ramets, heritability might have been on the order of .62.

In this test, clonal differences were a major source of variance, and the treatment × clone interaction was never significant. Therefore it is unlikely that a major error in selection would occur due to neglect of primary ramet effects. Moreover, if the purpose of a clonal test is to compare genetic variances of populations (e.g. as in a provenance test), mixing of material from primary ramets should not biase estimates as long as all clones are similarly produced. However, if the purpose of investigation is to estimate genetic variance and predict genetic gain, then the effect of cloning must be accounted for in experimental design.


### Acknowledgement

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# Allozyme Variation Among North Carolina Populations of Liriodendron tulipifera L.[1])


By J. V. Brotschol[2]), J. H. Roberds and G. Namkoong[3])



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### Abstract

Genetic variation among three mature-tree and six seedling populations of yellow-poplar *(Liriodendron tulipifera* L.) was investigated by analyzing six polymorphic allozyme loci. The populations analyzed were from six separate locations in North Carolina. They were found to be highly differentiated with respect to allele frequencies in both seedling and mature-tree populations. Mature-tree and seedling populations from the same location were found to have different genotypic distributions although they did not differ in allele frequencies. Frequency of homozygotes in the seedling populations was found to be greater than expected under the assumption of Hardy-Weinberg distribution of genotypes. In the mature-tree populations, however, frequency of homozygotes did not differ from Hardy-Weinberg expectations. Outcrossing rates (t) were estimated for one mountain population and two coastal plain populations and these values verify that yellow-poplar has a mixed mating system. The coastal plain populations had substantially lower values than the mountain population (t = 0.55 vs. t = 0.86) indicating that mating behavior varies among populations.



*Key words:* Population differentiation, outcrossing rate, yellow-poplar, isozyme, genotypic distribution, allele frequency.



### Zusammenfassung

Es wurde die genetische Variation zwischen 3 Altbaum- und 6 Sämlingspopulationen von *Liriodendron tulipifera* L. untersucht, indem 6 polymorphe Allozym-Loci analysiert wurden. Die untersuchten Populationen stammten von 6 separaten Standorten in Nord-Carolina. Es wurde herausgefunden, daß sie hinsichtlich der Allozymfrequenzen sowohl bei den Sämlings- als auch bei den Altbaumpopulationen stark unterschiedlich sind. Altbaum- und Sämlingspopulationen vom gleichen Standort hatten unterschiedliche genotypische Verteilungen, obwohl sie sich in den Allozymfrequenzen nicht unterschieden. Die Häufigkeit der Homozygoten in den Sämlingspopulationen war größer, als unter der Voraussetzung der Hardy-Weinberg-Verteilung für Genotypen erwartet wurde. Für eine Gebirgspopulation und zwei Populationen in der Küstenebene wurde die Fremdungsrate (t) geschätzt, wobei diese Werte bestätigen, daß *Liriodendron tulipifera* ein gemischtes Kreuzungsschema hat. Die Küstenpopulationen hatten erheblich geringere Werte als die Gebirgspopulation (t = 0,55 gegenüber t = 0,86), was anzeigt, daß das Kreuzungsverhältnis stark variiert.


### Introduction

Forest tree species are commonly reported to be polymorphic with multiple alleles at many electrophoretically variable enzyme loci (Tigerstedt, 1973; Lundkvist and Rudin, 1977; Yang *et al.*, 1977; Hamrick *et al.*, 1979; Lundkvist, 1979). Information about the genetic structure of tree populations is therefore available and can be used to infer the evolutionary history of trees. The patterns of allele frequency variations among and within populations can indicate if stands are genetically divergent or essentially uniform, and whether the variation patterns are consistent in different areas or in succeeding generations.

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For temperate zone coniferous species, patterns and the extent of populational divergence seem to vary considerably (NAMKOONG, 1984). In *Pseudotsuga menziesii,* (MIRB.) FRANCO, for example some populations show little divergence (YEH, 1981), while others display both subpopulation (CAMPBELL, 1979) and inter-population differences (MUHS, 1981). There is also variation in the levels of genetic diversity among stands of *Picea abies* (L.) KARST. (BERGMANN and GREGORIUS, 1979). In addition to spatial variations in genetic structure, there also exist temporal variations in structure as found between two generations of *Pinus taeda* L. (ROBERDS and CONKLE, 1984).

All of the above studies, and indeed most forest-tree population genetic studies, are on wind pollinated, temperate zone conifers. To gain a more complete picture of the genetic structure of forest trees requires not only that angiosperm species be studied, but also species with different mating structures. One such species is *Liriodendron tulipifera* L., yellow-poplar, which is an entomophilic species in the *Magnoliaceae* found in the eastern United States. It has been shown to be moderately heterozygous for isozyme genes but has not diverged far genetically from *Liriodendron chinense* (HENSL.) SARG. the only other extant species in the same genus (PARKS *et al.*, 1983).

Honeybees frequently serve as pollen vectors for yellow-poplar, although other insects are also active as pollinators. Bees that work on a single tree can induce self pollination even if pollen is mixed in the hive, but bees working several trees may transfer pollen by body contact in the hive (TAFT, 1961). By assuming complete mortality of selfed seed in yellow-poplar and complete viability of crossed seed, TAFT (1965) estimated that the outcrossing rate is in the 80 to 90% range. He also inferred that seedling populations were the result of completely random outcrossing, which would imply that Hardy-Weinberg genotypic frequencies should exist, at least locally.

Seed migration in yellow-poplar is limited (MCCARTHY, 1933) and, over large areas, the species is often found distributed as widely-separated stands. It is therefore reasonable to conjecture that there might exist strong genetic divergence among stands if not within stands. On the other hand, the present distribution pattern may be of recent origin, and a more continuous distribution of genes might have been the norm. If so, then little divergence among populations would be expected or there might be clinal patterns of allele frequencies due to the combined effects of selection and migration. In fact, clinal patterns of variation in morphological traits were observed in yellow-poplar populations in North Carolina (KELLISON, 1966, 1970) except for stands growing on highly acidic, wet, organic soils in the lower coastal plain. Such populations were deemed to belong to a distinct ecotype. Contrary to the above conjecture, and in spite of its restricted mating and migration potential, yellow-poplar might actually be predominantly outcrossing with generally little genetic diversity among stands.

In this paper, we investigate the structure of six North Carolina populations and two generations of yellow-poplar using allelic and genotypic frequencies for six isoenzyme loci. In three of these populations, allele frequency variation was studied in two different generations and rates of outcrossing were estimated.

## Materials and Methods

### *Description of Populations*

The populations investigated were natural stands from separate locations in North Carolina *(Fig. 1).* Seed was collected from individual trees and kept separated by parent tree in three populations: Bent Creek Experimental Forest (MTS), coastal plain organic soil (CP-ORG), and coastal plain mineral soil (CP-MIN). In three additional populations, Barnardsville (BARN), Poplar Cove (PC), and Duke Forest (DF) seed was gathered from either the ground or from felled trees. The MTS and BARN populations are from areas about 65 km apart in the mountains of Buncombe County. The PC population is located approximately 30 km south of MTS in the mountains of Henderson County. DF is a piedmont population located in Durham County and the two coastal plain populations (CP-ORG and CP-MIN) are from sites approximately 25 km apart in Columbus County in the southeastern portion of the state. These latter two populations are from two distinctly different soil types; CP-ORG is from a site with highly acid, deep peat, organic soils, whereas CP-MIN is from a mineral soil site.

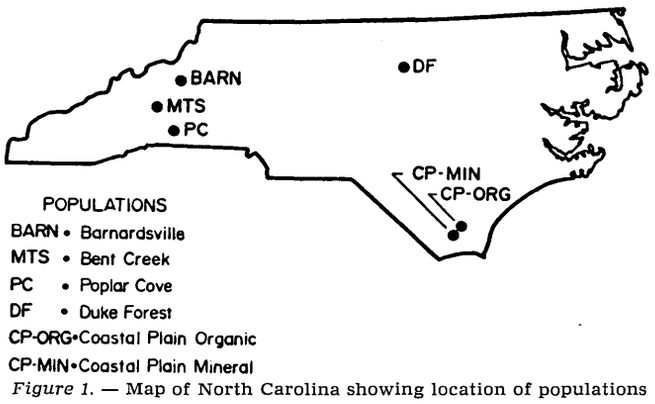

*Figure 1.* — Map of North Carolina showing location of populations investigated.

In the three populations in which seed was collected from individual trees, 10 to 20 (mostly 20) seedling progeny from each indiviual parent tree were sampled for electrophoretic analysis. Assays were made of seedlings from 33 trees from MTS, 37 trees from CP-ORG, and 44 trees from CP-MIN. For these populations, genotypes of mature trees were ascertained from their progeny by using the maximum likelihood method of BROWN *et al.* (1975). These determinations allowed genotypic and allelic frequencies to be estimated in mature-tree as well as in seedling populations. Hence genetic variation among stands of mature trees was compared with variation among populations composed of their seedling progeny. Furthermore, differences in the genetic constitution of the mature-tree populations and their corresponding seedling progeny populations were investigated.

In each of the three populations sampled by ground collections, 100 seedlings were randomly chosen for electrophoresis assay. Further details on collection procedures may be found in BROTSCHOL (1983).

### *Electrophoretic Methods*

Freshly germinated seedlings were individually homogenized in the extraction buffer for bud tissue described by CHELIAK and PITEL (1984). Homogenate supernatants were absorbed onto filter paper wicks which were placed in a gel mold with 46 g starch, 35 ml lithium borate electrode buffer pH 8.0), and 315 ml tris-citrate gel buffer (pH 8.0) (BROTSCHOL, 1983). Gels were subjected to a direct electric current of approximately 10 watts for 4 to 5 hours or until a dye marker reached 6 cm from the origin. Seedlings were then assayed for allozyme genotypes at six loci. Two acid phosphotase loci (ACPH1 and ACPH2) and one peroxidase (PER) locus, each with two alleles, were studied. One esterase (EST) locus with five alleles was scored. Two

*Table 1.* — Allele frequencies for mature-tree populations.

| Locus | Allele | Bent Creek (MTS) | C. Plain Organic (CP-ORG) | C. Plain Mineral (CP-MIN) | Homo $\chi^2$ | Degrees of freedom |
|---|---|---|---|---|---|---|
| ACPH1 | 1 | 0.561 | 0.162 | 0.352 | | |
| | 2 | .439 | .838 | .648 | | |
| | | | | | 38.707*** | 2 |
| ACPH2 | 1 | .894 | .986 | .966 | | |
| | 2 | .106 | .014 | .034 | | |
| | | | | | 7.132* | 2 |
| PER | 1 | .955 | .973 | .989 | | |
| | 2 | .045 | .027 | .011 | | |
| | | | | | 1.713 | 2 |
| EST | 1 | 0.0 | .419 | .227 | | |
| | 2 | .470 | .189 | .466 | | |
| | 3 | .439 | .216 | .216 | | |
| | 4 | .076 | .176 | .091 | | |
| | 5 | .015 | 0.0 | 0.0 | | |
| | | | | | 52.160*** | 8 |
| GOT1 | 1 | .121 | .041 | .114 | | |
| | 2 | .530 | .797 | .614 | | |
| | 3 | .076 | .027 | .114 | | |
| | 4 | .273 | .135 | .159 | | |
| | | | | | 15.417* | 6 |
| GOT2 | 1 | 1.0 | .892 | .727 | | |
| | 2 | 0.0 | .108 | .273 | | |
| | | | | | 24.194*** | 2 |
| Sample size | | 33 | 37 | 44 | | |

*** significant at 0.005 level
* significant at 0.05 level

glutamate oxaloacetate transaminase (GOT) loci were investigated, one (GOT1) with four alleles and a second (GOT2) with two alleles. Staining techniques used were modified from those described by Conkle *et al.* (1983). Further information about staining techniques and banding patterns for each locus are given in Brotschol (1983). Tests for Mendelian segregation were performed for the two multiple allelic loci, GOT1 and EST. Chi-square tests indicated no evidence for departures from the expected 1:1 ratios (Brotschol, 1983).

## Results

### *Allele Frequency Variation*

Allele frequencies were highly variable among populations in both mature-tree and seedling populations. Among the mature-tree populations all loci, with the exception of the PER locus, showed statistically significant variation in allele frequencies *(Table 1)*. For the ACPH1, EST, and GOT2 loci differences were significant at the .005 level. Allele 1 at the EST locus (EST-1) was not present in the sample from the MTS population, yet was found at substantial frequencies in the coastal plain populations (0.419 in CP-ORG and 0.227 in CP-MIN). Likewise, only the GOT2-1 allele was detected in the MTS population; however, GOT2-2 appeared at moderate frequencies in the coastal plain populations (0.108 in CP-ORG and 0.273 in in CP-MIN). Although both alleles at the ACPH1 locus are present in all three mature-tree populations, ACPH1-1 occurs at the greater frequency in the MTS population whereas the reverse pattern is true for the coastal plain populations. Furthermore, the two coastal plain populations were substantially different from each other in allele frequencies at all three of these loci. A similar variation pattern exists for the GOT1 locus, although frequency differences were not as large.

*Table 2.* — Allele frequencies in seedling populations.

| Locus | Allele | Barnardsville (BARN) | Bent Creek (MTS) | Poplar Cove (PC) | Duke Forest (DF) | C. Plain Organic (CP ORG) | C. Plain Mineral (CP MIN) | Homo. $\chi^2$ | DF |
|---|---|---|---|---|---|---|---|---|---|
| ACPH1 | 1 | 0.615 | 0.572 | 0.590 | 0.705 | 0.160 | 0.387 | | |
| | 2 | .385 | .428 | .410 | .295 | .840 | .613 | | |
| | | | | | | | | 637.465*** | 5 |
| ACPH2 | 1 | 1.0 | .955 | .950 | .740 | .989 | .977 | | |
| | 2 | 0.0 | .045 | .050 | .260 | .011 | .023 | | |
| | | | | | | | | 344.663*** | 5 |
| PER | 1 | .980 | .962 | .970 | .960 | .976 | .984 | | |
| | 2 | .020 | .038 | .030 | .040 | .024 | .016 | | |
| | | | | | | | | 15.872*** | 5 |
| EST | 1 | 0.0 | 0.0 | 0.0 | 0.0 | .461 | .237 | | |
| | 2 | .910 | .470 | .585 | .530 | .207 | .431 | | |
| | 3 | .080 | .448 | .380 | .245 | .207 | .231 | | |
| | 4 | .010 | .068 | .030 | .225 | .122 | .100 | | |
| | 5 | 0.0 | .014 | .005 | 0.0 | .001 | 0.0 | | |
| | | | | | | | | 1434.219*** | 20 |
| GOT1 | 1 | .095 | .109 | .165 | .240 | .036 | .110 | | |
| | 2 | .895 | .522 | .750 | .690 | .803 | .610 | | |
| | 3 | 0.0 | .107 | 0.0 | .050 | .034 | .134 | | |
| | 4 | .010 | .262 | .085 | .020 | .127 | .146 | | |
| | | | | | | | | 505.774*** | 15 |
| GOT2 | 1 | 1.0 | .999 | .895 | .730 | .873 | .697 | | |
| | 2 | 0.0 | .001 | .105 | .270 | .127 | .303 | | |
| | | | | | | | | 543.463*** | 5 |
| Sample size | | 100 | 629 | 100 | 100 | 699 | 826 | | |

***Significant at the 0.005 level.

Significant allele frequency variation among populations was found at all loci in the seedling populations *(Table 2)*. A similar pattern of variation to that found among the mature-tree populations was observed among the seedling populations in the three locations in which both mature-tree and seedling populations were studied. In these (MTS, CP-ORG, and CP-MIN), chi-square homogeneity tests indicated no significant departures from allele frequency homogeneity for the mature-tree and seedling populations. As was observed in the mature-tree populations, EST-1 was only detected in the coastal plain populations. Furthermore in the coastal plain populations, ACPH1-1 occurred at higher frequency than ACPH1-2 whereas the reverse pattern was found in all the remaining populations. In general, the pattern of large allele frequency variations among populations observed among the three mature-tree populations was also found for the more extensive sample of seedling populations.

An overall measure of differentiation among populations was obtained by using the differentiation measure introduced by Gregorius and Roberds (1986). Although this measure was developed specifically to study differentiation among subpopulations within a population, Gregorius (1985) pointed out that it can be used to measure differentiation among populations provided that the populations investigated are considered a closed set and not a sample drawn from the set of all populations. In the context of allelic differentiation among populations, the measure is based upon differences between each population and its complement population. The complement population for the $j^{th}$ population consists of the combined populations in the set of all populations excluding the $j^{th}$ population. A measure defined as

$$D_j = 0.5 \Sigma_j \left| p_i(j) - \bar{p}_i(j) \right|$$

represents the amount of allelic differentiation associated with the $j^{th}$ population. In this expression $p_i(j)$ is the frequency for the $i^{th}$ allele in the $j^{th}$ population, and $\bar{p}_i(j)$ is the frequency of the $i^{th}$ allele in its complement. This measure can be interpreted as the proportion of the effective number of alleles by which a population differs from the remainder of the populations (Gregorius and Roberds, 1986). The total differentiation among populations is obtained by computing the weighted average of the individual population differentiation measures, i.e. $\delta = \Sigma_j c_j D_j$ where $c_j$ is the weight for the $j^{th}$ population and depends on the relative population size. Thus $\delta$ is the proportion of the effective number of alleles by which the populations differ from their complements. Measures of differentiation for the six seedling populations are given in *Table 3* and

*Table 3.* — Allelic differentiation among seedling populations.

| Population | Loci | | | | | | |
|---|---|---|---|---|---|---|---|
| | ACPH1 | ACPH2 | PER | EST | GOT1 | GOT2 | ALL LOCI |
| | $D_j$ | | | | | | |
| Barnardsville | 0.132 | 0.078 | 0.010 | 0.465 | 0.220 | 0.161 | 0.178 |
| Bent Creek | .081 | .024 | .012 | .232 | .248 | .160 | .126 |
| Poplar Cove | .102 | .018 | .002 | .215 | .093 | .035 | .078 |
| Duke Forest | .240 | .234 | .014 | .168 | .137 | .163 | .159 |
| Coastal plain organic soil | .414 | .065 | .005 | .449 | .131 | .008 | .179 |
| Coastal plain mineral soil | .141 | .050 | .014 | .154 | .141 | .202 | .117 |
| $\delta$ | .185 | .078 | .010 | .281 | .162 | .122 | .139 |

are illustrated graphically in *Figure 2.* Since allele frequencies in the seedling and mature-tree populations did not differ, only differentiation measures for the seedling populations are given. The six populations studied were considered to be of approximate equal significance, thus we followed the suggestion of Gregorus (1985) and gave each population equal weight in determining $\delta$, i.e. each $c_j$ was assigned a value of 1/6. In addition to evaluating differentiation at each locus, the average differentiation over loci, which is the gene pool measure of differentiation for the six loci, was also determined. This gene pool measure reflects the proportion of effective alleles for which populations differ from the remainder of the populations in the set over the six loci. Among the populations studied, BARN and CP-ORG are the most differentiated. These populations each differ from the remainder of the populations at about 18% of the effective number of alleles. PC is the least differentiated population, $\delta = 0.078$. Among the loci, EST has the greatest differentiation, $\delta = 0.281$, and PER the least $\delta = 0.010$. The large amount of differentiation among populations is reflected by the value for the gene pool measure, $\delta_{ge} = 0.139$. Hence in the gene pool of the six loci analyzed, populations differed from their complement populations at approximately 14% of the effective number of alleles.

*Distribution of Genotypes*

Genotypes in the mature-tree and seedling populations were found to have different distribution patterns. In the mature-tree populations, no departures from Hardy-Weinberg proportions were found that could not be attributed to random causes *(Table 4).* Only one locus for one population out of the 18 locus-population combinations tested had a test value large enough to indicate statistical significance at the .05 level. However, in the seedling populations, statistically significant departures from Hardy-Weinberg proportions were found at two to six loci in five of the six populations investigated. In all cases these departures were due to an excess of homozygotes. Such a difference in genotypic distribution for the two types of populations might be due to differences in population structure which change over time or with stage of development. It may also be the result of natural selection that takes place during a developmental stage following the seedling stage we investigated.

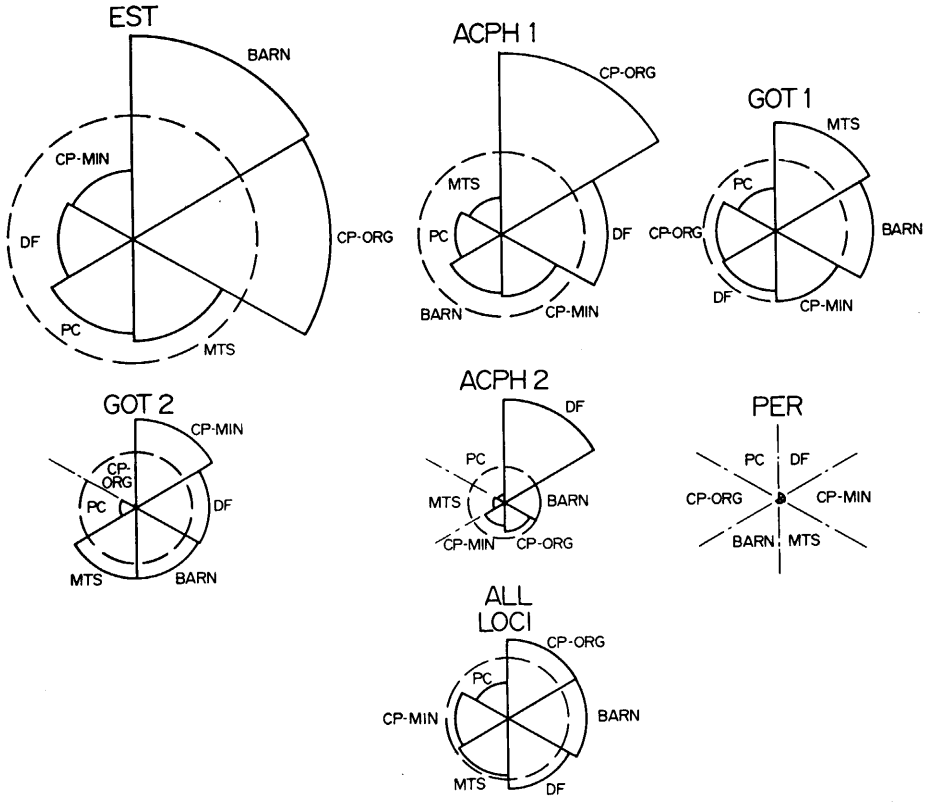


*Figure 2.* — Illustration of differentiation among yellow-poplar populations in allele frequencies. Each drawing depicts components of the $\delta$ measure of differentiation for a locus and each section represents the differentiation for a population ($D_j$). Radii of the sections are proportional to values for the $D_j$ and the radii of the dotted circles are proportional to the $\delta$'s. Angles for all sections equal 60° and reflect the value of $c_j$. Populations are designated as follows: BARN-Barnardsville; MTS-Bent Creek; PC-Poplar Cove; DF-Duke Forest; CP-ORG-Coastal plain organic soil; CP-MIN-Coastal plain mineral soil.

*Table 4.* — Proportion of heterozygotes in seedling and mating-tree populations.

| Population | Locus | Seedling populations: Expected frequency | Seedling populations: Observed frequency | Mature-tree populations: Expected frequency | Mature-tree populations: Observed frequency |
|---|---|---|---|---|---|
| Barnardsville (BARN) | ACPH1 | 0.474 | 0.450 | | |
| | ACPH2 | 0.0 | 0.0 | | |
| | PER | .039 | .040 | | |
| | EST | .165 | .140 | | |
| | GOT1 | .190 | .190 | | |
| | GOT2 | 0.0 | 0.0 | | |
| Bent Creek (MTS) | ACPH1 | .490 | .448** | 0.493 | 0.454 |
| | ACPH2 | .085 | .064*** | .190 | .212 |
| | PER | .073 | .054*** | .087 | .091 |
| | EST | .573 | .509*** | .580 | .636 |
| | GOT1 | .635 | .474*** | .624 | .667 |
| | GOT2 | .002 | .002 | 0.0 | 0.0 |
| Poplar Cove (PC) | ACPH1 | .484 | .420 | | |
| | ACPH2 | .095 | .020* | | |
| | PER | .059 | .060 | | |
| | EST | .512 | .520 | | |
| | GOT1 | .403 | .270*** | | |
| | GOT2 | .188 | .190 | | |
| Duke Forest (DF) | ACPH1 | .416 | .450 | | |
| | ACPH2 | .385 | .080*** | | |
| | PER | .077 | .080 | | |
| | EST | .608 | .610 | | |
| | GOT1 | .463 | .391** | | |
| | GOT2 | .394 | .300** | | |
| Coastal plain organic soil (CP-ORG) | ACPH1 | .269 | .246** | .272 | .324 |
| | ACPH2 | .021 | .010*** | .027 | .027 |
| | PER | .046 | .041** | .053 | .054 |
| | EST | .686 | .549*** | .711 | .892 |
| | GOT1 | .337 | .133*** | .344 | .189 |
| | GOT2 | .222 | .043*** | .193 | .216 |
| Coastal plain mineral soil (CP-MIN) | ACPH1 | .475 | .308*** | .456 | .386 |
| | ACPH2 | .045 | .017*** | .066 | .068 |
| | PER | .032 | .025*** | .022 | .023 |
| | EST | .694 | .540*** | .676 | .682 |
| | GOT1 | .577 | .206*** | .572 | .318* |
| | GOT2 | .422 | .240*** | .397 | .409 |

*** $\chi^2$ test indicates significant departure from Hardy-Weinberg proportions at 0.005 level.

** $\chi^2$ test indicates significant departure from Hardy-Weinberg proportions at 0.01 level.

* $\chi^2$ test indicates significant departure from Hardy-Weinberg proportions at 0.05 level.

*Outcrossing Rate*

Yellow-poplar had been previously reported to have approximately 80 to 90% outcrossing (Taft, 1965). Since yellow-poplar produces about 12% viable seed, it was thought that the selfed seed aborted and that most of the viable seed came from outcrossing. We estimated the proportion of ovules (t) resulting from outcrossing with the method of moments estimator described by Shaw *et al.* (1980). The proportion of self-pollinated ovules can then be estimated as s = 1-t. This estimator incorporates data from multiple loci and requires the following four assumptions: (1) the marker loci segregate independently, (2) allele frequencies in the outcross pollen pool are uniformly distributed over the population of maternal parents, (3) the probability of an outcross is independent of the maternal genotype, and (4) no selection acts on the marker loci between mating and the developmental stage at which progeny genotypes are ascertained. Our estimates for the outcrossing rate on a population basis are: MTS, 86.07 ± 2.6%; CP-ORG, 55.22 ± 2.8%; and CP-MIN, 54.70 ± 1.7%.

In the populations we studied, assumption 2 may be violated. Allele frequencies in populations of the male gametes that result in germinable zygotes vary among maternal parents and the differences are highly significant (Brotschol, 1983). Such allele frequency heterogeneity in the pollen pool probably resulted from random sampling of pollen parents with each maternal parent being mated with a restricted number of pollen parents. This type of sampling may well result when mating takes place among individuals in groups of limited size. Such mating behavior causes the proportion of homozygotes in the progeny population to be overestimated (Wahlund effect) and leads to a downward bias in estimates of t (Shaw and Allard, 1979). Mistaken classification of outcrossed progeny as selfed progeny is another source of downward bias for estimates of t. However such bias is reduced by use of the multiple-locus estimator. This etimator has the desirable property that as data from additional loci are used, identification of outcrossed progeny becomes more complete resulting in estimates of t with less bias.

## Discussion

*Population Differentiation*

It is apparent that natural populations of yellow-poplar are highly differentiated with respect to the isozyme loci we studied. The two most distant populations included in our investigation, CP-ORG and BARN, show the greatest differentiation from the remaining populations *(Fig. 2)* though possibly because of different reasons. As previously noted, CP-ORG is from an environment that differs greatly from those of the remaning populations. This population occupies a site with an organic soil that is highly acidic and water saturated. Populations found on such soils have been shown to distinctly differ in morphological characters from populations occupying less acidic, better drained, mineral soils (Kellison, 1966). In this respect, our results for isozyme allele frequencies are consistent with the morphological findings. Thus the differentiation observed for the iso-

zyme loci may be a reflection of differences in adaptation to rather different environments or to genetic sampling which took place during the past history of the population. At present it is not possible to decide between these two possibilities. On the other hand, the differentiation observed for the BARN population may have resulted from the manner in which seeds were sampled. Seed was gathered at the site of this population from wind dispersals on the forest floor or from entire fruits attached to branches on the ground. A large portion of the seed was collected in the vicinity of a large tree with a visible number of fruit remnants. Numbers of seed available for collection declined with distance from this large tree. Thus it seems likely that a substantial portion of the seed collected were from this single tree and that the sample obtained was not reflective of the genetic composition of the population. The greater similarity of allele frequencies for the MTS and PC populations than between the frequencies for these populations and the BARN population lends some support to this contention.

The coastal plain populations, while being differentiated from each other, also show dissimilarities to the remainder of the populations at two loci. EST-1 occurs at the intermediate frequencies in the coastal plain populations but was not detected at all in our samples of the mountain and piedmont populations. Substantial differences also occur between these two groups at the ACPH1 locus. In this case, there is a switch in the allele that is found with the highest frequency. Such drastic differences imply that there has been little recent gene flow between these two groups. It also suggests that either these two groups were derived from different ancestral sources or that the antecedents of the coastal plain populations became isolated from the interior populations and evolved independently.

*Genotypic Distributions*

Genotypic distributions were different in the seedling and mature-tree populations even though allele frequencies were uniform across the two age groups. Genotypic frequencies in the mature-tree populations did not depart from expectations for Hardy-Weinberg distribution of genotypes. Greater homozygosity than expected was, however, observed in the seedling populations.

In a species in which a considerable portion of the matings are not the result of random outcrossing, an excess of homozygote is expected due to inbreeding. The yellow-poplar mature-tree populations, however, do not fit such expectations. It thus appears that different factors are involved in influencing genotypic distributions in the two age groups. Since the mature-tree populations belong to the generation just prior to that of the seedling populations, it might appear that different mating systems were involved in producing the two generations. If this is the case, individuals in the mature-tree generation are almost exclusively the product of random outcrossing whereas individuals in the seedling generation resulted in part from other types of matings. If a pattern of variation in mating systems among generations is the norm, however, differences in mating systems for mature-tree populations in different generations would be expected. Such differences were not observed when mature-tree populations were separated into age classes which represent two or more generations (Brotschol, 1983). The apparent consistent fit of different mature-tree age classes to Hardy-Weinberg distributions, suggests that difference in genotypic distributions for seedling and mature populations are due to factors operating between the seedling and mature-tree state at which our observations were made, rather than with differences in mating systems across generations.

A factor that might have been reponsible for the different genotypic distributions observed is natural selection favoring heterozygotes. Such selection would have to occur during the life cycle in the interval between seed dispersal and reproductive age. Zygotes might be particularly exposed to selection pressure during germination or immediately following germination during sedling establishment. This type of selection was the explanation given for differences in outcrossing rates measured at the seed and seedling stages and for differences in heterozygosity observed in the progeny and adult stages of *Eucalyptus pauciflora* Sieb. ex. Spreng, (Phillips and Brown, 1977). Although selection favoring heterozygotes might be expected to reduce the frequency of homozygotes from the excessive levels generated by the mating system, it is difficult to understand why such selection would result in Hardy-Weinberg genotypic distributions in mature-tree populations rather than distributions characterized by excessive levels of heterozygosity. If selection is a factor in producing the observed differences in genotypic distributions, it is clear that our understanding of the way it operates is imcomplete.

Subdivision of the seedling populations into groups containing a small number of families is another factor that may have contributed to the differences in genotypic distributions observed. Evidence for such partitions in the seedling populations was observed by Brotschol (1983). Variation in allele frequencies across these partitions undoubtedly contributed to the excessive homozygosity observed because of the Wahlund effect. Severe reductions in population sizes due to the intense competition for limiting resources is believed to take place during the establishment of seedling populations. Random elimination of individuals irrespective of genotypic composition during this competition would be expected to result in residual populations with genotypic distributions in or near Hardy-Weinberg proportions.

At present it is not known which of these factors is most significant in causing the differences observed in genotypic distributions. Further investigation aimed at describing more completely the serial changes in genotypic distributions that occur over the history of individual populations is needed.

*Mating System*

Estimates of the rate of outcrossing were not uniform across populations. The value for the MTS population (86.07 ± 2.6%) was within the range previously reported for outcrossing in yellow-poplar (Taft, 1965). Values observed for the two coastal plain populations, while of about equal magnitude, were considerably smaller (55.22 ± 2.8% for CP-ORG, and 54.70 ± 1.7% for CP-MIN). In view of the possibility that the assumption of uniform allele frequency in the pollen pool may not hold for yellow-poplar populations, these t values reflect one of either two types of mating behavior. One interpretation of our results is that self-pollinations account for a significant proportion of the matings in natural populations of yellow-poplar and that, particularly in the coastal plain, the rate of natural selfing is much greater than previously thought. A second interpretation is that while some selfing occurs, the rate is lower than is indicated by strict interpretation of the t values as measures of outcrossing rate. Instead, according to this view, most mating in natural stands of yellow-poplar occurs substantially at random among individuals with-

in groups of limited size. As a consequence of the Wahlund effect, frequencies of homozygotes expected in the progeny population under the assumption of Hardy-Weinberg proportions are lower than the observed homozygote frequencies and thus estimated values of t are less than the true outcrossing rate. Both explanations are consistent with the observation of greater than expected levels of homozygosity. The first, however, provides a means for generating the excessive homozygosity required in the selection-favoring-heterozygotes hypothesis for reconciling differences in genotypic distributions in the mature-tree and seedling populations. The second is in accord with the subdivision-random-elimination hypothesis for seedling populations. We cannot determine from our data which of these two mating systems is the rule in yellow-poplar populations. The differences observed in t values between the MTS and coastal plain populations does indicate, however, that mating behavior is not uniform across populations regardless of which mating system exists. It is also apparent that trees in the mature-tree populations are not strongly segregated into groups of relatives since there was no evidence for subdivisions in these populations.

The large difference between mountain and coastal populations is striking and while the conditions that produce it are not understood, there are a number of factors that might have an effect. Differences in number of insects available for pollination, pollinator flight patterns and number of plant species being serviced by the pollinator all could contribute to variation in t values. Marked differences in mating behavior have often been found for populations from different habitats (Allard, 1970; Brown *et al.*, 1974, 1978). It therefore seems reasonable to suspect that environmental influences which differ markedly between the mountain and coastal plain habitats of yellow-poplar are responsible for the differences observed in estimates of t.

## Summary and Conclusions

Yellow-poplar populations from different geographic locations are highly differentiated with respect to the allozyme loci we studied. The coastal plain populations appear to be genetically distinct from the interior mountain and piedmont populations. Within both of these groups, populations are also differentiated, which implies very limited gene migration between locations. This pattern of variation differs from that found in most wind-pollinated tree species and is probably associated with the entomophilic mating behavior of this angiosperm. In the coastal plain, differences are particularly striking between populations from organic and mineral environments. The genetic distinctiveness in allozymes observed for the organic soils population is consistent with the pattern found for morphological traits (Kellison, 1966; 1970).

A distinctive difference was found in the genotypic distributions within the mature-tree and seedling populations even though these two types of populations did not differ in allele frequencies within locations. The mature-tree populations had genotypic frequencies in agreement with Hardy-Weinberg proportions whereas seedling populations were often found with homozygous frequencies in excess of Hardy-Weinberg expectations. Two hypotheses may explain these differences in genotypic distributions. One is that the excessive number of homozygotes in the seedling populations is the result of inbreeding and that, following seed germination, natural selection favoring heterozygotes is an important factor. The other hypothesis considers that the seedling populations are composed of family groups which vary in allele frequencies and the illusion of an excessive number of homozygotes is due to the Wahlund effect. According to this hypothesis, the original family cluster-structure is destroyed during development of the seedling populations by random elimination of individuals by competition, irrespective of genotype.

Our investigation of the outcrossing rates confirmed that the mating system of yellow-poplar is of the mixed mating type. Populations, however, were found to differ in estimates of outcrossing rate (t). The coastal plain populations had substantially lower t values than the mountain population studied. These results may be interpreted to indicate that the rate of outcrossing is much lower in some populations than previously thought, or alternatively that random mating within groups of trees of restricted size forms the primary mating pattern. According to the latter interpretation some selfing may occur but the rate of outcrossing is greater than suggested by the estimates of t. The variation in t values does indicate, however, that whatever the mating system, mating behavior is not uniform over populations.

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# Sugar Pine and its Hybrids

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**Summary**

Unlike most white pines, sugar pine *(Pinus lambertiana)* is severely restricted in its ability to hybridize with other species. It has not been successfully crossed with any other North American white pine, nor with those Eurasian white pines it most closely resembles. Crosses with the dissimilar *P. koraiensis* and *P. armandii*, both native to eastern Asia, have produced one and a few hybrids respectively. These Asian species are highly resistant to white pine blister rust *(Cronartium ribicola)*, and hybrids and hybrid derivatives of sugar pine × *P. armandii* show some enhancement of resistance to this disease.

*Key words: Pinus lambertiana*, white pines, blister rust, *Cronartium ribicola*, resistance.

**Zusammenfassung**

Anders als die meisten Kiefernarten der Sektion *Strobus* ist *Pinus lambertiana* in ihrer Fähigkeit, mit anderen Arten zu hybridisieren, stark eingeschränkt. Es gibt keine erfolgreichen Kreuzungen, weder mit irgendeiner anderen nordamerikanischen Kiefernart, noch mit den eurasischen Arten, denen sie am meisten ähnelt. Kreuzungen mit den unähnlichen Arten *Pinus koraiensis* und *Pinus armandii*, beide in Ostasien beheimatet, ergaben einen bzw. wenige Hybriden. Diese asiatischen Arten sind in hohem Maße gegen *Cronartium ribicola* resistent und Hybriden, sowie Hybrid-Nachkommenschaften von *P. lambertiana* × *P. armandii* zeigten eine erhöhte Resistenz gegen diese Krankheit.

## Introduction

Among the white pines (genus *Pinus*, section *Strobus)*, the least predictable in crossing behavior is sugar pine *(P. lambertiana* DOUGL.), a valuable timber species native to the Pacific Slope of North America between central Oregon and Baja California. Within section *Strobus*, sugar pine is grouped with other American and Eurasian white pines having winged seeds and cones that open and shed their seeds at maturity. It has not been successfully crossed with any other member of this morphologically coherent and otherwise highly crossable group, however. Verified hybrids have been produced with only two other species, both white pines native to eastern Asia: *P. armandii* FRANCH. and *P. koraiensis* SIEB. and ZUCC. (STONE and DUFFIELD 1950). SHAW (1914), in his landmark monograph of the pines, used just two characteristics to segregate the white pines into groups: presence or absence of seed wings and opening or "indehiscence" of mature cones. Both Asian species differ from sugar pine in having wingless seeds, and the indehiscent cone of *P. koraiensis* has scales that fail to separate sufficiently to permit seed shedding. Shaw placed each of these species in a different group within section *Strobus:* sugar pine in *Strobi*, *P. koraiensis* in *Cembrae*, and *P. armandii* in *Flexiles*. This classification is still widely used, often with minor modifications (e.g. LITTLE and CRITCHFIELD 1969), but the crossing behavior of sugar pine and its Asian relatives has helped to undermine the assumptions on which the classification is based.

The reproductive barriers that restrict or prevent crossing between species are quite different in the two principal groups of pines: the white pines of section *Strobus* and the hard pines of section *Pinus*. According to KRIEBEL (1972, 1975, pers. comm. Aug. 1981), there are no well-documented exceptions to the generalization that barriers between hard pines are expressed before fertilization and barriers between white pines after fertilization. KRIEBEL emphasized histological evidence in establishing the developmental stage at which reproductive processes break down, but routine crossing data (numbers of female strobili, cones, sound seeds, and hollow seeds) can also be used to determine whether breakdowns occur before or after fertilization. This is possible because the seeds of pines (BUCHHOLZ 1945), including sugar pine (KRUGMAN 1961), reach their final size by the time of fertilization and develop hard seedcoats then or shortly thereafter. Thus pre-fertilization barriers, which cause the abortion of conelets or individual