GEOGRAPHIC PATTERNS OF ALLOZYMIC VARIATION IN LOBLOLLY PINE [1/]

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Abstract.--Haploid maternal gametophytes from more than 200 loblolly pine (Pinus taeda L.) trees sampled from eight geographic provenances were assayed for six enzyme systems. Ten allozyme loci Caere identified having an average of 3.6 alleles per locus.

Results indicated considerable genetic differentiation has evolved within the loblolly pine species. Structuring of the species genetic variation may be a result of restriction to gene flow and varying selection regimes over the natural range of loblolly pine.

Additional keywords: Isozymes, environmental variation, gene frequency, regression, correlation.

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INTRODUCTION

It is well documented that the electrophoretic analysis of isozymes is applicable to the study of genetic variability in forest tree populations (Rudin, 1976). The direct estimation of Mendelian gene frequencies in populations of the Pinacea is readily obtained from the simply inherited, codominant isozyme gene markers using the haploid (1N) maternal gametophyte. This study addresses two basic questions using this tissue and the isozyme technique: (1) how much genetic variability can be detected in the loblolly pine (Pinus taeda L.) genome when sampled across its natural range; and (2) how is the detectable genic variation distributed?

MATERIALS AND METHODS

Sample Locations.--Gametophyte tissues from more than 200 loblolly pine trees were assayed in the study. These included 195 single-tree collections and two bulk-seed lots representing at least 5-10 trees each.

The range map (Figure 1) shows the locations of the 75 local populations from which >1 tree was analyzed. Eight geographic provenances of loblolly pine were available: Central Texas (TXC), East Texas (TXE), Arkansas (AR), Louisiana (LA), Mississippi (MS), Alabama (AL), Georgia (GA), and South Carolina (SC).

Mean longitude, latitude, and means of six environmental variables were calculated for each of the eight loblolly pine provenances (Table 1). These data were tabulated to describe the average environmental regime for a provenance.

Seed materials from contributors outside Texas were most helpful and are listed in the "Acknowledgments" of this paper. Seed collections were made from the two Texas provenances in the Fall of 1977.

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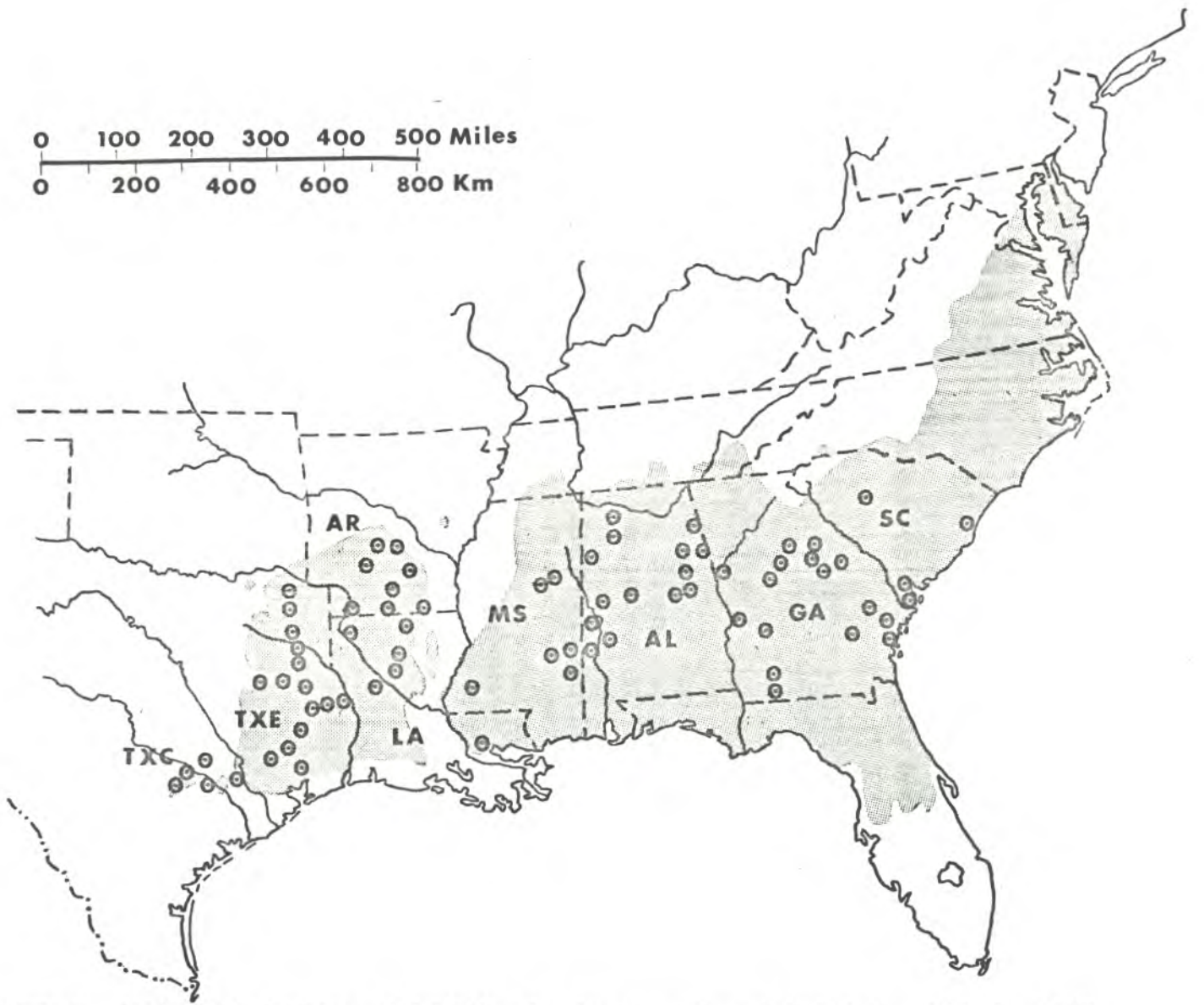


Figure 1. Seed sample locations of loblolly pine (range map from Critchfield and Little, 1966).

Table 1. Mean longitude, latitude and means for the environmental variables associated with each of the loblolly pine provenances.[1/]

Location	Long.	Lat.	Ann. Precip.[a]	T_{Max}[b]	T_{Min}[c]	$\frac{T_{Max} + T_{Min}}{2}$	$\frac{T_{Max}}{T_{Min}}$	Range ($T_{Max} - T_{Min}$)
TXC	97°12'	30°02'	29.7	86.3	42.6	64.4	2.03	43.7
TXE	94°54'	31°38'	41.6	83.9	37.6	60.8	2.23	46.3
LA	92°10'	31°40'	66.5	81.2	48.4	64.8	1.68	32.8
AR	92°30'	33°44'	58.1	80.7	45.5	63.1	1.77	35.2
MS	89°40'	32°26'	70.1	79.4	46.5	63.0	1.71	32.9
AL	86°55'	33°11'	71.6	78.1	44.6	61.3	1.75	33.5
GA	83°09'	32°21'	58.1	80.1	49.6	64.9	1.61	30.4
SC	80°21'	33°20'	56.5	80.2	49.0	64.6	1.64	31.2

a = annual precipitation (inches)

b = maximum monthly temperature (F°)

c = minimum monthly temperature (F°)

TXC = Central Texas

TXE = East Texas

LA = Louisiana

AR = Arkansas

MS = Mississippi

AL = Alabama

GA = Georgia

SC = South Carolina

[1/] Data Source: U.S. Dept. of Commerce, Climatological Data Summaries for the States, 1975.

Enzyme Analyses.--Seeds were stratified (4 °C) for 30 days on moist filter paper. They were germinated at room temperature and the gametophytes prepared for electrophoresis after radicle emergence (approx. 3-5 mm).

Standard horizontal starch gel (12% w/v, Sigma Chemical Co., St. Louis, MO) procedures were followed (e.g., Conkle, 1972; Guries and Ledig, 1978) using two discontinuous buffer systems. Acid phosphatase (AcP), glucosphosphate isomerase (GPI), malate dehydrogenase (MDH), and 6-phosphogluconate dehydrogenase (6-PGD) were assayed after procedures reported by Namkoong, et al. (1979). Glutamate oxaloacetate transaminase (GOT) and glutamate dehydrogenase (GDH) were analyzed from the "Poulik" system of Schaal and Anderson (1974).

Enzymatic activity was identified with methods from the following sources: AcP (Shaw and Prasad, 1970); GPI, MDH, and 6-PGD (Guries and Ledig, 1978); GOT, GDH, (Schaal and Anderson, 1974).

Inheritance at allozymic loci was confirmed by testing for 1:1 segregations of allelic variants contained in the gametophytes of heterozygous trees. Comparisons were also available from other enzyme studies of loblolly pine (Adams and Coutinho, 1977; Conkle and Adams, 1977; Hunter [unpublished]).

Genetic loci were assigned capital letters, A, B, C,... with A being the most rapidly migrating. Alleles were numbered, 1, 2, 3...n, the number 1 allele being the fastest migrating for a given locus. Relative migration distances between alleles were referenced against the consistent band patterns of the hybrid yellow corn, "Truckers' Favorite" (Service Seed Co., Crystal Springs, Miss.), and the most frequent allele at a locus.

Data Analyses.--Allelic variation at loci having >3 alleles was combined such that each locus could be analyzed as having two alleles. This permitted a more straightforward treatment of the binomial properties of independently segregating alleles within a population, i.e., p = most frequent allele plus rare alleles, q = 1-p = frequency of the second most frequent allele, such that p + q = 1 (Li, 1955).

Allele frequencies (transformed to arcsin percent) were entered into simple regression analysis and Pearson's correlation coefficients calculated to test the null hypothesis that genic variability in loblolly pine was randomly distributed over the eight provenances. The independent variables were those shown in Table 1.

RESULTS

Ten (10) allozyme loci and 36 alleles (avg. 3.6 alleles/locus) were identified for the six enzymes assayed among the eight loblolly provenances. Loci and their allele frequencies are shown in Table 2.

Closer inspection of the data in Table 2 prompts these observations:

(a) Total alleles recorded for a particular locus were not present in all provenances. For those alleles at low frequency (i.e.,<.100), absence is likely a function of sampling error.

(b) Considerable heterogeneity exists among provenances for a given allele at a locus, e.g., MDH - B_1 = .731 in Central

TABLE 2. Allele Frequencies Calculated for the Ten Allozyme Loci in Loblolly Pine from Eight Geographic Provenances.

Locus	Allele	TXC	TXE	LA	AR	MS	AL	GA	SC	Overall Freq.
AcP	1	0	0	0	0	.038	0	.017	.111	.015
	2	.308	.354	.150	.067	.238	.250	.133	.278	.248
	3	.654	.594	.650	.833	.676	.562	.667	.556	.646
	4	.038	.021	.050	0	.038	0	0	.056	.024
	5	0	.031	.150	.100	.010	.188	.183	0	.067
GDH	1	.987	.906	1.000	.967	.971	.979	.817	1.000	.943
	2	0	.021	0	0	.010	0	.033	0	.011
	3	.013	.073	0	.033	.019	.021	.117	0	.042
	4	0	0	0	0	0	0	.033	0	.004
GOT-A	1	0	0	0	0	.011	.062	.083	0	.020
	2	.218	.065	.400	.300	.130	.188	.300	.222	.246
	3	.782	.635	.600	.700	.859	.750	.617	.778	.734
GOT-B	1	.038	.167	.150	.100	.087	.062	.133	.056	.099
	2	.962	.812	.850	.900	.913	.938	.833	.889	.890
	3	0	.021	0	0	0	0	.033	.055	.011
GPI	1	1.000	.990	1.000	.967	1.000	.979	.983	.944	.989
	2	0	.010	0	.033	0	.021	.017	.056	.011
MDH-A	1	0	0	0	.033	0	0	0	0	.002
	2	.974	.969	.850	.934	.990	.938	.950	.944	.961
	3	.026	.031	.150	.033	.010	.042	.033	.056	.033
	4	0	0	0	0	0	.020	.017	0	.004
MDH-B	1	.731	.667	.750	.833	.457	.562	.533	.389	.604
	2	.244	.281	.150	.167	.476	.417	.467	.611	.358
	3	.013	.031	.100	0	.067	021	0	0	.031
	4	.012	.021	0	0	0	0	0	0	.007
MDH-C	1	0	0	0	0	0	.021	.017	0	.004
	2	1.000	1.000	1.000	1.000	1.000	.979	.983	1.000	.996
6-PGD-A	1	0	.011	0	0	0	0	.050	.111	.013
	2	.397	.375	.400	.400	.419	.500	.233	.278	.367
	3	.029	.023	0	.067	.181	.042	.233	0	.090
	4	0	.023	0	0	.029	.062	.017	0	.018
	5	0	0	0	0	0	0	0	.111	.004
	6	.574	.568	.600	.533	.371	.396	.467	.500	.508
6-PGD-B	1	.897	.875	.900	.900	.959	.979	.717	.944	.897
	2	.013	.031	0	0	.027	0	.267	0	.048
	3	.090	.094	.100	.100	014	.021	.016	.056	.055
N[a]		39	48	15	10	≥30[b]	24	30	9	ΣN≥205

a = Number of trees sampled /provenance
b = Contained bulked-tree samples

Texas and .389 in South Carolina. The variance test for homogeneity of a binomial distribution showed there to be significant heterogeneity among provenances at $P < .05$ (Snedecor and Cochran, 1973; p. 240) at all loci except GPI and MDH-C.

(c) Trends in gene frequencies and the presence or absence of alleles at a locus are somewhat partitioned west of the Mississippi River (TXC, TXE, AR, LA) and to the east (MS, AL, GA, SC). This observation was confirmed by correlation and regression analysis (note Tables 3 and 4).

(d) East Texas (TXE) and Georgia (GA) provenances contained the highest proportions of the total alleles recorded per locus over all provenances, 80.5 and 88.8 percent, respectively.

Pearson correlation coefficients and simple regression analyses were used as statistical procedures to determine the strength of association between provenance variables in Table 1 (x = independent variable) and gene frequency (y = dependent variable). These results are summarized in Tables 3 and 4.

When gene frequencies for all loci were tested over all provenances, only MDH-B had a marginally significant relationship with longitude. Visual inspection of gene frequency plotted against each provenance variable prompted all subsequent analyses to be performed with the data treated as either Western (TXE,TXC, AR, LA) or Eastern (MS, AL, GA, SC). Regression following this method of classification improved considerably the fit of the MDH-B data and was generally more informative for more loci. It also seemed consistent with the natural partition of the loblolly pine range (note Figure 1).

Table 3.--Significant Pearson Correlation Coefficients between most frequent allele per allozyme locus and provenance variables.

Provenances	Locus	Provenance Variable	Correlation Coefficient (r) [a/]
Western	GDH	$T_{max} + T_{min}/2$	+.932
	MDH-A	Latitude	-.922
	MDH-B	T_{min}	+.961
	MDH-B	T_{max}/T_{min}	-.994
	MDH-B	$T_{max}-T_{min}$	-.998
	6-PGD-A	$T_{max}+T_{min}/2$	-.904
	6-PGD-B	Longitude	+.972
	6-PGD-B	Ann. Precip.	-.958
	6-PGD-B	T_{max}	+.973
Eastern	GPI	Longitude	+.901
	6-PGD-A	T_{min}	+.924
	6-PGD-A	T_{max}/T_{min}	-.948
	6-PGD-A	$T_{max}-T_{min}$	-.967

[a/] Significance level: $P \leq .05$

Table 4.--Summary of significant regressions of the most frequent allele per allozyme locus against provenance variables.

Provenances	Prov. Var. (x)	Locus (y)	r^2 [a/]	$\hat{y}$
Western	T_{min}	MDH-B	.92	23.04 + .92(x)
	T_{max}/T_{min}	MDH-B	.99	174.92 -1.35(x)
	$T_{max}-T_{min}$	MDH-B	.99	89.42 - .67(x)
	Longitude	6-PGD-B	.98	54.03 + .19(x)
	Ann. Precip.	6-PGD-B	.96	73.28 - .03(x)
	T_{max}	6-PGD-B	.98	57.72 + .17(x)
Eastern	Ann. Precip.	6-PGD-A	.95	147.65 -1.54(x)
	$T_{max}-T_{min}$	6-PGD-A	.93	315.12 -8.32(x)

a/ Significance level: $P \leq .05$

DISCUSSION

The results presented above confirm the observations made by others in concluding that loblolly pine possesses considerable genetic variability at allozymic loci (Conkle and Adams, 1977; Hunter [unpub.]). Most striking is the degree to which the genic variation in loblolly pine has become organized in different regions of its distribution. It seems appropriate to believe that the genetic evolution of the species has been significantly influenced by the Mississippi River disjunction and subsequent development of environ-

NT east sx..c1 west.

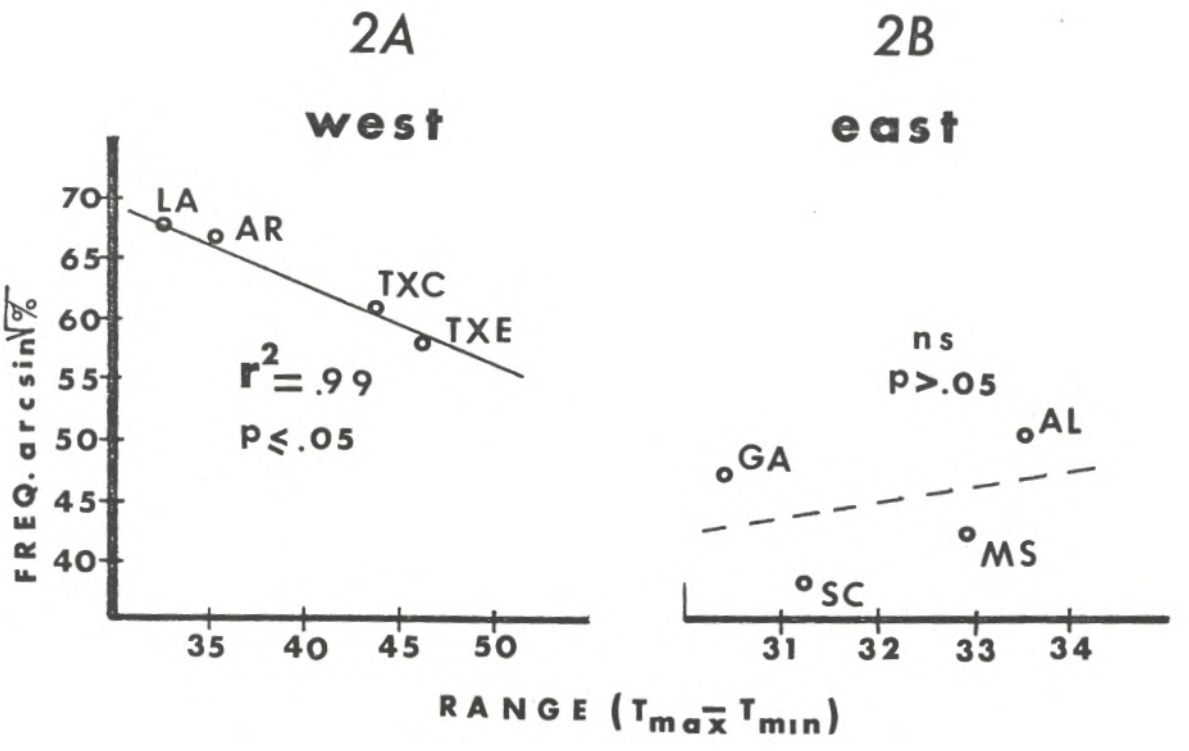
Some of the most obvious generalizations from the results of this study are:

(a) The overall environmental regimes in the western provenances of loblolly pine are more extreme in temperature and moisture. The eastern environment is somewhat more equable and tends to reflect more of a coastal influence.

(b) Three of the 10 allozyme loci, MDH-B, 6-PGD-A, and 6-PGD-B offer potential as gene markers of gene flow restriction and differential selection among the varying environments across the range of loblolly pine.

The observations made in (b) above are strikingly displayed in Figures 2A and 2B, showing the most frequent allele frequency for the MDI{-B locus regressed against temperature range ($T_{max}-T_{min}$). These two representations graphically show the differential response of the same locus interacting with different sets of environmental patterns and likely, the MDH-B alleles having considerably different genetic backgrounds among the provenances.

Sampling strategy of this study maximized area of the natural loblolly range, rather than local genic variation and population structure. Therefore, many of the differences among populations within provenance have not been detected. However, it is significant to note that the relationships encountered



Figures 2A and 2B.--Regressions of the most frequent MDH-B allele against temperature range for loblolly pine provenances east and west of the Mississippi River.

in the present study coincide closely with the conclusions drawn from provenance studies of drought resistance (Knauf and Bilan, 1974), variation in height growth, volume, seedling survival and resistance to fusiform rust (Grigsby, 1977; Rink and Thor, 1971; Wells and Wakeley, 1966). If our results prove to be indicative of the allyzymic variation among local populations of loblolly pine, allozymic markers offer considerable potential during basic adaptational studies and monitoring selection efforts of tree improvement programs.

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LITERATURE CITED

Adams, W. T. and S. Coutinho. 1977. Isoenzyme genetic markers useful for studies of the Pinus rigida x P. taeda hybrid. Northeast For. Tree Improv. Cong. Proc. 24:1-13.

Conkle, M. T. 1972. Analyzing genetic diversity in conifers--isozyme resolution by starch gel electrophoresis. USDA For. Serv. Res. Note PSW-264, 5p.

Conkle, M.T. and W. T. Adams. 1977. Use of isoenzyme techniques in forest genetics research. South. For. Tree Improv. Conf. Proc. 14:219-226.

Critchfield, W. B. and E. L. Little, Jr. 1966. Geographic distribution of the pines of the world. USDA U. S. For. Serv., Misc. Pub. 991, 97p.

Grigsby, H. C. 1977. A 16-year provenance test of loblolly pine in southern Arkansas. South. For. Tree Improv. Conf. Proc. 14:261-268.

Curies, R. P. and F. T. Ledig. 1978. Inheritance of some polymorphic isoenzymes in pitch pine (Pinus rigida Mill.). Heredity 40:27-32.

Hunter, S. C. (unpub.) An electrophoretic analysis of isoenzyme variation in a Piedmont loblolly pine seed orchard. M.S. Thesis (1977), Sch. of For., North Carolina State Univ., Raleigh, 48p.

Knauf, T. A. and M. V. Bilan. 1974. Needle variation in loblolly pine from mesic and xeric seed sources. For. Sci. 20:88-90.

Li, C. C. 1955. Population Genetics. Univ. of Chicago Press, Chicago & London, 366p.

Namkoong, G., J. H. Roberds, L. B. Nunnally, H. A. Thomas. 1979. Isozyme variations in populations of southern pine beetles. For. Sci. 25:197-203.

Rink, G. and E. Thor. 1971. Results from ten loblolly pine provenance tests in Tennessee. South. For. Tree Improv. Conf. Proc. 11:158-164.

Rudin, D. 1976. Isozymes as markers guiding selection for advanced generation breeding. I.U.F.R.O. Joint Meeting on Advanced Generation Breeding, Bordeaux, June 14-18, 13p.

Schaal, B. A. and W. W. Anderson. 1974. An outline of techniques for starch gel electrophoresis of enzymes from the American oyster Crassostrea virginica Gmelin. Georgia Marine Science Center, Tech. Rep. Ser. No. 74-3, 19p.

Shaw, C. R. and R. Prasad. 1970. Starch gel electrophoresis of enzymes--a compilation of recipes. Bio-chem Genet. 4:297-320.

Snedecor, G. W. and W. G. Cochran. 1973. Statistical Methods (sixth ed.). Iowa State Univ. Press, Ames, 593p.

Wells, O. O. and P. C. Wakeley. 1966. Geographic variation in survival, growth and fusiform rust infection of planted loblolly pine. For. Sci. Monogr. 11, 40p.