Allozyme Diversity of Selected and Natural Loblolly Pine Populations

By R. C. SCHMIDTLING[1)2)], E. CARROLL[1)3)] and T. LAFARGE[1)4)]

(Received 19th January 1999)

Summary

Loblolly pine (*Pinus taeda* L.) megagametophytes and embryos were examined electrophoretically to compare the extent and distribution of genetic variability in allozymes of selected and wild populations. Range-wide collections of three different types were investigated in this study. These consisted of seed sampled from, 1. a provenance test established in 1953, 2. bulk seed sampled from collections obtained from natural stands, and 3. seed harvested from clones used to produce improved seed in a tree improvement program.

All 18 loci tested were found to be polymorphic. The average number of alleles overall (N_a) was 3.8. Expected heterozygosities (H_e) varied from 0.193 in the 70-year old orchard clones, to 0.174 in the 40-year-old provenance test samples, to 0.163 in the embryos of the bulk collections. The maximum F_{ST} was 0.066 for the provenance test populations, which indicates that only a small proportion (6.6 %) of the total variation in allozymes was attributed to population differences. In spite of this, the populations were well differentiated in multivariate analysis.

In controlled-pollinated progeny tests of the orchard selections, there was a negative association between growth and the presence of rare alleles in the parent. A rare allele at the IDH locus was associated with slower growth, probably because it indicated hybridization with the slower-growing shortleaf pine (*P. echinata* MILL.).

Allozyme variation as well as variation in cortical monoterpenes and fusiform rust resistance suggests that loblolly pine resided in two refugia during the Pleistocene; one in south Texas / northeast Mexico and one in south Florida / Caribbean. The two populations migrated to the northern Gulf Coastal Plain at the beginning of the Holocene and merged just east of the Mississippi River.

Key words: Pinus taeda, allozymes, geographic variation, Pleistocene, tree improvement, genetic variation, hybridization.

FDC: 165.52; 165.3; 165.71; 174.7 *Pinus taeda.*

Introduction

Forest tree populations in the southeastern United States have undergone a great deal of change after the height of the Wisconsin glaciation 13,000 years ago (WATTS, 1983). As populations migrated northward in the wake of the retreating glacier, following optimum environments for the species, they evolved in response to their new environment. Adaptations in response to these environmental changes are probably still taking place because forest trees are long lived, have a slow generation turnover, and exist in a constantly changing climate.

Recently, man has greatly accelerated the rate of genetic change. Starting before the turn of the century, clear-cutting and high-grading, followed by sporadic natural regeneration left many areas devoid of forest or sparsely populated by a few genotypes that were inferior to the harvested stands. In the reforestation carried out by the Civilian Conservation Corps in the 1930's, an effort was made to use native seed sources, but nursery records[5)] show that the source of seed was sometimes disregarded; the result was a random mixing of foreign provenances with the native populations. During reforestation, species composition shifted significantly. Vast acreages of slash pine (*Pinus elliottii* ENGELM.) were planted within, as well as outside its natural range. Longleaf pine (*P. palustris* MILL.), once the predominant species on the Coastal Plain, was largely replaced by slash and loblolly pines (*P. taeda* L.) because longleaf pine was very difficult to plant and slow in early growth (CROKER, 1990). Shortleaf pine (*P. echinata* MILL.) was largely replaced by the faster growing loblolly pine in the southern part of its range.

Beginning in the 1950's, the mixing of populations became much more widespread and systematic in loblolly, the most frequently planted southern pine. Tremendous quantities of seedlings from Livingston Parish, Louisiana, and east Texas have been planted in Georgia, Alabama, and north Florida because they are resistant to fusiform rust (WELLS, 1985). In the other direction, great quantities of seedlings from the Coastal Plain of the Carolinas have been planted in Arkansas, because they greatly surpass the local sources in growth, although there is increased risk for crop failure (LAMBETH *et al.,* 1984).

The plantations resulting from these moves are genetically different from the local sources (WELLS, 1985). Even if all of these plantations are clear-cut, their genes will persist as advanced natural regeneration or as pollen contamination in seed produced in surrounding native populations.

Tree improvement programs also bring about genetic change; this is their sole purpose. The widespread use of genetically improved material may also affect genetic diversity.

Studies of allozyme variation can be very useful in defining genetic variation in forest trees (HAMRICK *et al.,* 1992). There have been few published studies of allozyme variation and diversity for range-wide loblolly pine. FLORENCE and RINK (1979) reported on variation that did not include samples from the northeastern part of the range. They noted differences between populations east of the Mississippi River and those west of the river. In the present study, loblolly pine seed were examined electrophoretically to compare the extent, distribution and magnitude of possible change in genetic variability for selected and wild populations of various origins. Progeny test data were also examined to determine the relationship between genetic variability (as measured by allozymes) and height growth.

[1)] Geneticists, USDA Forest Service

[2)] Southern Institute of Forest Genetics, 23332 Hwy 67, Saucier, MS 39574, USA, tel: 228-832-2747, fax: 228-832-0130, e-mail: schmidtl@datasync.com

[3)] National Forest Genetic Electrophoresis Lab., Camino, CA 95709, USA

[4)] Southern Region, Atlanta, GA 30367, USA

[5)] Nursery records on file at the Ashe Nursery, USDA Forest Service, Brooklyn, MS.

Materials and Methods

Seed Sources

Range-wide collections of seed of four different types were used in the study. Seed were collected from: 1. The Southwide Southern Pine Seed Source Study (SSPSSS), 2. Bulk woods-run collections, and 3. and 4. Collections from orchard clones from a tree improvement program.

1. The Southwide Southern Pine Seed Source Study (SSPSSS) is the most extensive set of provenance tests ever installed in the United States (WELLS, 1969). The SSPSSS was a very large undertaking involving many cooperators coordinated by the Southern Forest Tree Improvement Committee. Seed from numerous natural stands of slash, loblolly, longleaf, and shortleaf pines was collected across the entire southern pine range in the early 1950's. The loblolly pine plantings were established in 1954.

Seed for electrophoresis were collected from 9 to 17 trees per source from 14 sources in two of the SSPSSS loblolly plantings located in south Mississippi *(Fig. 1, Table 1).* Only megagametophytes were used for analysis, since the provenance of the pollen parent is indeterminate.

2. The wild, or bulk seed lots were from 10 locations across the natural range *(Fig. 1).* These were obtained from various cooperators and were collections from large numbers of trees in natural stands for general planting use. Embryos were analyzed.

3. The orchard clones are from the USDA Forest Service tree improvement program in the Southern Region (R-8). The R-8 loblolly pine program divides the southeastern US into 5 breeding zones, containing one or more 50-clone sources. Breeding zone 1 contained interior sources 11, 12, and 13 from north AL, GA and MS, respectively *(Fig. 1).* Zone 2 consists of the western sources 21 and 22 from west LA and TX. Zone 3 is number 32 from southwest MS. Zone 4 consists of numbers 41 and 44 from the coastal Carolinas. Zone 5 consists of piedmont SC number 53. A total of 329 clones from 9 sources were used in this analysis. Each clone was genotyped at 18 loci by analyzing 10 megagametophytes from each clone.

4. A simulated „orchard output" was constructed for each of the 9 sources by mixing equal numbers of seed from each clone within each source and analyzing embryos, to study the effects of tree improvement on genetic diversity.

Enzyme Electrophoresis

Isozyme band patterns were investigated using megagametophyte and embryo tissues. Intact seeds were sterilized for five minutes in calcium hypochlorite solution and then spread on petri plates lined with filter paper moistened with 1% hydrogen peroxide. Seeds were placed in a germinator at 20°C to 21°C with a 8-hr photoperiod, until radicles were 5 mm to 10 mm in length, which normally occurred within 5 to 7 days.

For the SSPSSS and orchard clonal material, extracts were prepared by crushing an excised megagametophyte in two drops of 0.20 M phosphate buffer (pH 7.5), absorbing it onto 2 mm wide paper wicks and freezing the wicks at -70°C. For the bulk and orchard output samples, the excised embryos were placed in two drops of 0.20 M phosphate buffer (pH 7.5), and frozen at -70°C.

Prior to electrophoresis, the embryo samples were thawed, macerated and absorbed on 2.5 mm wicks for electrophoresis.

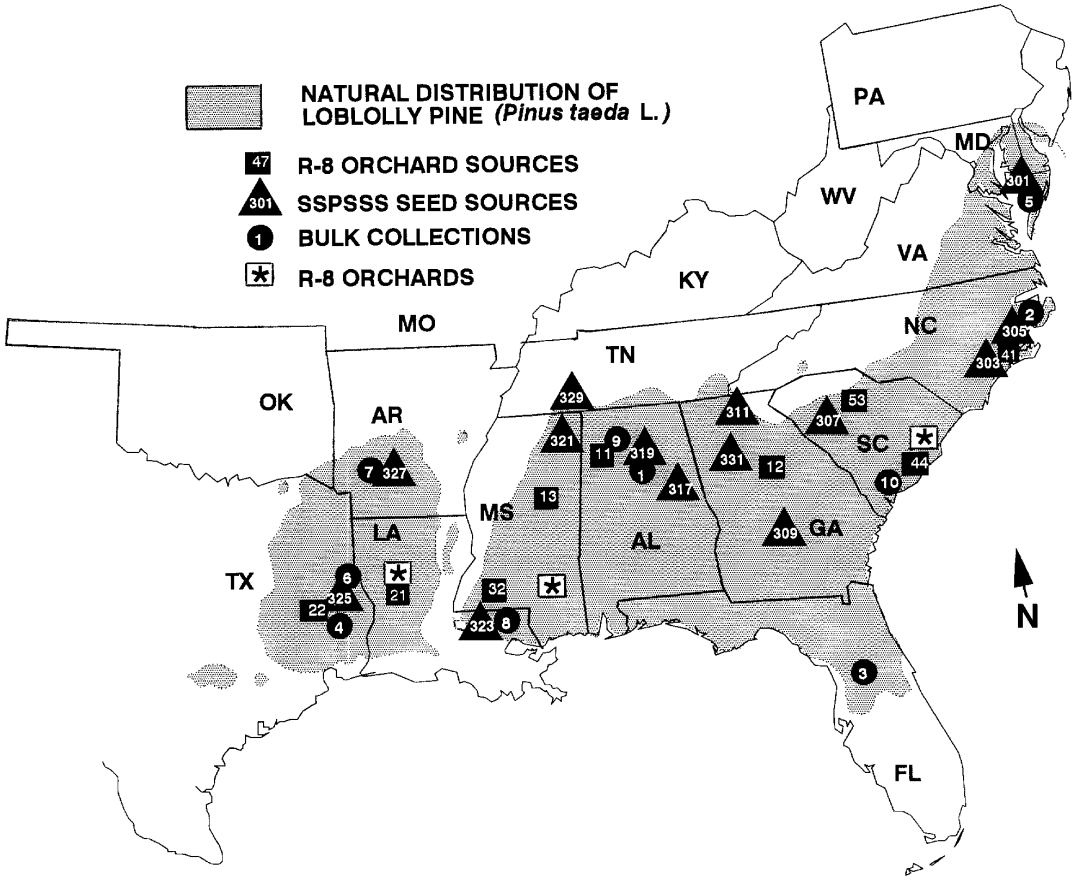

Figure 1. – Map of the southeastern United States showing the natural range of loblolly pine *(Pinus taeda* L.) and the locations of the sampled populations.

Table 1. – Population origins, number of trees sampled per population, mean number of alleles per polymorphic loci (N_a), percent loci polymorphic (P_1), observed heterozygosity (H_o), and expected heterozygosity (H_e) at 12 loci.

Population Source	County	State	Sample Size	N_a	P_1[1] 100%	P_1[1] 95%	H_o	H_e
Bulk (Woods-Run)			Seed					
1	Jefferson	AL	68	2.00(0.28)[2]	66.7	33.3	0.142(0.056)	0.154(0.061)
2	Beaufort	NC	63	2.42(0.36)	75.0	50.0	0.171(0.060)	0.174(0.065)
3	Marion	FL	61	2.58(0.38)	83.3	41.7	0.158(0.065)	0.174(0.068)
4	Polk	TX	66	1.83(0.21)	66.7	41.7	0.152(0.061)	0.144(0.056)
5	Worchester	MD	67	2.42(0.38)	83.3	41.7	0.157(0.057)	0.173(0.064)
6	Angelina	TX	64	2.08(0.19)	83.3	41.7	0.143(0.051)	0.157(0.058)
7	Howard	AR	66	2.33(0.38)	83.3	50.0	0.160(0.063)	0.173(0.062)
8	Livingston	LA	69	2.17(0.32)	75.0	33.3	0.127(0.056)	0.137(0.061)
9	Cullman	AL	67	2.33(0.31)	83.3	50.0	0.175(0.060)	0.173(0.057)
10	Charleston	SC	66	2.25(0.41)	66.7	50.0	0.172(0.064)	0.171(0.065)
Mean				**2.24**	**77.5**	**43.3**	**0.156**	**0.163**
SSPSSS Sources			Trees					
301	Somerset	MD	17	2.17(0.39)	58.3	58.3	–[3]	0.203(0.066)
303	Onslow	NC	17	2.33(0.31)	75.0	50.0		0.184(0.057)
305	Pamlico	NC	9	2.00(0.17)	83.3	41.7		0.149(0.048)
307	Newberry	SC	10	1.83(0.21)	66.7	66.7		0.169(0.053)
309	Wilcox	GA	10	1.67(0.22)	50.0	41.7		0.154(0.060)
311	Clarke	GA	10	2.00(0.25)	66.7	66.7		0.200(0.055)
317	Clay	AL	11	2.42(0.36)	75.0	75.0		0.238(0.060)
319	Jefferson	AL	11	2.33(0.28)	83.3	66.7		0.213(0.062)
321	Prentiss	MS	10	1.50(0.23)	33.3	25.0		0.119(0.064)
323	Livingston	LA	17	1.83(0.30)	50.0	50.0		0.181(0.066)
325	Angelina	TX	10	1.58(0.23)	41.7	33.3		0.110(0.055)
327	Clark	AR	15	2.08(0.19)	83.3	58.3		0.169(0.051)
329	Hardeman	TN	10	2.08(0.23)	75.0	66.7		0.218(0.056)
331	Spalding	GA	10	1.83(0.21)	66.7	33.3		0.135(0.055)
Mean				**1.98**	**64.9**	**52.4**		**0.174**
Seed Orchard Clones			Trees					
11	N.-Central	AL	53	2.67(0.31)	91.7	75.0	0.156(0.051)	0.159(0.052)
12	N.-Central	GA	19	2.58(0.31)	83.3	75.0	0.228(0.064)	0.216(0.057)
13	N.-Central	MS	30	2.42(0.29)	91.7	66.7	0.161(0.053)	0.176(0.062)
21	Central	LA	51	2.50(0.19)	100	50.0	0.196(0.064)	0.180(0.056)
22	S.-East	TX	50	2.33(0.19)	91.7	66.7	0.202(0.054)	0.196(0.050)
32	S.-West	MS	39	2.25(0.25)	83.3	58.3	0.182(0.058)	0.177(0.058)
41	Coastal	NC	33	2.58(0.38)	91.7	58.3	0.240(0.064)	0.235(0.060)
44	Coastal	SC	26	2.50(0.42)	75.0	58.3	0.250(0.072)	0.231(0.066)
53	Piedmont	SC	48	2.58(0.35)	83.3	58.3	0.167(0.056)	0.166(0.055)
Mean				**2.49**	**88.0**	**60.2**	**0.198**	**0.193**
Seed Orchard Output			Seed					
11	N.-Central	AL	46	1.83(0.34)	50.0	16.7	0.118(0.064)	0.110(0.059)
12	N.-Central	GA	54	2.08(0.26)	75.0	41.7	0.167(0.067)	0.164(0.062)
13	N.-Central	MS	39	1.92(0.25)	66.7	41.7	0.132(0.056)	0.147(0.065)
21	Central	LA	43	2.25(0.30)	83.3	41.7	0.154(0.061)	0.154(0.058)
22	S.-East	TX	47	1.92(0.19)	75.0	50.0	0.158(0.054)	0.160(0.053)
32	S.-West	MS	43	2.00(0.17)	83.3	33.3	0.155(0.069)	0.158(0.064)
41	Coastal	NC	45	2.08(0.34)	66.7	50.0	0.165(0.063)	0.173(0.066)
44	Coastal	SC	40	2.33(0.31)	83.3	50.0	0.177(0.061)	0.178(0.057)
53	Piedmont	SC	44	2.25(0.35)	66.7	41.7	0.133(0.056)	0.148(0.059)
Mean				**2.07**	**72.2**	**40.8**	**0.151**	**0.155**

[1]) At the 100% criterion, a locus is considered polymorphic if more than one allele was detected. At the 95% criterion, the most common allele must have a frequency of 0.95 or less for a locus to be considered polymorphic.

[2]) Standard errors of the estimates are in parenthesis.

[3]) The seed were bulked and approximately 50 megagametophytes per source were genotyped. Since individual tree data are not available, observed heterozygosities were not computable.

Table 2. – Enzyme systems and buffers used.

Enzyme	Abbreviation	Buffers	
		Megagametophyte	Embryo
Aconitase	ACO-1	LB	LB
Fluorescent esterase	FEST-2	LB,MC6	LB
Glutamic oxaloacetate transaminase	GOT-2	SB	
	GOT-3	SB	SB
Isocitrate dehydrogenase	IDH-1	MC8	MC8
Leucine aminopeptidase	LAP-1,2	LB	LB[2]
Malate dehydrogenase	MDH-1	MC6	MC6
	MDH-2	MC6,MC8	MC6,MC8
	MDH-3	MC8[1]	
	MDH-4	MC8	MC8
Malic enzyme	ME-7	LB	LB
Phosphoglucose isomerase	PGI-1	LB	LB[2]
Phosphoglucose isomerase	PGI-2	LB	LB
Phosphoglucomutase	PGM-1	LB	LB
Shikimate dehydrogenase	SKD-1,2	MC6,MC8	MC6,MC8[2]
6-phosphogluconate dehydrogenase	6PGD-1	SB	SB,MC6,MC8
	6PGD-2	MC6,MC8	MC6,MC8
Uridine diphosphoglucose pyrophosphorylase	UGP-1	SB[1]	

[1]) Dropped due to inconsistent resolution.
[2]) Dropped due to unreliable scoring results.

Paper wicks from the megagametophyte samples were also thawed for electrophoresis, and the wicks for all samples were inserted into 11% starch gels (Sigma Chemical Co.) that accommodated 48 samples. The preparation and running of the gels are modifications of ADAMS *et al.* (1990) and CONKLE *et al.* (1982). Four buffer systems were used: 'LB' (gel and tray buffer 'A' of ADAMS *et al.* (1990)), 'SB' (a modification of gel and tray buffer 'B' of ADAMS *et al.* (1990), where the electrode buffer was pH 8.0), 'MC8' (a modification of gel and tray buffer 'C' of ADAMS *et al.* (1990), where the stock solution was adjusted to pH 8.0), and 'MC6' (the same as 'MC8' except that the pH is 6.1) *(Table 2).*

Stain recipes follow ADAMS *et al.* (1990) and CONKLE *et al.* (1982). After a dye marker migrated 8 cm, gels were cut horizontally into four to seven slices, stained and data was recorded for enzyme bands representing alleles for individual gene loci.

Growth Data

Data was obtained on 5-year progeny heights of 235 orchard tree selections. Progeny were from controlled crosses made in a disconnected diallel design (LAFARGE, 1989). The resulting seedlings were planted in randomized designs in 3 to 5 plantings within their respective breeding zones. Data is available from zones 1, 2, 3, and 4 (sources 11 to 13, 21 to 22, 32, and 41 to 44, respectively, *Fig. 1*). Since none of the plantings contained a substantial proportion of the total number of families, the Best Linear Prediction (BLP) process (WHITE and HODGE, 1989) was used to estimate overall heights and compute breeding values. In a pioneering species such as loblolly pine, rapid early height growth is an important measure of adaptability (BAKER, 1950). The breeding values for height provide one estimate of adaptability over a wide range of growing conditions. These values were compared to allozyme data at 18 loci by linear regression to test for the effects of diversity, heterozygosity, and individual allozyme variation on growth.

Statistical Analysis

Allozyme data were used to provide several estimates of genetic variation using BIOSYS I (SWOFFORD and SELANDER, 1989): Mean number of alleles per polymorphic loci (N_a), percent loci polymorphic (P_l, 100% and 95% criteria), observed heterozygosity (H_o), and expected heterozygosity (H_e).

BIOSYS also provided F statistics F_{IS}, F_{IT} and F_{ST} (WRIGHT, 1965; NEI, 1977). Gene flow was estimated using WRIGHT's (1931) formula:

$$Nm = (1 - F_{ST}) / 4F_{ST} \quad (1)$$

where N is the effective population size of the recipient population and m is the rate of gene flow. Nm estimates the number of migrants per generation. F_{ST} is considered to be equivalent to G_{ST} (WRIGHT, 1978). Additionally, the number of rare alleles per tree (N_r) was computed (a rare allele being defined here as one which occurs at a frequency of 0.05 or less in the overall population).

Diploid genotypes were also transformed for multivariate analysis using the technique of SMOUSE and WILLIAMS (1982). For each allele at a locus (minus one), the value of 0.5 was assigned when the allele was present and 0 when the allele was absent. The score when the allele at the locus is in the homozygous state would be 0.5 + 0.5 = 1.0, when it is in the heterozygous state, 0.5 + 0.0 = 0.5. For individuals without the allele the score would be 0. Data sets with more than ten alleles can be assumed to have a normal distribution (SMOUSE and WILLIAMS, 1982). Transformed data were analyzed using SAS (1985) multivariate analysis of variance and canonical discriminant analysis.

To test for the relationship between growth and heterozygosity, the following linear model was tested:

$$Y_i = \beta_0 + \beta_1 H_{i1} + \beta_2 H_{i2} + ... + \beta_{18} H_{i18} + \epsilon_i \quad (2)$$

where:

β_0 ... β_{18} = slope and intercept parameters fitted by the regression procedure,

Y_i = BLP estimate of breeding value for height at age 5 for progeny from clone *i,*

H_{i1} ... H_{i18} – for locus 1 through 18 for clone *i,* = 0 if the locus is homozygous and =1 if the locus is heterozygous, and

ϵ_i = residual.

As one approach to examining the hypothesis that allozyme variants are selectively neutral, it was first assumed that if allozymes are not selectively neutral, than the „fitness" of an allozyme may be related to its frequency in the population. At each locus for each allele, the allozyme codes were replaced with the frequency of the subject allozyme in the population, and then tested in a linear model relating these frequencies to breeding values for height:

$$Y_i = \beta_0 + \beta_1 A_{i1} + \beta_2 a_{i1} + \beta_3 A_{i2} + \beta_4 a_{i2} + ... + \beta_{35} A_{i18} + \beta_{36} a_{i18} + \epsilon_i \quad (3a)$$

or

$$Y_i = \beta_0 + \beta_1 A_{i1} + \beta_2 A_{i2} + ... + \beta_{18} A_{i18} + \epsilon_i \quad (3b)$$

where:

β_0 ... β_{36} = slope and intercept parameters fitted by the regression procedure,

Y_i = BLP estimate of breeding value for height at age 5 for progeny from clone *i*,

A_{i1} ... A_{i18} – for locus 1 through 18, = the frequency of the allele for the *most* common allele at the subject locus for clone *i,*

a_{i1} ... a_{i18} – for locus 1 through 18, = the frequency of the allele for the *least* common allele at the subject locus for clone *i,* and

ϵ_i = residual.

Two models were tested, the „full" or additive model that included all the terms, ie, both the „A" and „a" terms *(3a),* and the „dominance model" *(3b),* which consisted only of the „A" terms, or the frequency of only the most common allele at the locus (this assumes that the most common allele is dominant to the least common allele).

Results and Discussion

Allozyme Variation

All 18 loci were found to be polymorphic in at least one population. The average number of alleles overall (N_a) was 3.8 for 18 loci. For the 12 loci common to all four groups of data, the mean number of alleles per locus (N_a) was 3.9. On a population basis, N_a averaged 2.19, the percentage of polymorphic loci (P_l, 100% criterion) averaged 76.1%, and mean expected heterozygosity (H_e), averaged 0.171 *(Table 1).* These values are similar to those estimated in an unpublished loblolly pine study (using different loci) – 1.93, 72.7%, and 0.218, respectively (cited in EDWARDS and HAMRICK, 1995).

The four types of sampled populations, ie, bulk, SSPSSS, orchard or orchard output, vary somewhat in diversity parameters *(Table 1).* The variation can be attributed to differences in tissue type, sample size and age of the material. The total number of alleles per locus (N_a) in the types using megagametophyte tissue was 3.67 and 3.75 for the orchard and the SSPSSS material, respectively. The orchard type sampled a much greater number of trees (349) than the SSPSSS (167), but the SSPSSS covered a greater part of the natural range *(Fig. 1).*

The effect of differences in sample size among populations within types is evident in the population diversity parameters *(Table 1).* Since N_a and P_l are dependent on sample size, the SSPSSS populations have lower values, because the samples originated from only 9 to 17 trees per population. H_e is little affected by sample size, and the value for this parameter in the SSPSSS is more in agreement with the other three classes.

Some of the other differences among the four classes can be explained by the relative ages of the sampled populations. Heterozygosity and allozyme diversity generally increase from embryo to seedling to mature tree, probably because of excess mortality in inbred individuals under the increased stress of competition (LEDIG, 1986).

Table 3. – Summary of F-statistics at 12 and 18 loci.

Allozyme Locus	Orchard Clones			Bulk Collections			Orchard Output			SSPSSS
	F_{IS}	F_{IT}	F_{ST}	F_{IS}	F_{IT}	F_{ST}	F_{IS}	F_{IT}	F_{ST}	F_{DT}
ACO-1	-0.051	-0.043	0.007	-0.005	0.020	0.025	-0.012	-0.001	0.011	0.044
FEST-2	-0.007	0.012	0.019	-0.004	0.024	0.028	0.151	0.163	0.016	0.056
GOT-3	0.068	0.093	0.026	0.013	0.033	0.020	0.033	0.059	0.026	0.070
IDH-1	0.165	0.191	0.031	-0.023	-0.009	0.013	0.314	0.335	0.031	0.079
MDH-1	-0.040	-0.024	0.015	0.315	0.325	0.014	-0.023	-0.002	0.020	0.036
MDH-2	-0.059	-0.035	0.022	-0.029	0.010	0.038	0.056	0.070	0.015	0.068
MDH-4	-0.012	-0.004	0.008	0.661	0.666	0.016	-0.010	-0.001	0.009	0.041
ME-7	-0.173	-0.137	0.031	0.616	0.627	0.030	-0.020	-0.004	0.016	0.083
PGI-2	0.017	0.057	0.041	0.013	0.025	0.013	-0.005	0.017	0.022	0.081
PGM-1	-0.084	-0.057	0.025	0.032	0.127	0.099	0.107	0.121	0.016	0.032
6PGD-1	-0.029	0.013	0.040	0.079	0.109	0.032	-0.018	0.019	0.036	0.082
6PGD-2	-0.057	-0.043	0.013	-0.019	-0.007	0.012	-0.027	0.014	0.040	0.056
Mean	-0.041	-0.014	**0.026**	0.037	0.068	**0.033**	0.013	0.037	**0.024**	**0.066**
GOT-2	-0.054	-0.036	0.017							
LAP-1	-0.019	-0.003	0.016							
LAP-2	0.010	0.028	0.018							
PGI-1	-0.069	-0.019	0.047							
SKD-1	-0.021	0.037	0.057							
SKD-2	0.215	0.235	0.025							
Mean	-0.032	-0.003	**0.028**							

In the bulk collections and the seed orchard output, embryos are genotyped; they have the lowest values for N_a, P_l, and H_e *(Table 1).* Since megagametophytes were genotyped for the orchard clones and the SSPSSS collections, these represent genotypes of the parent trees. The seed orchard clones, which have the highest values for these parameters, have been selected for their size and averaged around 70 years of age at the time of selection. They are presumably well-adapted. The SSPSSS samples, which are from trees 40 years old, are intermediate in H_e, the one parameter that is comparable to the other classes. H_e varies from 0.193 in the 70-year old orchard clones, to 0.174 in the 40-year-old SSPSSS samples, to 0.163 and 0.155 in the bulk collections and embryos of the seed orchard output, respectively.

Although the seed orchard clones have the greatest genetic variability of any of the four classes, the open-pollinated seed from these clones, the seed orchard output, has slightly less genetic variability than the comparable bulk collections *(Table 1).* This may be a reflection of the size of the orchard sources, which is limited to 50 clones, and not all clones from a given source were represented in some of our samples.

The observed heterozygosities (H_o) are very close to the expected heterozygosities (H_e) indicating that the populations are close to HARDY-WEINBERG equilibrium *(Table 1).* H_e is slightly higher than H_o in the bulk and seed orchard output classes indicating a slight degree of inbreeding; the opposite is true in the orchard clones. The excess of homozygotes in the bulk and seed orchard classes in verified by the slightly positive values for F_{IS} and F_{IT} *(Table 3).* These values are slightly negative in the seed orchard clones, indicating an excess of heterozygotes.

Geographic Variation

F_{ST}, a quantification of population differentiation, was 0.033 for the bulk populations, and 0.066 for the SSPSSS populations (the two types representing the broadest range of sampling) which indicates that only a small proportion (3.3% to 6.6%) of the total variation in allozymes was attributed to population differences *(Table 3).* F_{ST} values for the orchard clones and orchard output were lower (0.026 and 0.024, respectively), probably because the orchards did not include populations from the extremities of the range, Arkansas and Maryland *(Fig. 1).*

Estimates of gene flow using WRIGHT's formula (1) ranges from Nm = 3.54 migrants per generation in the bulk collections to Nm = 9.37 in the orchard sources. These values bracket the number of 4 migrants per generation which WRIGHT (1931) considered the minimum to prevent differentiation due to drift. This is not surprising considering the extensive long-distance pollen flow demonstrated in studies of pollen contamination in loblolly pine seed orchards (FRIEDMAN and ADAMS, 1985).

In spite of the low values for F_{ST}, the multivariate analysis of transformed diploid data of the orchard, bulk, and SSPSSS seed sources showed significant differences among populations ($P < 0.001$). Plots of the first two canonical functions showed

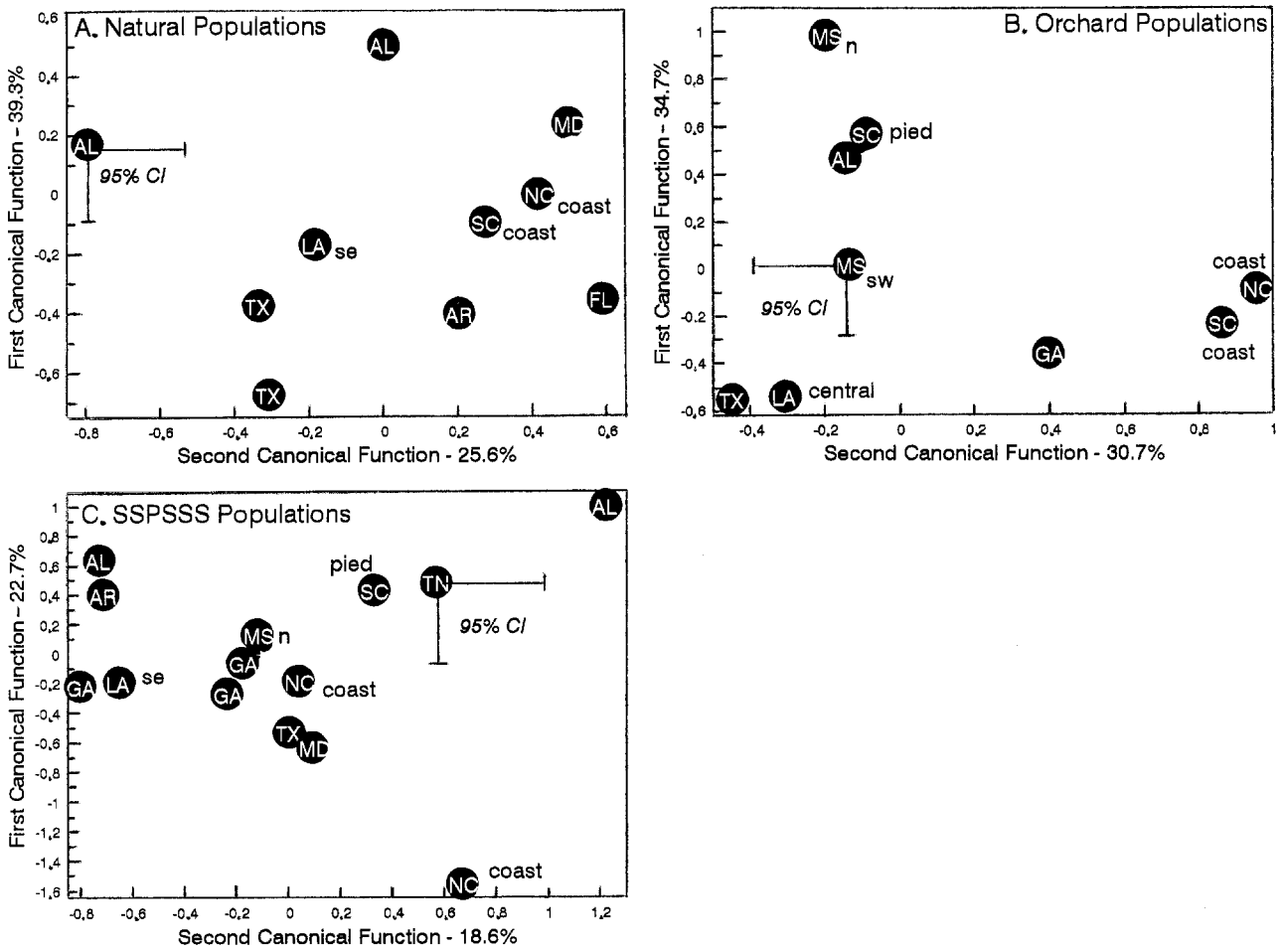

Figure 2. – Plot of the first and second canonical functions from the discriminant analysis for the (A) bulk natural populations, (B) the orchard populations, and (C) the SSPSSS populations.

reasonable distributions and groupings for the orchard and bulk samples *(Fig. 2A* and *2B*). Sources from west of the Mississippi River tend to group together in the lower left corner in both plots.

In the plot of the orchard populations *(Fig. 2B)*, the Texas and central Louisiana sources group very close together in the lower left corner, the Atlantic coastal sources are arrayed to the right, and the inland sources are arrayed toward the upper left. The southwest Mississippi source, which is from just east of the Mississippi River, is in a transitional position relative to the other three groupings.

The plot of the bulk natural populations is similar to the plot of the orchard populations *(Fig. 2A).* In this case the southeast Louisiana source, which originates from just south of the southwest Mississippi source *(Fig. 1),* is in a transitional position. The one anomaly in the plot of the natural sources is the position of the Arkansas source, which plots closer to the Atlantic sources than the other western sources. This source was collected around 1991, and the affinity for the coastal sources may be an indication of pollen contamination from non-local Atlantic Coastal Plain sources which have been planted in great numbers in Arkansas (LAMBETH *et al.,* 1984).

The plot of the SSPSSS populations shows a more confused picture of geographic variation, perhaps because of the small sample sizes *(Fig. 2C, Table 1).* The first two canonical functions account for only 41% of the variation in the SSPSSS, compared to 65% and 66% for the first two functions in the orchard and bulk samples, respectively. The two most distant sources geographically, Texas and Maryland, are adjacent to one another in a plot of the first two functions, although they are well separated in the third and fourth functions (not shown). The Texas source was derived from only ten trees *(Table 1),* and appears out of place. There is a tendency for a separation of eastern and western sources, as well as some differentiation between coastal and interior sources in the eastern populations.

One interesting feature of *figure 2C* as well as *figure 2B* is the wide separation of the two Alabama sources. All of the Alabama sources originate from a relatively small part of the natural range, in northern Alabama *(Fig. 1).* Although more than 50 years separates the times of the original collections for the SSPSSS and the bulk samples, they both seem to show a great deal of variation on a relatively small geographic scale.

In spite of these differences, the populations do not differ obviously in allelic frequencies at most loci. The average frequency of the most common allele is 0.87 for all loci over all populations.

There did appear to be some differences in the occurrence of rare alleles among populations. Twenty of the rare alleles were detected only in the eastern populations, whereas only two were found exclusively in the western populations. Nearly all the alleles that are found in the western populations can be found in the eastern populations, but many of the alleles found in the eastern populations were not found in the western populations. Only one private allele was found, a third allele at the IDH-1 locus in the Florida bulk population.

Allele number 7 of enzyme 6PGD-1 is relatively common in many populations of loblolly pine east of the river, having a frequency as high as 0.29 in one population in Maryland *(Fig. 3).* This allele is very rare west of the river, and was not detected in most populations. The presence of this allele in the Arkansas natural bulk population is another indication of possible contamination with coastal Carolina sources. The allele was not detected in the SSPSSS collection from a nearby county *(Fig. 3).* The original SSPSSS collections were made in the early

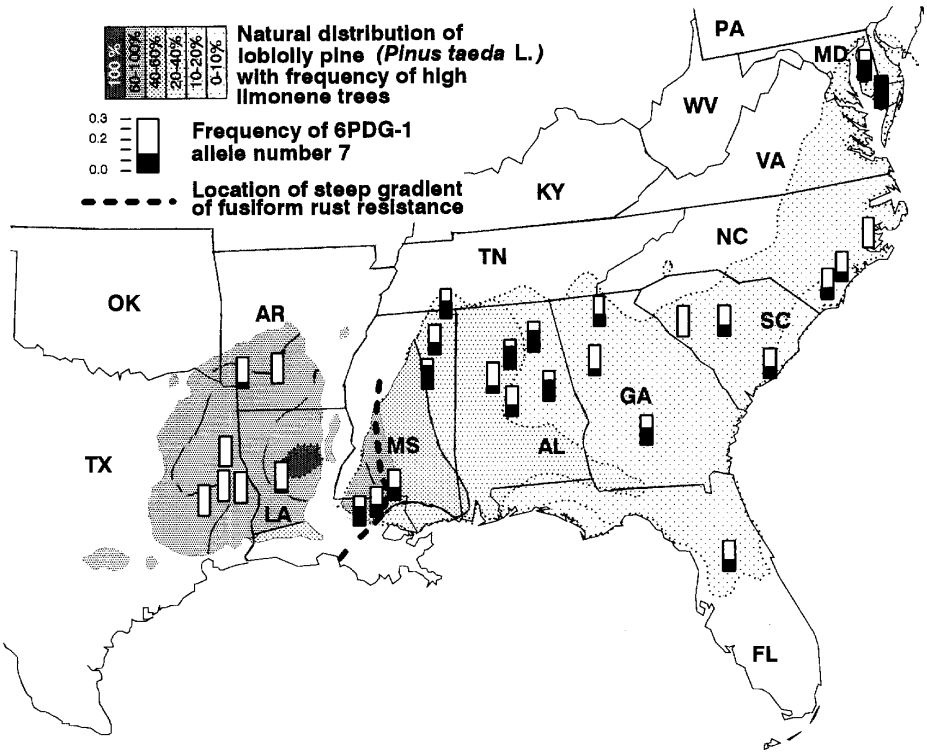

Figure 3. – Map of the southeastern United States showing the natural range of loblolly pine, the frequency of trees with high limonene (adapted from SQUILLACE and WELLS, 1981), and the frequency of locus number 7 of the enzyme 6PGD-1 in the sampled populations.

1950's, before these mass transfers began (LAMBETH *et al.,* 1984). The early results of the SSPSSS were, in fact, instrumental in bringing about these transfers (WELLS and WAKELEY, 1966). The Arkansas natural bulk collection was made from mature trees on the National Forest where such seed transfers have not occurred.

The distribution of 6PGD-1 allele number 7, as well as the distribution of the rare alleles suggests that gene flow in the westerly direction across the Mississippi River is restricted. The six populations sampled from the western edge of the eastern populations, *ie.,* western Tennessee, north Mississippi, southwest Mississippi and southeast Louisiana, all possess the seventh allele in appreciable proportions, with an average frequency around 0.10 to 0.15 *(Fig. 3).* In populations west of the river, the allele was found in only two of the seven populations at a frequency of less than 0.02.

Although the east-west differences in allozyme frequencies tend to be subtle, there are well-defined differences between eastern and western populations of loblolly pine in adaptive characters. Western sources are slower growing, have better survival, have greater resistance to fusiform rust *(Cronartium quercuum* (BERK.) MIYABE ex SHIRAI f.sp. *fusiforme*), and have greater concentrations of limonene in cortical gum than eastern sources (SQUILLACE and WELLS, 1981, *Fig. 3*). Perhaps as a result of differences in monoterpenes, western sources appear to be less susceptible than eastern sources to southern pine beetle (*Dendroctonus frontalis* ZIMM., POWERS *et al.,* 1992) and Nantucket pine tip moth (*Rhyacionia frustrana* COMSTOCK, SCHMIDTLING and NELSON, 1996). The area where SQUILLACE and Wells (1981) found that 100% of loblolly pine had high limonene concentrations in their cortical gum (central Louisiana – *Fig. 3*), corresponds closely to the only area in the southeastern USA identified as a „hazard area" for three insect pests; southern pine beetle, loblolly saw fly (*Neodiprion taedae linearus* ROSS), and Texas leaf-cutting ant (*Atta texana* BUCKL.) (USDA, 1969).

The isolating effect of the pineless Mississippi River Valley has been proposed as the reason for differences between eastern and western sources (WELLS and WAKELEY, 1966). The well-known resistance to fusiform rust of the western sources of loblolly pine is also present, however, in a seed source from east of the river, in Livingston Parish, Louisiana. The division between resistant western sources and susceptible eastern sources is well east of the river (WELLS *et al.,* 1991, *Fig. 3*). When geographic variation in limonene concentration and fusiform rust resistance are considered *(Fig. 3)* the division between western and eastern sources also appears to be east of the Mississippi River.

The continuous clinal variation in limonene content and fusiform rust resistance across the Mississippi River (SQUILLACE and WELLS, 1981; WELLS *et al.,* 1991, *Fig. 3*) suggests that there is no barrier to gene flow in the eastern direction across the valley. The allozyme data presented here, however, suggest that gene flow in the western direction is restricted. Prevailing winds since the beginning of the Holocene 14,000 years ago may be a factor in this predominately one-way gene flow.

Pleistocene Refugia

There was no obvious geographic trend in the amount of genetic variation in our data, unlike the situation found in longleaf pine. In a range-wide study of allozyme variation in longleaf pine (SCHMIDTLING and HIPKINS, 1998) a linear decrease from west to east was observed in the mean number of alleles per polymorphic loci (N_a), percent loci polymorphic (P_l), observed and expected heterozygosity (H_o and H_e) and number of rare alleles N_r. In a linear regression, longitude of the seed source explained 65% of the variation in H_e at 22 loci. This was taken as evidence that longleaf pine was located in a single refugium in south Texas or north Mexico and migrated northward and eastward since the last glacial maximum.

The current natural range of longleaf and loblolly pines is largely overlapping. Loblolly pine occurs in 13 southeastern states *(Fig. 1).* Longleaf pine is the more austral of the two species, occurring further south into peninsular Florida, but not occurring naturally in Oklahoma, Arkansas, Tennessee, Maryland and New Jersey (CRITCHFIELD and LITTLE, 1966). The two species are closely related. They sometimes hybridize naturally (CHAPMAN, 1922), and creating the artificial hybrid is not difficult if longleaf pine is the female parent (SNYDER and SQUILLACE, 1966).

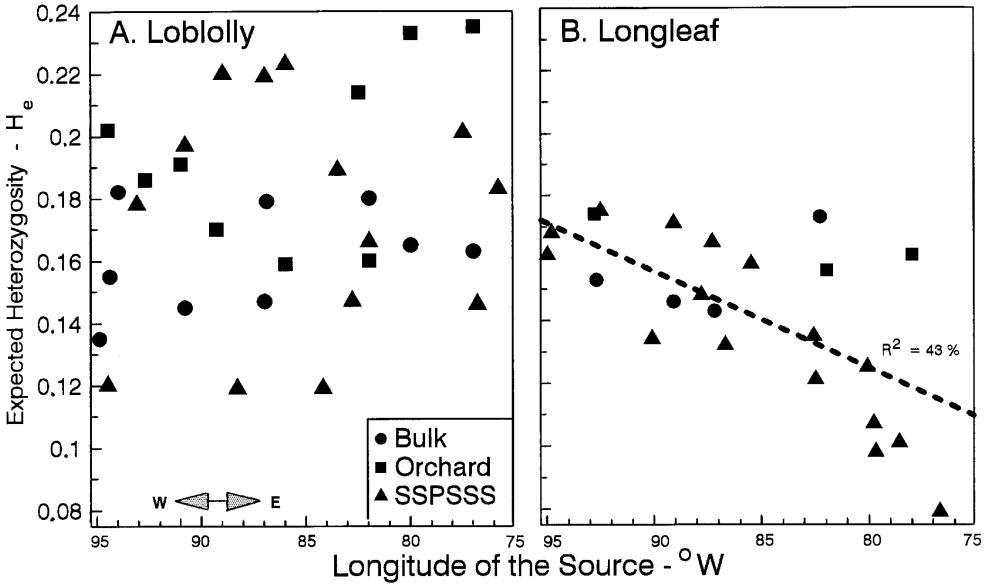

Figure 4. – Regression of expected heterozygosity on longitude of the seed source. A. Loblolly pine, and B. Longleaf pine. Longleaf data adapted from SCHMIDTLING and HIPKINS 1998. Based on the 11 loci common to both studies.

The pattern of east-west variation is different for the two species, however. A comparison of the east-west variation in H_e for the 11 loci that are common to the current study and the longleaf study (SCHMIDTLING and HIPKINS, 1998) shows clearly the trend in variation with longitude in longleaf pine *(Fig. 4B)* but not in loblolly pine *(Fig. 4A)*. This could be taken as negative evidence for a single refugium for loblolly pine during the Pleistocene, and positive evidence for two refugia, one in south Texas / northeast Mexico and the other in south Florida / Caribbean as proposed by WELLS *et al.* (1991). The dashed line east of the Mississippi, in southeast Louisiana and west Mississippi *(Fig. 3)* shows the location of a very steep gradient in fusiform rust resistance (WELLS *et al.,* 1991) in loblolly pine populations. There is no corresponding change in climate or soils (USDA, 1969) or in abundance of the oak *(Quercus sp.)* species (SQUILLACE and WILHITE, 1977) which are the alternate hosts of fusiform rust. The part of the natural range of loblolly pine having the highest rust infection is in south Georgia, where very little resistance is found in the natural populations. The gradient in fusiform rust resistance just east of the Mississippi River can best be explained by assuming the confluence of two populations. This, as well as the previously mentioned gradient in terpene concentration in the same location suggests the merging of two populations after the retreat of the Wisconsin glaciation.

There are some interesting similarities between longleaf pine and *western* sources of loblolly pine. Like western sources of loblolly pine, longleaf pine is resistant to fusiform rust and much less susceptible to tip moth and southern pine beetle (SNYDER *et al.,* 1977). This suggests that western loblolly populations and all longleaf populations shared an environment at some time in the past where selection for resistance to these pests was important. The proposal that longleaf pine and western sources of loblolly pine both originated in a common refugia in south Texas / northeast Mexico fits the circumstantial evidence. The present climate in south Texas is too dry for pines, but was probably much wetter during the Pleistocene (WATTS, 1983). Other pines occur just south of the border in Mexico, at high elevations (CRITCHFIELD and LITTLE, 1966).

Growth and Allozymes

Variation at allozyme loci is considered non-adaptive (ENDLER, 1986), but such variation may reflect variation at other loci. Therefore it may be useful to compare allozyme variation, especially heterozygosity, with the adaptive trait of height growth. The progeny test data for 5-year height from 235 of the orchard selections was compared to allozyme heterozygosity at 18 loci. Heterozygosity of the parent was not related to height growth of the progeny. The linear model (2) showed no relationship between heterozygosity and height (p=0.43). Age 5 may be too soon to show much effect, however, since the stress associated with competition would be minimal. It may also be important that these progeny are all from controlled pollinations among unrelated select trees. Although the gains in height growth are relatively modest in the R-8 tree improvement program, 5% to 10%, the select trees are higher in heterozygosity and are more diverse in general than the average tree *(Table 1)*. Although the progeny were not genotyped, it is safe to assume that controlled crosses among the unrelated select trees would result in much less inbreeding and greater than average heterozygosity and diversity in the progeny than in open-pollinated, woods run progeny. The populations of progeny measured, therefore, would represent a somewhat truncated population, and might not give an accurate measure of the relationship between heterozygosity and growth.

STRAUSS and LIBBY (1987) suggested that at greater levels of heterozygosity, rare alleles are a major contribution to this heterozygosity, and the less frequent alleles, or tightly linked loci, tended to have deleterious effects. Such an effect is apparent in our data if growth is compared to the presence of rare alleles *(Fig. 5)*. The presence of four rare alleles in a parent resulted in a reduction in height growth of nearly 10% in its progeny, compared to progeny of those with no rare alleles.

A direct effect of allozymes on fitness has been suggested on the basis of catalytic functions (MITTON and GRANT, 1984). If we look at allozymes simply as enzymes catalyzing (mainly) respiratory reactions, we can accept that some allozymes will have higher activity than others. A „dominance" model would assume that one copy of a gene coding for an enzyme with

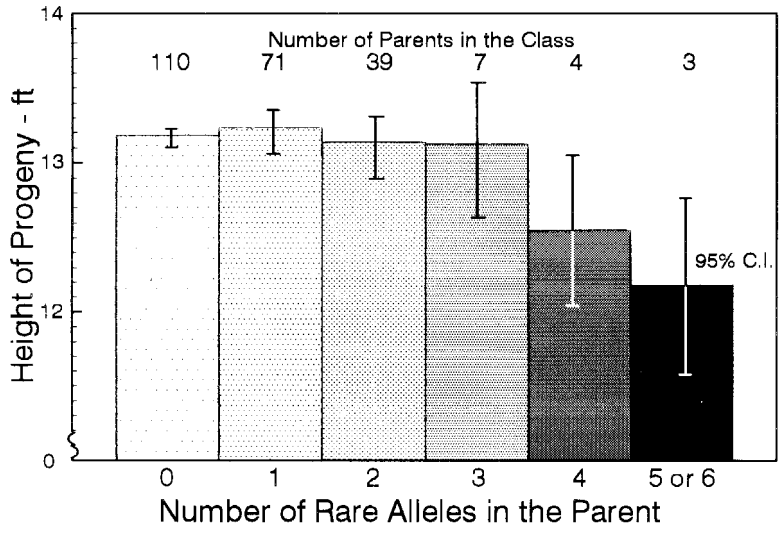

Figure 5. – Relationship of height growth with the presence of rare allozyme alleles in progeny test data from the orchard selections.

optimum activity would be sufficient for optimum catalysis. There is a possibility that „more is better“, or there may also be competitive effects between allozymes of differing efficiency.

Our linear models (3a and 3b) assumed that the „fitness“ of an allozyme, measured here as height, is related to its frequency in the population. The „dominance“ model (3b) did not show a significant relationship between height and allozyme frequency (p=0.34). The „additive“ model (3a), on the other hand, showed a highly significant relationship between allozymes and height (p=0.006). A step-wise regression, however, revealed that only one locus, IDH-1, was responsible for the statistical significance. Polymorphisms at this locus have previously been linked to hybridization with shortleaf pine (HUNEYCUTT and ASKEW, 1989). A comparison between our loblolly data and shortleaf allozyme data from another study (SCHMIDTLING *et al.,* 1996) confirmed that the probable origin of the rare allele at this locus is from shortleaf pine.

Shortleaf pine grows slower than loblolly pine, and the hybrid is intermediate in growth (SCHMITT, 1968). We would expect, therefore, that hybridization with shortleaf pine would result in slower growth. Six of our orchard clones possess the „shortleaf“ allele at the IDH-1 locus, three from Texas, one from Louisiana and two from north Mississippi. One of the Texas clones is homozygous for the shortleaf allele. Progeny from the clone homozygous for the shortleaf allele averaged 3.56 m, progeny from clones heterozygous for the shortleaf allele averaged 3.68 m, and those from clones homozygous for the loblolly allele averaged 4.02 m in height after 5 years. The differences are statistically significant ($p < 0.001$).

If this locus is indeed an indicator of hybridization between shortleaf and loblolly pines, than the converse relationship should obtain. That is, shortleaf possessing the „loblolly“ allele should grow faster than those not possessing the allele. Allozyme data and growth data from studies involving controlled crosses of 22 shortleaf clones from central Arkansas (SCHMIDTLING, 1996; SCHMIDTLING *et al.,* 1996) revealed just such a relationship. Four of the 22 clones were heterozygous for the loblolly allele, their progeny averaged 2.90 m, progeny from the other 18 clones averaged 2.82 m. The difference, though small, was statistically significant ($p = 0.023$).

Conclusions

1. Genetic parameters measured by allozymes indicate that loblolly pine is slightly more diverse than longleaf pine but comparable to other southern pines.

2. Selections from a loblolly pine tree improvement programs had greater average allozymic diversity and heterozygosity than natural populations, but the seed output from the orchards is very much like the natural populations in diversity.

3. The assumption that allozymes are selectively neutral may not be correct. Rare alleles are rare for a reason; trees with the alleles appear to be slightly less fit. Differences in fitness among the more common alleles, if they exist, were too subtile to be detected in our tests.

4. It is suggested that loblolly pine was located in two refugia during the Pleistocene, one in Texas / Mexico and one in Florida / Caribbean as proposed by WELLS *et al.* (1991).

5. Using the Mississippi River Valley as the dividing line between western and eastern populations is contraindicated by this study. There appears to have been regular gene flow across the southern end of the Valley, at least in the eastward direction.

6. There was some evidence that seed movement by forest managers is affecting the genetic makeup of natural populations.

7. The usefulness of using the IDH locus to indicate hybridization between loblolly and shortleaf pines is supported.

Literature Cited

ADAMS, W.T., NEALE, D.B., DOERKSEN, A.H. and SMITH, D.B.: Inheritance and linkage of isozyme variants from seed and vegetative bud tissue in coastal Douglas-fir [*Pseudotsuga menziesii* var. *menziesii* (MIRB.) FRANCO]. Silvae Genet. **39**: 153–167 (1990). — BAKER, F.S.: Principles of Silviculture. McGraw-Hill, New York (1950). — CHAPMAN, H.H.: A new hybrid pine *(Pinus palustris* x *Pinus taeda).* J. For. **20**: 729–734 (1922). — CONKLE, M.T., HODGKISS, P.D., NUNNALLY, L.B. and HUNTER, S.C.: Starch gel electrophoresis of conifer seeds: a laboratory manual. USDA For. Serv., Pacific Southwest For. Range Exp. Sta., Berkeley, CA, 18 p. (1982). — CRITCHFIELD, W.B. and LITTLE Jr., E.L.: Geographic distribution of the pines of the world. USDA For. Serv. Misc. Pub. 991. 97 p. (1966). — CROKER Jr., T.C.: Longleaf pine – myths and facts. In: Proceedings of a symposium on the management of longleaf pine. USDA For. Serv., South. For. Exp. Sta., Gen. Tech. Rep. SO-75. New Orleans, LA, p. 2–10 (1990). — EDWARDS, M.A. and HAMRICK, J.L.: Genetic variation in shortleaf pine, *Pinus echinata* MILL. (Pinaceae). For. Genet. **2**: 21–28 (1995). — ENDLER, J.A.: Natural selection in the wild. Princeton Univ. Press, Princeton, NJ (1986). — FLORENCE, L.Z. and RINK, G.: Geographic patterns of allozymic variation in loblolly pine. In: Proc. 15th South. For. Tree Improv. Conf., Mississippi State, MS. p. 33–41 (1979). — FRIEDMAN, S.T. and ADAMS, W.T.: Estimation of gene flow into two seed orchards of loblolly pine (*Pinus taeda* L.). Theor. Appl. Genet. **69**: 609–615 (1985). — HAMRICK, J.L., GODT, M.J.W. and SHERMAN-BROYLES, S.L.: Factors influencing levels of genetic diversity in woody plant species. New Forests **6**: 95–124 (1992). — HUNEYCUTT, M. and ASKEW, G.R.: Electrophoretic identification of loblolly pine-shortleaf pine hybrids. Silvae Genet. **38**: 95–96 (1989). — LAFARGE, T.: Applications of best linear prediction to the analysis of five full-sib loblolly pine progeny tests. In: Proc. 20th South. For. Tree Improv. Conf., Charleston, SC. p. 315–322 (1989). — LAMBETH, C.C., DOUGHERTY, P.M., GLADSTONE, W.T., MCCULLOUGH, R.B. and WELLS, O.O.: Large-scale planting of North Carolina loblolly pine in Arkansas and Oklahoma: A case of gain versus risk. J. Forestry **82**: 736–741 (1984). — LEDIG, F.T.: Heterozygosity, heterosis, and fitness in outbreeding plants. In: M.E. SOULE, ed.. Conservation biology: The science of scarcity and diversity. Sinauer Associates, Sunderland, MA. p. 77–104 (1986). — MITTON, J.B. and GRANT, M.C.: Associations among protein heterozygosity, growth rate, and developmental homeostasis. Annu. Rev. Ecol. Syst. **15**: 479–499 (1984). — NEI, M.: F-statistics and analysis of gene diversity in subdivided populations. Ann. Human Genet. **41**: 225–233 (1977). — POWERS Jr., H.R., BELANGER, R.P., PEPPER, W.D. and HASTINGS, F.L.: Loblolly pine seed sources differ in susceptibility to the southern pine beetle in South Carolina. South. J. Appl. For. **16**: 169–174 (1992). — SAS Institute, Inc.: SAS User's Guide: Statistics. SAS Institute Inc., Cary, NC. 956 p. (1985). — SCHMIDTLING, R.C.: Reproductive environment affects growth of shortleaf pine. Proc. Tree Improv. Sustainable Tropical Forestry – QFRI – IUFRO Conference, Caloundra, Queensland, Australia, Oct. 1996. p. 261–264 (1996). — SCHMIDTLING, R.C. and HIPKINS, V.: Genetic diversity in longleaf pine (*Pinus palustris* MILL.): Influence of historical and prehistorical events. Can. J. For. Res. **28**: 1135–1145 (1998). — SCHMIDTLING, R.C., HIPKINS, V. and CARROLL, E.: After effects of reproductive environment in shortleaf pine (*Pinus echinata* MILL). Proc. IUFRO Diversity and adaptation in forest ecosystems in a changing world. Aug. 1996. UBC, Vancouver, BC, Canada. p. 54 (1996). — SCHMIDTLING, R.C. and NELSON, C.D.: Interprovenance crosses in loblolly pine using selected parents. For. Genet. **3**: 53–66 (1996). — SCHMITT, D.: Performance of southern pine hybrids in south Mississippi. USDA For. Serv., South. For. Exp. Sta., Res. Paper SO-36. 15 p. (1968). — SMOUSE, P.E. and WILLIAMS, R.C.: Multivariate analysis of HLA-disease associations. Biometrics **38**: 757–768 (1982). — SNYDER, E.B., DINUS, R.J. and DERR, H.J.: Genetics of longleaf pine. USDA For. Serv. Res. Paper WO-33, 24 p. (1977). — SNYDER, E.B. and SQUILLACE, A.E.: Cone and seed yields from controlled breeding of southern pines. USDA For. Serv., South. For. Exp. Sta., Res. Paper SO-22. 7 p. (1966). — SQUILLACE, A.E. and WELLS, O.O.: Geographic variation of monoterpenes in cortical oleoresin of loblolly pine. Silvae Genet. **30**: 127–135 (1981). — SQUILLACE, A.E. and WILHITE, L.P.: Influence of oak abundance and distribution on fusiform rust. In: DINUS, R.J. and SCHMIDT, R.A.: Proc. Symp. Manag. Fusiform Rust in Southern Pines. Univ. FL, Gainesville, p. 59–70 (1977). — STRAUSS, S.H. and LIBBY, W.J.: Allozyme heterosis in radiata pine is poorly explained by overdominance. Amer. Nat. **130**: 879–890 (1987). — SWOFFORD, D.L. and SELANDER, R.B.: BIOSYS-1, a computer program for the analysis of allelic variation in population genetics and biochemical systematics. Release 1.7. IL Nat. Hist. Surv., Champaign, IL. (1989). — USDA: A Forest Atlas of the South. USDA For. Serv., South. For. Exp. Stat., 27 p. (1969). — WATTS, W.A.: A vegetational history of the eastern United States 25,000 to 10,000 years ago. In: S.C.

PORTER (ed.). The late Pleistocene. p. 294–310. Vol. I. Late-Quaternary environments of the United States. University of Minnesota Press, Minneapolis (1983). — WELLS, O.O.: Results of the southwide pine seed source study through 1968–69. In: Proc. 10th South. For. Tree Improv. Conf., Houston, TX. p. 117–129 (1969). — WELLS, O.O.: Use of Livingston Parish, Louisiana loblolly pine by forest products industries in the Southeast. South. J. Appl. For. **9**: 180–185 (1985). — WELLS, O.O., SWITZER, G.L. and SCHMIDTLING, R.C.: Geographic variation in Mississippi loblolly pine and sweetgum. Silvae Genet. **40**: 105–118 (1991). — WELLS, O.O. and WAKELEY, P.C.: Geographic variation in survival, growth and fusiform rust infection of planted loblolly pine. For. Sci. Monograph **11**, 40 p. (1966). — WHITE, T.L. and HODGE, G.R.: Predicting breeding values with applications in forest tree improvement. Kluwer Academic Publishers, Dordrecht, Netherlands. 367 p. (1989). — WRIGHT, S.: Evolution in MENDELian populations. Genetics **16**: 97–159 (1931). — WRIGHT, S.: The interpretation of population structure by F-statistics with special regard to systems of mating. Evolution **19**: 395–420 (1965). — WRIGHT, S.: Evolution and the Genetics of Populations. Vol. 4. Variability Within and Among Natural Populations. Univ. Chicago Press, Chicago (1978).

Clonal Multiplication of *Syzygium alternifolium* (WIGHT.) WALP., Through Mature Nodal Segments

By P. S. SHA VALLI KHAN[1)2)], J. F. HAUSMAN[1)3)] and K. R. RAO[2)]

(Received 18th November 1998)

Abstract

Clonal multiplication of *Syzygium alternifolium* (WIGHT.) WALP., (Myrtaceae), a rare medicinal tree was achieved through mature nodal segments on modified MURASHIGE and SKOOG's (MS) medium. Shoot initiation was dependent on cytokinin supply, but the synergistic combination of 4.0 mg/l N^6 benzyladenine and 0.5 mg/l 1-naphthaleneacetic acid induced the highest percentage of nodal segments sprouting (69.5 ± 2.9%), number of shoots per node (3.9 ± 0.1), shoot length (2.9 ± 0.2 cm), the number of new nodal segments generated per active explant (2.2 ± 0.1) and the multiplication co-efficient (1.5) within 6 weeks. Explants harvest period influenced the shoot initiation in nodal segments. Repeated subculturing through five passages of nodes of shoot cultures enabled continuous production of healthy shoots. At the end of the 5th passage, 72% to 73% of nodal segments produced 8 to 9 shoots, each having 4 cm height and 3 to 4 nodes. Multiplication co-efficient was also increased from the 1st subculture (1.5) to the 5th subculture (2.3). Rooting involved a two step method: root initiation on solidified half-strength MS medium supplemented with 1.0 mg/l indole-3-butyric acid (IBA), and root elongation following transfer to half-strength MS medium devoid of growth regulators. Repeating this two step sequence yielded up to 70% of rooted shoots. Shoots derived from subcultures exhibited better rooting response than those from cultures of shoot initiation. About 70% of the rooted plants were established in 20 cm diameter pots containing a mixture (1:1) of soil and sand after 3 weeks of hardening.

Key words: in vitro, medicinal plant, micropropagation, Myrtaceae, nodal segments, *Syzygium alternifolium.*

FDC: 165.441; 161.4; 232.328.1; 176.1 *Syzygium alternifolium;* (540).

Abbreviations: BA: N^6 benzyladenine; KN: kinetin; IAA: indole-3-acetic acid; NAA: 1-naphthaleneacetic acid; MS: MURASHIGE and SKOOG's medium (1962); IBA: indole-3-butyric acid; PAR: Photosynthetically Active Radiation; RI: root initiation; RE: root elongation.

[1)] CRP-Centre Universitaire, CREBS, 162A, Av. de la Faïencerie, L-1511 Luxembourg, Grand-Duchy of Luxembourg

[2)] Plant tissue culture lab, Department of Botany, Sri Venkateswara University, Tirupati-517502, A.P., India

[3)] Author for correspondence

Introduction

Syzygium alternifolium (WIGHT.) WALP., (Myrtaceae), popularly known as 'Mogi', is a rare fruit tree of great medicinal and economical importance. It's distribution is restricted to certain forested areas in the Southern part of India (MADHAVACHETTY and RAO, 1990). This species yields nutritious, edible fruits and durable timber. The ripe fruits are also used in making squashes and jellies. Timber is used for making furniture and agricultural implements (The Wealth of India, 1976). Blossoms yield honey and possess antibiotic properties. An alcoholic extract of seeds posses antidiabetic activity and tender shoots have been traditionally used by villagers and tribal folks to treat bacillary dysentery (RAJA REDDY *et al.,* 1989; NAGARAJU and RAO, 1990).

S. alternifolium is out breeder and routinely propagated by seeds. As seed progenies are not uniform, no standard varieties are available. Seed production is sporadic, the seeds cannot be stored for long periods due to short viability and insect attack. Vegetative propagation has not been successful. To date only one report has been published on micropropagation of *S. alternifolium* using seed-derived explants (SHA VALLI KHAN *et al.,* 1997). Considerable progress has been made for the *in vitro* micropropagation of other species of the genus *Syzygium* like *S. aromaticum* (MATHEW and HARIHARAN, 1990) and *S.cuminii* (YADAV et al., 1990) using seed-derived explants. The use of mature explants was fraught with difficulties like dark-brown phenolic-exudation and severe contamination (YADAV *et al.,* 1990). The present study was undertaken to establish a method for clonal multiplication of *S. alternifolium* using mature nodal segments.

Materials and Methods

Plant material

A 10-year-old tree *(S. alternifolium)* with a large trunk (~ 40 cm diameter), well-spread canopy and regular flowering and fruiting characteristics, growing in the protected forest area of Tirumala hills of Chittoor district, India served as the source for explants. Stem cuttings with the youngest four to