alleles. The allele that was deficient migrated to a location between the two alleles that had excesses when in heterozygous combination. Although bands for this locus were very clear, the closeness in position of these bands may have been partly responsible for the excess numbers observed.

Every locus evaluated in longleaf pine had at least two electrophoretic variants (Figure 2) although the second variant (allele) at several loci was very rare. The reason for this observation may be that samples from a large portion of the longleaf pine natural range in Alabama, Florida, Georgia, and Mississippi were included in the open-pollinated seed collection, thus constituting a very broad sample.

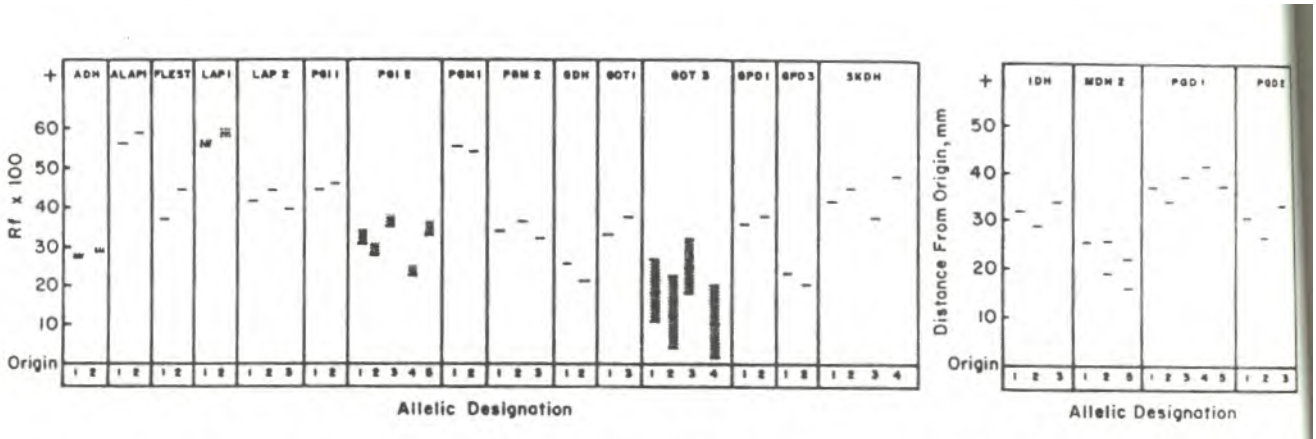


**Figure 2.-- Band patterns and their allelic designations for 19 loci in longleaf pine.**

An important utility of isoenzymal analyses to applied tree breeding has been the identification of parents (Adams and Jolly 1980). Offspring genotypes and frequencies have been predicted from genotypes of the parents involved in control-crosses. If each parent involved had a unique genotype, then the parents of any particular cross should be verifiable. The number of loci evaluated in this study was sufficient to allow for the determination of unique genotypes for each parent involved in control-crosses (Table 2). These multi-locus genotypes were suitable for evaluations of male and female contributions to hybrid embryos and the exact establishment of parentage (Table 3). The discovery that 62 of a total of 68 parents had unique genotypes was reassuring. If a large enough number of loci were included in evaluations, determinations of unique genotypes for applied breeding utilization is feasible.

Table 2.-- Unique genotypes of the five parents used in control crosses

| Parent | Genotype (by locus) ADH | ALAP | FEST | LAP1 | LAP2 | PGI1 | PGI2 | PGM1 | PGM2 | GDH | GOT1 | GOT3 | GPD1 | GPD3 | SKDH | IDH | MDH2 | PGD1 | PGD2 | Number of alleles by which two parents differ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 1 | 2 | 1 | 2 | 1 | 1 | 1,3 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1,2 | 1 | 1,5 | 1,5 | 1 | 6, 6, 1, 7, 6, 3 |
| 2 | 1 | 1 | 1 | 1 | 1 | 1 | 1,2 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1,2 | 1,4 | 1 | |
| 3 | 1 | 2 | 1 | 2 | 1 | 1 | 1,3 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1,2 | 1 | 2,5 | 1,5 | 1 | |
| 4 | 1 | 1,2 | 1 | 1,2 | 1 | 1 | 1 | 1 | 1 | 1 | 1,3 | 1,2 | 1 | 1 | 1 | 1 | 1,5 | 1,5 | 1 | 7, 4 |
| 5 | 1 | 1 | 1 | 1 | 1 | 1 | 1,3 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 2,5 | 1 | 1 | 7 |

Table 3.--Observed genotypes from ten control crosses used in hybrid verification

| Cross | | Genotype (by locus) ADH | ALAP | FEST | LAP1 | LAP2 | PGI1 | PGI2 | PGM1 | PGM2 | GDH | GOT1 | GOT3 | GPD1 | GPD3 | SKDH | IDH | MDH2 | PGD1 | PGD2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 x 2 | ♀/♂ | 1/1 | 2/1 | 1/1 | 2/1 | 1/1 | 1/1 | 1,3/1,2 | 1/1 | 1/1 | 1/1 | 1/1 | 1/1 | 1/1 | 1/1 | 1,2/1 | 1/1 | 1,5/1,2 | 1/1,4 | 1/1 |
| 1 x 3 | ♀/♂ | 1/1 | 2/2 | 1/1 | 2/2 | 1/1 | 1/1 | 1,3/1,3 | 1/1 | 1/1 | 1/1 | 1/1 | 1/1 | 1/1 | 1/1 | 1,2/1,2 | 1/1 | 1,5/2,5 | 1,5/1,5 | 1/1 |
| 1 x 4 | ♀/♂ | 1/1 | 2/1,2 | 1/1 | 2/1,2 | 1/1 | 1/1 | 1,3/1 | 1/1 | 1/1 | 1/1 | 1/1,3 | 1/1,2 | 1/1 | 1/1 | 1,2/1 | 1/1 | 1,5/1,5 | 1,5/1,5 | 1/1 |
| 1 x 5 | ♀/♂ | 1/1 | 2/1 | 1/1 | 2/1 | 1/1 | 1/1 | 1,3/1,3 | 1/1 | 1/1 | 1/1 | 1/1 | 1/1 | 1/1 | 1/1 | 1,2/1 | 1/1 | 1,5/2,5 | 1/1 | 1/1 |
| 2 x 3 | ♀/♂ | 1/1 | 1/2 | 1/1 | 1/2 | 1/1 | 1/1 | 1,2/1,3 | 1/1 | 1/1 | 1/1 | 1/1 | 1/1 | 1/1 | 1/1 | 1/1,2 | 1/1 | 1,2/2,5 | 1,4/1,5 | 1/1 |
| 2 x 5 | ♀/♂ | 1/1 | 1/1 | 1/1 | 1/1 | 1/1 | 1/1 | 1,2/1,3 | 1/1 | 1/1 | 1/1 | 1/1 | 1/1 | 1/1 | 1/1 | 1/1 | 1/1 | 1,2/2,5 | 1,4/1 | 1/1 |
| 3 x 4 | ♀/♂ | 1/1 | 2/1,2 | 1/1 | 2/1,2 | 1/1 | 1/1 | 1,3/1 | 1/1 | 1/1 | 1/1 | 1/1,3 | 1/1,2 | 1/1 | 1/1 | 1,2/1 | 1/1 | 2,5/1,5 | 5/5 | 1/1 |
| 3 x 5 | ♀/♂ | 1/1 | 2/1 | 1/1 | 2/1 | 1/1 | 1/1 | 1,3/1,3 | 1/1 | 1/1 | 1/1 | 1/1 | 1/1 | 1/1 | 1/1 | 1,2/1 | 1/1 | 5/2,5 | 1,5/1 | 1/1 |
| 4 x 1 | ♀/♂ | 1/1 | 1,2/2 | 1/1 | 1,2/2 | 1/1 | 1/1 | 1/1,3 | 1/1 | 1/1 | 1/1 | 1,3/1 | 1,2/1 | 1/1 | 1/1 | 1/1,2 | 1/1 | 1,5/1,5 | 1,5/1,5 | 1/1 |
| 4 x 2 | ♀/♂ | 1/1 | 1,2/1 | 1/1 | 1,2/1 | 1/1 | 1/1 | 1/1,2 | 1/1 | 1/1 | 1/1 | 1,3/1 | 1,2/1 | 1/1 | 1/1 | 1/1 | 1/1 | 1,5/1,2 | 1,5/1,4 | 1/1 |

The utility of isoenzymes in genetic studies has been described previously (Adams 1979, Allard et al. 1975). Results on the inheritance of isoenzymes in longleaf pine allow for its addition to the list of species for which isoenzymal evaluations can enhance genetic analyses.

Part II

Allele frequencies were determined for each population as a first measure of genetic variation (Table 4). At least two loci were observed in each population sample, although several loci were essentially monomorphic. A single allele was more frequent in all populations for 16 of the 19 loci. Contingency chi-square analysis indicated significant differences for all loci except GPD-3 and PGI-1 (Table 5). Thus, in the allele frequency data, an appreciable amount of genetic variation was reflected among populations, but the distribution was not readily apparent.

Table 4.-- Allele frequencies at 19 loci in natural populations of longleaf pine

| Locus | Allele | Populations | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 3 | 5 | 7 | 9 | 11 | 13 | 16 | 19 | 23 | 25 | 29 | 30 | 31 | 32 | 36 | 37 | 38 | 41 | 42 | 43 | 45 | 46 | 50 | 51 |
| ADH | 1 | 1.00 | 1.00 | 1.00 | 0.97 | 0.94 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 0.98 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| | 2 | | | | 0.03 | 0.06 | | | | | | | | | 0.02 | | | | | | | | | | |
| ALAP | 1 | 0.80 | 0.95 | 0.94 | 1.0 | 0.86 | 0.80 | 0.64 | 0.85 | 0.91 | 1.00 | 0.95 | 0.89 | 0.95 | 0.97 | 0.92 | 0.96 | 0.89 | 0.91 | 0.97 | 0.92 | 0.86 | 0.80 | 0.88 | 0.62 |
| | 2 | 0.20 | 0.05 | 0.06 | | 0.14 | 0.20 | 0.36 | 0.15 | 0.09 | | 0.05 | 0.11 | 0.05 | 0.03 | 0.08 | 0.04 | 0.11 | 0.09 | 0.03 | 0.08 | 0.14 | 0.20 | 0.12 | 0.38 |
| PLEST | 1 | 0.97 | 0.93 | 0.97 | 0.87 | 0.95 | 0.94 | 0.89 | 0.90 | 0.88 | 0.89 | 0.97 | 0.83 | 0.86 | 0.95 | 1.00 | 0.91 | 1.00 | 1.00 | 0.82 | 0.95 | 0.98 | 0.97 | 0.93 | 0.90 |
| | 2 | 0.03 | 0.07 | 0.03 | 0.13 | 0.05 | 0.06 | 0.11 | 0.10 | 1.12 | 0.11 | 0.03 | 0.17 | 0.14 | 0.05 | | 0.09 | | | 0.18 | 0.05 | 0.02 | 0.03 | 0.07 | 0.10 |
| LAP-1 | 1 | 0.91 | 0.71 | 0.92 | 0.86 | 0.89 | 0.74 | 0.69 | 0.64 | 0.98 | 1.00 | 0.94 | 0.83 | 0.81 | 0.91 | 1.00 | 0.83 | 0.89 | 0.94 | 0.88 | 0.95 | 0.82 | 0.86 | 0.55 | 0.69 |
| | 2 | 0.09 | 0.29 | 0.08 | 0.14 | 0.11 | 0.26 | 0.31 | 0.36 | 0.02 | | 0.06 | 0.17 | 0.19 | 0.09 | | 0.17 | 0.11 | 0.06 | 0.12 | 0.05 | 0.18 | 0.14 | 0.45 | 0.31 |
| LAP-2 | 1 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 0.88 | 0.98 | 1.00 | 0.94 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 0.98 | 0.95 | 0.98 | 1.00 | 1.00 |
| | 2 | | | | | | | | | 0.12 | 0.02 | | 0.06 | | | | | | | | | | 0.02 | | |
| | 3 | | | | | | | | | | | | | | | | | | | | 0.02 | 0.05 | | | |
| PGI-1 | 1 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 0.98 | 1.00 | 1.00 | 1.00 | 1.00 | 0.98 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| | 2 | | | | | | | | | | | | | | 0.02 | | | | | 0.02 | | | | | |
| PGI-2 | 1 | 0.50 | 0.34 | 0.42 | 0.33 | 0.41 | 0.35 | 0.56 | 0.14 | 0.29 | 0.41 | 0.17 | 0.42 | 0.30 | 0.63 | 0.75 | 0.42 | 0.46 | 0.60 | 0.38 | 0.41 | 0.34 | 0.79 | 0.78 | 0.74 |
| | 2 | 0.42 | | | | | | | 0.07 | | 0.03 | 0.04 | 0.04 | | | | | | 0.09 | | 0.04 | | | | 0.02 |
| | 3 | 0.03 | 0.53 | 0.48 | 0.63 | 0.44 | 0.54 | 0.17 | 0.61 | 0.42 | 0.35 | 0.65 | 0.33 | 0.65 | 0.21 | 0.08 | 0.32 | 0.18 | 0.09 | 0.33 | 0.24 | 0.31 | 0.08 | 0.05 | 0.19 |
| | 4 | 0.05 | | | | | | | | | | | | | 0.03 | | 0.03 | | | 0.03 | 0.04 | | | 0.02 | 0.02 |
| | 5 | | 0.12 | 0.10 | 0.04 | 0.15 | 0.11 | 0.27 | 0.18 | 0.29 | 0.21 | 0.14 | 0.20 | 0.05 | 0.13 | 0.17 | 0.23 | 0.36 | 0.22 | 0.25 | 0.26 | 0.34 | 0.13 | 0.15 | 0.02 |
| PGM-1 | 1 | 1.00 | 1.00 | 1.00 | 0.95 | 0.91 | 1.00 | 1.00 | 0.97 | 1.00 | 1.00 | 1.00 | 0.97 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| | 2 | | | | 0.05 | 0.09 | | | 0.03 | | | | 0.03 | | | | | | | | | | | | |
| PGM-2 | 1 | 1.00 | 1.00 | 1.00 | 0.88 | 0.94 | 0.86 | 0.99 | 1.00 | 0.92 | 0.97 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 0.96 | 0.96 | 1.00 | 1.00 | 1.00 |
| | 2 | | | | 0.12 | 0.06 | 0.12 | 0.01 | | 0.08 | 0.03 | | | | | | | | | | | 0.04 | | | |
| | 3 | | | | | | 0.02 | | | | | | | | | | | | | | 0.04 | | | | |
| GDH | 1 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 0.94 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| | 2 | | | | | | | | 0.06 | | | | | | | | | | | | | | | | |
| GOT-1 | 1 | 0.97 | 0.94 | 0.91 | 0.85 | 0.97 | 1.00 | 0.94 | 0.97 | 0.86 | 0.89 | 0.91 | 0.83 | 0.96 | 1.00 | 1.00 | 1.00 | 0.97 | 0.94 | 0.97 | 0.95 | 0.86 | 0.97 | 0.95 | 0.88 |
| | 3 | 0.03 | 0.06 | 0.09 | 0.15 | 0.03 | | 0.06 | 0.03 | 0.14 | 0.11 | 0.09 | 0.17 | 0.14 | | | | 0.03 | 0.06 | 0.03 | 0.05 | 0.14 | 0.03 | 0.05 | 0.12 |
| GOT-3 | 1 | 0.91 | 0.97 | 0.95 | 0.91 | 0.94 | 0.77 | 0.97 | 0.97 | 0.97 | 0.91 | 0.91 | 0.97 | 0.79 | 0.97 | 1.00 | 0.97 | 1.00 | 0.88 | 1.00 | 0.94 | 0.89 | 0.94 | 0.95 | 0.98 |
| | 2 | 0.09 | | 0.03 | 0.06 | 0.03 | 0.20 | 0.03 | 0.03 | 0.03 | 0.06 | 0.06 | 0.03 | 0.21 | 0.03 | | 0.03 | | 0.12 | | 0.06 | 0.08 | 0.06 | 0.02 | |
| | 3 | | 0.03 | 0.02 | 0.03 | 0.03 | 0.03 | | | | 0.03 | 0.03 | | | | | | | | | | 0.03 | | 0.02 | |
| | 4 | | | | | | | | | | | | | | | | | | | | | | | | 0.02 |
| GPD-1 | 1 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 0.97 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| | 2 | | | | | | | | 0.03 | | | | | | | | | | | | | | | | |
| GPD-3 | 1 | 1.00 | 0.98 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 0.98 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| | 2 | | 0.02 | | | | | | | | | | | | | | | | 0.02 | | | | | | |
| SKDH | 1 | 0.69 | 0.46 | 0.71 | 0.58 | 0.67 | 0.73 | 0.81 | 0.48 | 0.45 | 0.70 | 0.53 | 0.80 | 0.88 | 0.67 | 0.92 | 0.73 | 0.68 | 0.56 | 0.79 | 0.74 | 0.64 | 0.61 | 0.86 | 0.83 |
| | 2 | 0.31 | 0.54 | 0.26 | 0.42 | 0.33 | 0.27 | 0.14 | 0.52 | 0.48 | 0.27 | 0.47 | 0.11 | 0.12 | 0.24 | 0.08 | 0.27 | 0.29 | 0.44 | 0.15 | 0.24 | 0.27 | 0.39 | 0.05 | 0.12 |
| | 3 | | | 0.03 | | | | | | 0.03 | 0.03 | | 0.09 | | | | | 0.03 | | 0.06 | | | | | 0.02 |
| | 4 | | | | | | | 0.06 | | 0.03 | | | | | 0.09 | | | | | | 0.02 | 0.09 | | 0.09 | 0.02 |
| IDH | 1 | 1.00 | 0.94 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 0.94 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| | 2 | | 0.03 | | | | | | | | | 0.06 | | | | | | | | | | | | | |
| | 3 | | 0.03 | | | | | | | | | | | | | | | | | | | | | | |
| MDH | 1 | 0.48 | 0.35 | 0.30 | 0.52 | 0.47 | 0.70 | 0.42 | 0.42 | 0.50 | 0.50 | 0.39 | 0.56 | 0.50 | 0.59 | 0.54 | 0.39 | 0.56 | 0.17 | 0.36 | 0.38 | 0.27 | 0.48 | 0.57 | 0.45 |
| | 2 | 0.32 | 0.08 | 0.34 | 0.33 | 0.41 | 0.18 | 0.26 | 0.15 | 0.26 | 0.36 | 0.20 | 0.35 | 0.33 | 0.27 | 0.33 | 0.27 | 0.41 | 0.38 | 0.44 | 0.29 | 0.33 | 0.52 | 0.17 | 0.07 |
| | 5 | 0.20 | 0.58 | 0.36 | 0.15 | 0.12 | 0.12 | 0.32 | 0.42 | 0.24 | 0.14 | 0.41 | 0.09 | 0.17 | 0.14 | 0.12 | 0.34 | 0.03 | 0.45 | 0.20 | 0.33 | 0.41 | | 0.26 | 0.48 |
| PGD-1 | 1 | 0.89 | 0.82 | 0.77 | 0.89 | 0.88 | 0.73 | 0.94 | 0.79 | 0.56 | 0.80 | 0.79 | 0.97 | 0.24 | 0.77 | 0.88 | 0.81 | 0.80 | 0.83 | 0.62 | 0.88 | 0.82 | 0.76 | 0.76 | 0.88 |
| | 2 | | | 0.04 | | | 0.04 | 0.04 | 0.03 | | 0.03 | 0.03 | | 0.10 | 0.06 | 0.04 | 0.16 | 0.14 | 0.03 | | 0.09 | | 0.03 | 0.07 | |
| | 3 | 0.06 | 0.15 | | 0.08 | 0.09 | 0.09 | 0.01 | 0.14 | 0.17 | 0.02 | 0.14 | 0.03 | 0.07 | 0.09 | 0.08 | 0.03 | 0.06 | 0.08 | | | 0.17 | 0.06 | 0.02 | 0.07 |
| | 4 | 0.02 | 0.03 | | | | | | 0.03 | 0.03 | | | | | | | | | 0.03 | | | 0.02 | | | |
| | 5 | 0.03 | | 0.18 | 0.03 | 0.03 | 0.14 | | 0.02 | 0.24 | 0.15 | 0.04 | | 0.60 | 0.08 | | | | 0.03 | 0.38 | 0.03 | | 0.15 | 0.14 | 0.05 |
| PGD-2 | 1 | 1.00 | 0.97 | 0.98 | 1.00 | 0.97 | 0.97 | 0.97 | 1.00 | 1.00 | 1.00 | 0.97 | 0.97 | 0.95 | 0.94 | 0.92 | 1.00 | 1.00 | 1.00 | 0.97 | 0.98 | 0.97 | 1.00 | 1.00 | 1.00 |
| | 2 | | | | | 0.03 | 0.03 | 0.03 | | | | 0.03 | 0.03 | 0.05 | 0.03 | 0.08 | | | | 0.03 | | | | | |
| | 3 | | 0.03 | 0.02 | | | | | | | | | | | 0.03 | | | | | | 0.02 | 0.03 | | | |

Table 5.--Contingency chi-square analysis for 19 loci pooled over populations

| Locus | No. of alleles | Chi-square | D.F. | P |
|---|---|---|---|---|
| ADH | 2 | 69.95 | 23 | 0.000 |
| ALAP | 2 | 161.89 | 23 | 0.000 |
| FLEST | 2 | 58.71 | 23 | 0.000 |
| LAP-1 | 2 | 175.73 | 23 | 0.000 |
| LAP-2 | 3 | 195.06 | 46 | 0.000 |
| PGI-1 | 2 | 23.22 | 23 | 0.448 |
| PGI-2 | 5 | 725.86 | 92 | 0.000 |
| PGM-1 | 2 | 91.03 | 23 | 0.000 |
| PGM-2 | 3 | 178.91 | 46 | 0.000 |
| GDH | 2 | 98.96 | 23 | 0.000 |
| GOT-1 | 2 | 69.98 | 23 | 0.000 |
| GOT-3 | 4 | 166.73 | 69 | 0.000 |
| GPD-1 | 2 | 48.60 | 23 | 0.001 |
| GPD-3 | 2 | 27.66 | 23 | 0.229 |
| SKDH | 4 | 320.74 | 69 | 0.000 |
| IDH | 3 | 136.56 | 46 | 0.000 |
| MDH | 3 | 272.28 | 46 | 0.000 |
| PGD-1 | 5 | 517.87 | 92 | 0.000 |
| PGD-2 | 3 | 79.20 | 46 | 0.002 |

As a second measure of isoenzymal genetic variation, the average number of alleles per locus, the percentage of polymorphic loci, and the average heterozygosity per individual were compared among populations (Table 6). Although 2 or more alleles were found for every locus over all populations, the range in number of alleles per locus for individual populations was only 1.5 to 1.9. There were no clear-cut differences between any two populations in number of alleles, but it was evident from the data that populations differed in the presence of specific alleles and in the frequencies of these alleles. The percentage of polymorphic loci per population ranged from 31.6 to 57.9 percent and was correlated with latitude of the populations ($r = -0.63$, $P < 0.002$). The more southern populations had a higher percentage of polymorphic loci.

Table 6.--Variation of isoenzyme characteristics in 24 natural populations of longleaf pine

| Population | | Average number of alleles per locus | Percentage of loci polymorphic[a] | Percent heterozygosity per individual |
|---|---|---|---|---|
| 3 | Washington, FL | 1.7 ± 0.2 | 36.8 | 13.8 ± 4.7 |
| 5 | Okaloosa, FL | 1.8 ± 0.2 | 47.4 | 15.8 ± 4.8 |
| 7 | Santa Rosa, FL | 1.8 ± 0.2 | 36.8 | 14.1 ± 4.9 |
| 9 | Escambia, FL | 1.8 ± 0.2 | 47.4 | 16.2 ± 4.5 |
| 11 | Baldwin, AL | 1.9 ± 0.2 | 57.9 | 16.2 ± 4.5 |
| 13 | Mobile, AL | 1.8 ± 0.2 | 47.4 | 17.9 ± 4.8 |
| 16 | Stone, MS | 1.8 ± 0.2 | 42.1 | 16.0 ± 5.1 |
| 19 | Worth, GA | 1.9 ± 0.2 | 42.1 | 17.6 ± 5.1 |
| 23 | Geneva, AL | 1.9 ± 0.2 | 47.4 | 18.6 ± 5.6 |
| 25 | Pike, AL | 1.8 ± 0.2 | 36.8 | 14.3 ± 5.0 |
| 29 | Conecuh, AL | 1.9 ± 0.2 | 42.1 | 14.8 ± 4.7 |
| 30 | Washington, AL | 1.8 ± 0.2 | 42.1 | 15.6 ± 4.6 |
| 31 | Wayne, MS | 1.7 ± 0.2 | 42.1 | 17.2 ± 4.9 |
| 32 | Perry, MS | 1.9 ± 0.2 | 31.6 | 13.5 ± 4.6 |
| 36 | Lawrence, MS | 1.5 ± 0.2 | 31.6 | 8.8 ± 3.8 |
| 37 | Taylor, GA | 1.6 ± 0.2 | 31.6 | 14.0 ± 5.2 |
| 38 | Meriwether, GA | 1.6 ± 0.2 | 31.6 | 12.6 ± 4.8 |
| 41 | Autauga, AL | 1.8 ± 0.3 | 42.1 | 14.0 ± 4.9 |
| 42 | Bibb, AL | 1.7 ± 0.2 | 31.6 | 15.2 ± 5.3 |
| 43 | Hale, AL | 1.9 ± 0.2 | 31.6 | 14.1 ± 5.0 |
| 45 | Scott, MS | 1.9 ± 0.2 | 42.1 | 18.0 ± 5.2 |
| 46 | Tallapoosa, AL | 1.7 ± 0.2 | 36.8 | 13.6 ± 4.2 |
| 50 | Marion, FL | 1.8 ± 0.2 | 36.8 | 13.9 ± 4.4 |
| 51 | Richmond, NC | 1.8 ± 0.3 | 42.1 | 15.0 ± 4.5 |

a) Considered polymorphic if the frequency of the most common allele does not exceed 0.95.

As a third measure of genetic variation, the genetic distance between pairs of populations was determined (Table 7). Genetic distance coefficients combined over all loci ranged from 0.0 for 2 populations (populations 37 and 43) from the same latitude but separated in longitude, to *a* high of 0.048 between the North Carolina population (population 51) and a Mississippi population (population 31). In general the genetic distance coefficients were small and of the same order as those for pitch pine (Guries and Ledig 1982), but were much larger than those for Douglas-fir (Yeh and O'Malley 1980). Distribution of the largest coefficients was essentially random although for some pairs, such as 31 and 51, they also were separated by a large geographic distance.

Genetic distance coefficients also were calculated for each locus separately to evaluate specific locus contributions to overall coefficients. There were six loci (ALAP, LAP-1, MDH, PGD-1, PGI-2, and SKDH) that seemed to contribute the most to the overall coefficients. Of these six, PDG-i and PGI-2 both had coefficients ranging from 0.0 to approximately 0.98, and were the largest contributors to overall distances. The wide variation attributable to separate loci indicated the necessity for evaluating large numbers of loci in order to correctly evaluate population differentiation. Although these analyses identify substantial variation, the distribution among populations suggested a generally random distribution with only slight population differentiation.

Table 7.-- Genetic distance[1] coefficients between longleaf pine populations

| Population | 3 | 5 | 7 | 9 | 11 | 13 | 16 | 19 | 23 | 25 | 29 | 30 | 31 | 32 | 36 | 37 | 38 | 41 | 42 | 43 | 45 | 46 | 50 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 3 | ***** | | | | | | | | | | | | | | | | | | | | | | |
| 5 | 0.027 | ***** | | | | | | | | | | | | | | | | | | | | | |
| 7 | 0.015 | 0.011 | ***** | | | | | | | | | | | | | | | | | | | | |
| 9 | 0.022 | 0.011 | 0.007 | ***** | | | | | | | | | | | | | | | | | | | |
| 11 | 0.011 | 0.015 | 0.004 | 0.003 | ***** | | | | | | | | | | | | | | | | | | |
| 13 | 0.020 | 0.019 | 0.012 | 0.008 | 0.007 | ***** | | | | | | | | | | | | | | | | | |
| 16 | 0.014 | 0.023 | 0.015 | 0.025 | 0.012 | 0.016 | ***** | | | | | | | | | | | | | | | | |
| 19 | 0.028 | 0.001 | 0.014 | 0.010 | 0.013 | 0.013 | 0.022 | ***** | | | | | | | | | | | | | | | |
| 23 | 0.024 | 0.015 | 0.009 | 0.009 | 0.010 | 0.015 | 0.029 | 0.015 | ***** | | | | | | | | | | | | | | |
| 25 | 0.012 | 0.020 | 0.003 | 0.005 | 0.002 | 0.011 | 0.018 | 0.021 | 0.006 | ***** | | | | | | | | | | | | | |
| 29 | 0.025 | 0.004 | 0.006 | 0.005 | 0.009 | 0.014 | 0.029 | 0.005 | 0.007 | 0.010 | ***** | | | | | | | | | | | | |
| 30 | 0.013 | 0.024 | 0.009 | 0.009 | 0.004 | 0.012 | 0.009 | 0.022 | 0.018 | 0.004 | 0.019 | ***** | | | | | | | | | | | |
| 31 | 0.047 | 0.043 | 0.020 | 0.029 | 0.030 | 0.019 | 0.047 | 0.038 | 0.024 | 0.023 | 0.031 | 0.033 | ***** | | | | | | | | | | |
| 32 | 0.009 | 0.020 | 0.007 | 0.012 | 0.004 | 0.011 | 0.013 | 0.024 | 0.013 | 0.003 | 0.018 | 0.007 | 0.031 | ***** | | | | | | | | | |
| 36 | 0.011 | 0.036 | 0.013 | 0.024 | 0.010 | 0.021 | 0.012 | 0.042 | 0.027 | 0.008 | 0.032 | 0.008 | 0.043 | 0.002 | ***** | | | | | | | | |
| 37 | 0.012 | 0.010 | 0.002 | 0.009 | 0.004 | 0.011 | 0.009 | 0.011 | 0.012 | 0.004 | 0.009 | 0.006 | 0.030 | 0.004 | 0.009 | ***** | | | | | | | |
| 38 | 0.011 | 0.025 | 0.010 | 0.014 | 0.004 | 0.013 | 0.012 | 0.023 | 0.013 | 0.004 | 0.019 | 0.006 | 0.037 | 0.003 | 0.006 | 0.005 | ***** | | | | | | |
| 41 | 0.011 | 0.016 | 0.008 | 0.021 | 0.012 | 0.028 | 0.016 | 0.024 | 0.017 | 0.011 | 0.017 | 0.020 | 0.048 | 0.011 | 0.015 | 0.007 | 0.012 | ***** | | | | | |
| 42 | 0.021 | 0.025 | 0.005 | 0.016 | 0.009 | 0.016 | 0.018 | 0.024 | 0.011 | 0.004 | 0.019 | 0.010 | 0.013 | 0.009 | 0.015 | 0.007 | 0.010 | 0.018 | ***** | | | | |
| 43 | 0.009 | 0.015 | 0.002 | 0.011 | 0.004 | 0.014 | 0.009 | 0.017 | 0.012 | 0.002 | 0.010 | 0.006 | 0.033 | 0.004 | 0.006 | 0.0 | 0.004 | 0.005 | 0.008 | ***** | | | |
| 45 | 0.015 | 0.009 | 0.004 | 0.011 | 0.006 | 0.015 | 0.008 | 0.011 | 0.010 | 0.007 | 0.008 | 0.010 | 0.035 | 0.010 | 0.016 | 0.003 | 0.008 | 0.005 | 0.012 | 0.002 | ***** | | |
| 46 | 0.011 | 0.033 | 0.016 | 0.022 | 0.009 | 0.020 | 0.015 | 0.035 | 0.020 | 0.011 | 0.032 | 0.015 | 0.041 | 0.005 | 0.008 | 0.014 | 0.006 | 0.011 | 0.016 | 0.013 | 0.017 | ***** | |
| 50 | 0.021 | 0.032 | 0.023 | 0.034 | 0.023 | 0.021 | 0.009 | 0.035 | 0.039 | 0.024 | 0.043 | 0.016 | 0.040 | 0.012 | 0.013 | 0.015 | 0.018 | 0.026 | 0.021 | 0.019 | 0.021 | 0.017 | ***** |
| 51 | 0.018 | 0.025 | 0.020 | 0.032 | 0.022 | 0.022 | 0.003 | 0.029 | 0.037 | 0.027 | 0.035 | 0.017 | 0.049 | 0.018 | 0.017 | 0.016 | 0.024 | 0.022 | 0.028 | 0.017 | 0.018 | 0.022 | 0.008 |

[1] Unbiased genetic distance (D) according to Nei, 1978.

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