Variation in needle traits in provenance tests of Pinus taeda and P. echinata

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Introduction

Loblolly pine (Pinus taeda L.) and shortleaf pine (P. echinata Mill.) are economically important species that occur in the Southeastern United States. They are sympatric over much of their ranges (*Fig.* 1) and thus are subjected to many of the same selection pressures exerted by physiography and climate. Provenance tests have shown that loblolly pines originating near the Atlantic and Gulf Coasts tend to grow faster, be more prone to ice breakage, and do not survive planting as well as those of more northern and continental origin (Wells and Wakeley 1966, Jones and Wells 1969, Kraus 1967, North Carolina State University 1971). In addition, differences in fruiting habit in loblolly pine were pointed out by Wakeley (1947) and Brender (1958), both of whom observed that seed yields were heavier and more consistent near the coast than inland. Shortleaf pine from the northern and southern extremities of the species range also performs very differently in provenance tests (Wells and Wakeley 1970).

In addition to coastal versus continental variation, populations of both species from west of the Mississippi River differ in several traits from those of more eastern origin. Compared to eastern loblolly, western loblolly from the same latitude is more rust resistant (Henry 1959), survives planting better, grows slower (Wells and Wakeley 1966), and has thinner seed coats and fewer stomata per unit area (Thorbjornsen 1961). Western loblolly contains certain proteins which are also found in shortleaf pine but not in eastern loblolly (Hare and Switzer 1969). Compared to eastern shortleaf pine, western shortleaf pine begins growth and sheds pollen earier in the spring (Wells 1973, Schmidtling 1971).

Some of the east-west difference in both species seem due to the selection pressures exerted by the drier western environment and the reproductive isolation barrier afforded by the Mississippi River. Most of them are manifested only as slight divergences from the eastern populations, but variation in others, particularly seed protein composition and rust resistance in loblolly is very large or discrete. The differences in seed protein composition are qualitative, *i.e.*, certain electrophoretic bands found in shortleaf and western loblolly do not appear in eastern loblolly.

The variation in rust resistance in loblolly is so striking that, on the basis of it alone, western loblolly appears intermediate between eastern loblolly and shortleaf. Introgression seems the most plausible mechanism for the transfer of discrete or substantive amounts of variation from one species to another, but intermediacy in some traits and lack

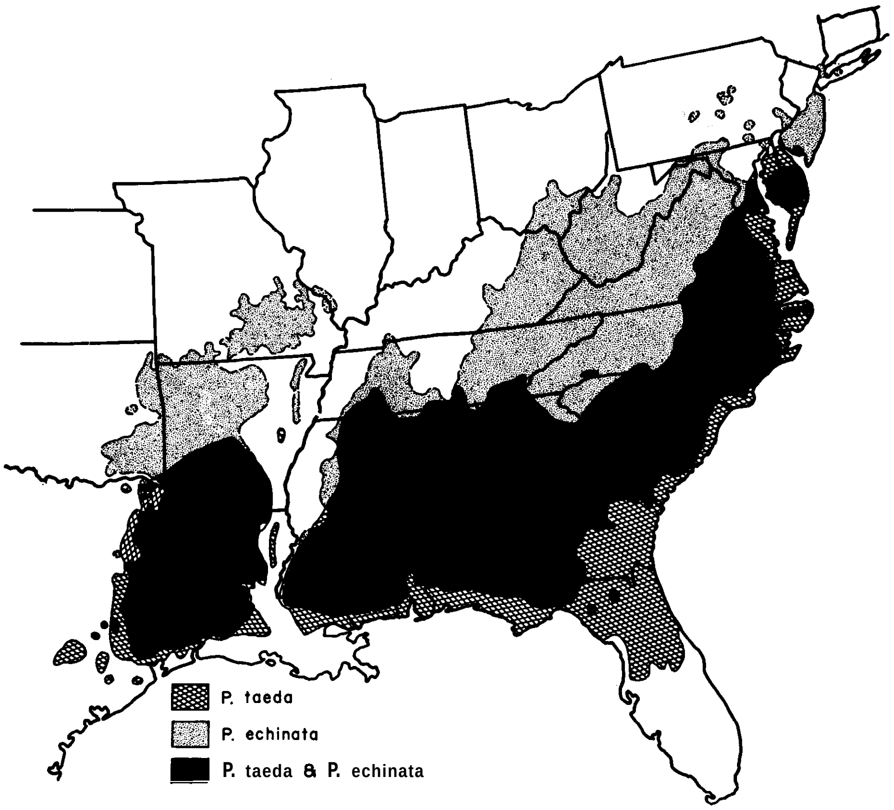

Figure 1. — Distribution of P. *taeda* and *P. echinata.*

of it in others leaves the question of introgression unsettled. Indeed, the subject has been controversial since Zobel's original observations (1953) that hybrids may be common west of the Mississippi River to the recent conclusions of Cotton *et al.* (1975) that true intermediates are rare in east Texas.

The present experiment is an attempt to further elucidate the genecology of the species and their environments by means of foliage measurements made on trees growing in several provenance plantings of shortleaf and loblolly. If introgressants are present in the population, they should manifest themselves as intermediates when the two species are grown together in provenance tests.

Materials and Methods

Material

The four provenance plantings in southern Mississippi and Louisiana which were sampled are part of the Southwide Pine Seed Source Study (Wakeley 1961). The parent trees had been selected in natural stands, and seed from at least 20 trees from a single geographic location was mixed together. A total of 37 geographic locations (provenances) was sampled: 23 of *P. echinata* and 14 of *P. taeda* (*Fig. 2*). Parent trees were selected in 1951 by many people working independently throughout the Southeastern United States. The study was organized by species; so very likely there was some selection for specimens typical of each species and against intermediates or extremes. Comparisons between species were not anticipated when the study was designed, but the plantings at Millard and Derby, Mississippi, were fortuitously arranged so that statistically valid comparisons between species could be made. We organized the material into three experiments on the basis of commonality of provenances, age and planting location (*Table 1*).

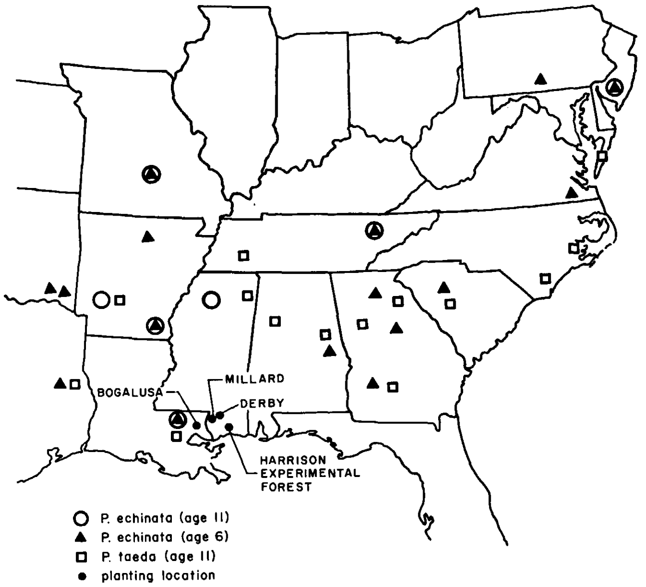

Figure 2. — Areas where seed was collected in 1951 and 1955 and locations where it was grown and where needle samples were collected.

Experiment I is represented by the plantings located at Millard and Derby, Mississippi. Both plantings are identical with respect to design (two randomized complete blocks), experimental material (14 rangewide *P. taeda* provenances and 7 rangewide *P. echinata* provenances), and year of establishment (1953). The soil is a fine sandy loam, low in organic matter and infertile. It originally supported a pure stand of longleaf pine (*P. palustris* Mill).

Experiment II is represented by the Bogalusa, Louisiana, planting, which has four randomized complete blocks and contains the same 14 *P. taeda* provenances that are in Experiment I. The planting was also established in 1953. The soil is also a fine sandy loam, but is much more fertile than Millard-Derby. The area had once been a tung (*Aleurites* spp.) orchard and had been fertilized.

Experiment III is represented by the Harrison Experimental Forest (HEF) planting, which has four randomized complete blocks and contains 16 rangewide *P. echinata* provenances from different seed collections than those in Experiment I. The planting was established in 1955 on a *P. palustris* site similar to that at Millard-Derby.

Measurements

Foliage samples were collected in May 1964, when the HEF planting was 6 years old and all other plantings were 11 years old. A healthy lateral branch was cut from the mid-crown of five randomly selected trees of each plot in each plantation. Three mature fascicles were removed from each branch and preserved in FAA in a 5 : 5 : 90 ratio by volume.

The following traits were studied:

1. Needle length — measured directly on the longest needle in each fascicle with a cm scale.
2. Needle width — measured with a stage micrometer graduated to 0.1 mm at 35X.
3. Number of serrations — counted along one side of a 5-mm section at 35X.
4. Number of rows of stomates — counted on the outer surface of each needle at 35X.
5. Number of stomates per row — counted in the two outer rows on each side of a 5-mm section at 35X. Incomplete rows were rejected, and the next interior complete row was utilized.
6. Number of resin canals — determined by direct count on microtome sections cut at 10 μ and stained with Safranin and Fast Green.

All measurements were made on 60 needles (15 needles from each of four plots) of each provenance in each experiment. The mean of 15 needles for each plot is the basic unit of analysis in this study.

Table 1. — Experiments, plantation locations, and provenance groups included in each.

Provenance group	Age when sampled	Represented in: Experiment 1 at Millard	Experiment 1 at Derby	Experiment 2 at Bogalusa	Experiment 3 at Harrison Exp. For
Rangewide *P. taeda*	11	x	x	x	
Rangewide *P. echinata*	11	x	x		
Rangwide *P. echinata*	6				x

Statistical Analysis

Experiment I: The 21 provenances represented in Experiment I include 14 rangewide *P. taeda* provenances and 7 rangewide *P. echinata* provenances. Instead of a single analysis with a partitioning of the 20 degrees of freedom (df) between provenances into 1 df for species effect and 19 df for provenance-within-species effect, we elected to perform two separate analyses based on the following subsets of the provenances:

Subset 1: 14 rangewide *P. taeda* provenances
Subset 2: 14 rangewide *P. taeda* provenances plus the 7 rangewide *P. echinata* provenances

In the analysis of subset 2, we chose not to contrast species but rather to contrast provenances regardless of their species. In this way, provenances were allowed to cluster on the basis of their morphological characteristics rather than on the basis of preconceived species designations.

The data is distinctly multivariate — consisting of mean values for six needle traits for each experimental plot. A common approach to the statistical analysis of such data is to apply univariate statistical methods to each variable in turn. This approach ignores the information contained in the covariances among the traits and concentrates on the variance for each trait. But, in the data at hand, there are many more covariances (15) than variances (6), and we chose not to ignore the information they contain.

Therefore, we applied standard multivariate procedures (multivariate analysis of variance and discriminant function analysis) which utilize the covariances and variances alike (Dempster 1969, Seal 1964, Morrison 1967).

The multivariate model hypothesized for Experiment I is:

$$Y_{ijkc} = \mu_c + \delta_{ic} + \beta_{i(j)c} + \varrho_{kc} + (\delta\varrho)_{ikc} + \varepsilon_{ijkc}$$

where: μ_c = mean of the c^{th} character measured
δ_{ic} = location effect for c^{th} character
$\beta_{i(j)c}$ = effect on j^{th} block within i^{th} location for the c^{th} character
ϱ_{kc} = effect of k^{th} provenance for c^{th} character
$(\delta\varrho)_{ikc}$ = interaction of i^{th} location with k^{th} provenance for the c^{th} character
ε_{ijkc} = error associated with ijk^{th} plot for the c^{th} character

and

$i = 1,2$; $j = 1,2$; $k = 1,14$ for subset 1; $k = 1,22$ for subset 2; and $c = 1,6$

and $\{\varepsilon_{ijkc}\} \sim MVN(O,\Sigma)$.

All components of the model are random with the exception of μ_c and ϱ.

Following the procedures (and notation) of Smith *et al.* (1962), the multivariate analysis of variance for this model is:

Source	Degrees of freedom Subset 1	Degrees of freedom Subset 2	Matrix of sums of squares and cross products
Total (T)	55	83	Y'Y
Planting location (L)	1	1	$S_{H0\delta}$
Blocks/location (B)	2	2	S_{E_1}
Provenances (P)	13	20	$S_{H0\rho}$
Provenance × Location (PL)	13	20	$S_{H0\delta\rho}$
Error	26	10	S_{E_2}

The hypotheses of interest are:

$H_0\delta\varrho$: Planting location by provenance interaction is zero over all responses

$H_0\varrho$: Provenance effect is zero over all responses. These were tested using the likelihood ratio test criterion developed by Wilks (1932):

$$\text{for } H_0\delta\rho\text{:}\quad |S_{E_2}|/|S_{H0\delta\rho} + S_{E_2}| = \Delta_{H0\delta\rho}$$

$$\text{for } H_0\rho\quad |S_{E_2}|/|S_{H0\rho} + S_{E_2}| = \Delta_{H0\rho}$$

In these test criteria, a function of Δ, say f (Δ), is given by Bartlett (1947) as:

$$f(\Delta) = -2.3026\ (N - 1 - (p + k)/2 \log \Delta$$

where

N = total number of plot means in sample
K = number of planting location × provenance classes in sample
P = number of characters measured

and f (Δ) is distributed approximately as chi-square with P degrees of freedom and coefficient equal to (K — 1).

If the null hypothesis $H_0\delta\varrho$ is rejected, it is necessary to compute the orthogonal linear discriminant functions which best separate the interaction class means. These functions correspond to the eigenvectors of the determinantal equation

$$|S_{H_0\delta\rho} - \lambda S_{E_2}| = 0.$$

The hypothesis of whether all the successive roots after the m^{th}, for example, are zero may be tested according to the following statistic (Bartlett 1947):

$$Z = (N - 1 - (p + K)/2) \ln \left(\prod_{j=m+1}^{M} (1 + \lambda_j) \right)$$

where

$$Z \cong \chi^2\ (p - m) \times (K - m - 1)$$

and

M is the rank of $S_{H0}\delta\varrho$. λ_j is the j^{th} root of the determinantal equation.

If $H_0\delta\varrho$ is not rejected and $H_0\varrho$ is, then the discriminant functions based on the determinantal equation

$$|S_{H0\rho} - \lambda E_2| = 0$$

are applicable, with the same test statistics applied as before.

Experiment II and III: The model hypothesized for Experiments II and III is:

$$Y_{jk(c)} = \mu_c + \beta_{jc} \pm \varrho_{kc} + \varepsilon_{jkc}$$

where:

μ_c = mean of the c^{th} character measured
β_{jc} = j^{th} block effect for the c^{th} character, j = 4 for both experiments
ϱ_{kc} = k^{th} provenance effect for the c^{th} character, with k = 14 for Experiment II and k = 16 for Experiment III

and $\{\varepsilon_{jk}\} \sim MVN(O,\Sigma)$.

ϱ is considered fixed, whereas β and ε are random effects. The analysis of variance table for this model is:

Source	Degrees of freedom Exp. II	Exp. III	Matrix of sums of squares and cross products
Total (T)	55	63	
Blocks (B)	3	3	$S_{H0\beta}$
Provenances (P)	13	15	$S_{H0\rho}$
Error (E)	39	45	S_{E_1}

where the hypothesis of interest and corrseponding test statistic are:

$H_{0\varrho}$: Provenance effect is zero for all characters

$\Delta_\rho = |S_{E_1}| / |S_{E_1} + S_{H0\rho}|$.

The appropriate test statistic and its approximate distribution are given in the preceding section. Also, discriminant functions based on the determinal equation

$|S_{H0\rho} - \lambda S_{E_1}| = 0$

are computed if the null hypothesis $H_{0\varrho}$ is rejected. A test of the hypothesis that the last M-m roots are zero is also described in the preceding section. The 0.05 level of significance was used in all tests.

Results and Discussion

When data from the rangewide collections of *P. taeda* and *P. echinata* at Millard-Derby (Experiment I, Subset 2) were analyzed, it was evident that the provenance × planting location interaction was small, the provenance effect was strong, and the between-species variation was independent of the within-species variation. The two species were widely separated on the first discriminate axis *(Fig. 3)*. As expected, the two species separate strongly on the basis of needle length, but all traits except ratio of needle length to needle width contributed substantially to the discrimination.

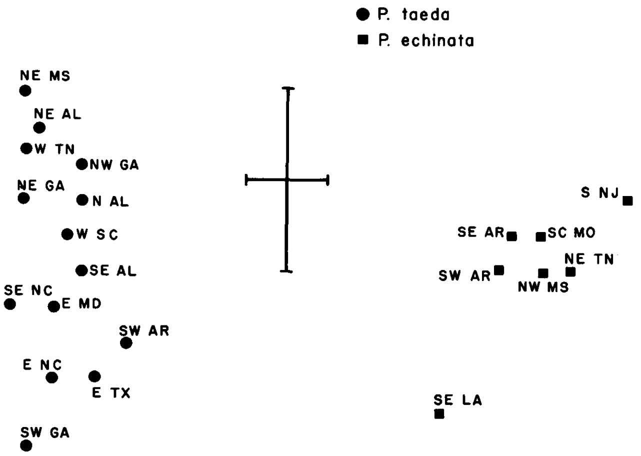

Figure 3. — Discriminate analysis of Millard-Derby rangewide *P. taeda* and rangewide *P. echinata* samples. Horizontal bar of cross indicates least significant difference on first discriminate axis; vertical bar, on second discriminate axis.

Most of the within-species variation in both species was on the second discriminate axis, independent of the first, except for the New Jersey and southeastern Louisiana *P. echinata* samples which differed from one another on both axes.

Loblolly Pine

The *P. taeda* population varied in a clinal geographic pattern which is basically a contrast of coastal and continental populations *(Fig. 3)*. It is noteworthy that samples originating in the continental portions of *P. taeda's* range (northwestern Mississippi, Georgia, and Alabama) have a lower ratio of needle length to needle width (are stubbier) than those originating near the Atlantic or Gulf Coast. The two samples from west of the Mississippi River plot in the coastal part of the array are nearer shortleaf on the first axis than the other *P. taeda* samples, although neither is significantly different from the average of the *P. taeda* population.

When the rangewide Millard-Derby *P. taeda* data (Subset 1) were analyzed by themselves *(Fig. 4)*, the two western samples plotted very close together and showed a tendency, although again nonsignificant, to diverge from the rest of the *P. taeda* population. Their most obvious divergent feature is their smaller numbers of stomata per row.

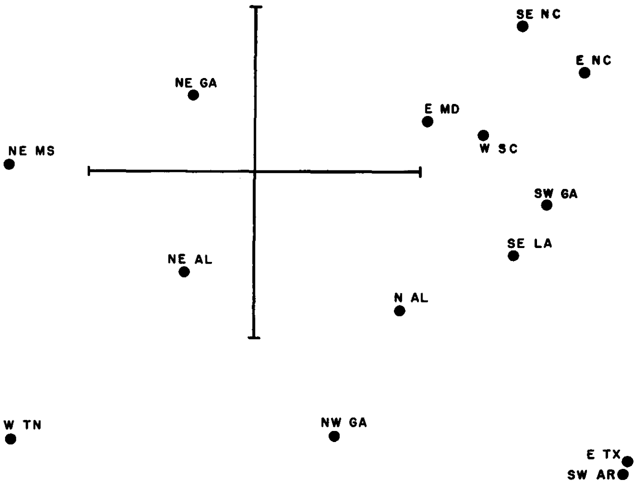

Figure 4. — Discriminate analysis of Millard-Derby rangewide *P. taeda*. Horizontal bar of cross indicates least significant difference on first discriminate axis: vertical bar, on second discriminate axis.

THORBJORNSEN (1961) sampled cones, seed, and needles of *P.. taeda* "in place". i.e., not grown in a common environment as in the present experiment, and also found that populations west of the Mississippi River had fewer stomates than eastern ones. This variation could easily be attributed to selection pressure exerted by the drier western environment except that unlike *P. taeda, P. echinata* from west of the Mississippi River does not have fewer stomates per row than eastern *P. echinata* ((*Table 2*). If decreased stomatal frequency resulted from natural selection for drought resistance, one would except similar trends in both species as they are subject to similar environmental stress.

Also evident in *Figure 4* as in *Figure 3* is the clinal separation of coast and continental *P. taeda*. When data from the same *P. taeda* material grown near Bogalusa, Louisiana (Experiment 2), were analyzed, there was less significant variation in all traits both in the univariate and multivariate cases. The general loss of statistical significance was due to much larger block × treatment interaction (er-

Table 2. — Measurements of six needle characteristics of *P. taeda* and *P. echinata* pines grown in provenance tests in southern Louisiana and Mississippi.

Provenance	Needle characteristics					
	Length (cm)	Length/width	Serrations per cm	Rows of stomates	Stomates per row	Resin canals
Harrison Experimental Forest rangewide shortleaf planting at age 6 years						
Southern NJ, C-453	7.75	65.18	26.75	12.87	57.20	2.53
South-central PA., C-451	6.05	52.28	29.58	12.42	60.68	2.65
Southeastern VA., C-455	7.93	69.11	26.13	12.10	58.08	3.12
Northeastern TN., C-487	7.29	64.95	27.68	12.17	58.61	2.48
Western SC, C-457	8.69	77.58	25.82	12.15	58.33	2.57
Northern GA., C-463	8.24	74.71	25.63	11.37	57.23	2.20
Northern GA., C-461	8.70	74.39	25.47	12.82	58.92	2.78
Southwestern GA., C-465	9.24	82.66	26.88	11.73	59.87	2.43
East-central AL., C-467	8.49	75.10	26.92	12.13	59.03	2.23
Southeastern LA., C-473	8.80	83.62	25.93	11.20	59.23	2.42
Eastern TX., C-475	8.09	74.62	24.42	11.80	58.00	2.35
Southeastern OK., C- 477A	8.87	83.16	24.77	11.48	59.31	2.15
Southeastern AR., C-481	8.76	76.95	25.17	12.25	60.24	2.32
Southeastern OK., C-477B	8.15	77.44	26.42	11.80	60.62	2.45
Northern AR., C-483	8.77	74.54	25.60	13.57	59.28	2.88
South-central MO., C-485	8.24	69.23	27.08	12.30	61.23	2.47
Millard-Derby rangewide shortleaf planting at age 11 years						
Southern NJ, C-403	7.50	65.99	25.10	11.83	56.61	4.15
Northeastern TN., C-435	7.96	66.11	25.25	12.58	58.01	3.40
Northwestern MS., C-419	8.47	73.56	23.47	11.82	58.14	3.55
Southeastern LA., C-421	8.93	84.02	24.27	11.07	59.91	2.88
Southeastern AR., C-429	8.69	80.40	24.68	11.52	63.74	3.88
Southwestern AR., C-427	8.97	78.52	23.97	11.65	60.09	3.53
South-central MO., C-433	8.16	68.10	26.50	12.98	61.71	3.35
Millard-Derby rangewide loblolly planting at age 11 years						
Eastern MD., C-301	14.86	113.64	26.67	11.50	66.97	2.38
Southeastern NC, C-303	16.08	123.05	24.83	10.63	65.67	2.73
Eastern NC, C-305	14.57	117.08	25.62	10.82	66.49	2.28
Western SC, C-307	15.01	114.86	26.00	10.97	66.72	2.75
Southwestern GA., C-309	15.60	120.31	24.50	10.85	62.79	2.12
Northeastern GA., C-311	15.19	114.12	27.77	10.90	68.20	2.53
Northwestern GA., C-331	14.94	106.84	25.22	11.23	64.04	2.58
Northeastern AL., C-317	16.14	110.19	26.50	12.12	65.75	2.88
Northern AL., C-319	14.76	108.71	25.33	11.25	65.40	2.52
Northeastern MS., C-321	15.32	108.15	28.98	11.83	69.15	2.63
Southeastern LA., C-323	14.12	111.03	25.43	10.48	66.02	2.45
Eastern TX., C-325	15.05	112.65	25.28	11.70	61.17	2.65
Southwestern AR., C-327	14.74	109.16	25.42	12.02	62.04	2.70
Western TN., C-329	14.97	109.09	28.48	10.55	64.73	2.40
Bogalusa rangewide loblolly planting at age 11 years						
Eastern MD., C-301	13.11	111.97	27.20	10.38	67.93	2.47
Southeastern NC, C-303	15.10	130.91	26.52	10.18	66.85	2.78
Eastern NC, C-305	14.21	123.58	27.98	10.20	65.21	2.45
Western SC, C-307	15.16	123.36	27.88	10.37	69.17	3.20
Southwestern GA., C-309	14.52	124.71	28.93	9.37	66.02	2.67
Northeastern GA., C-311	14.17	121.20	30.83	10.18	69.91	3.15
Northwestern GA., C-331	15.13	127.20	27.82	10.35	66.52	3.13
Northeastern AL., C-317	15.41	125.06	28.37	10.55	66.73	2.88
Northern AL., C-319	13.25	112.96	27.52	10.23	67.18	2.55
Northeastern MS., C-321	14.02	117.67	30.35	11.12	68.98	2.70
Southeastern LA., C-323	15.92	138.69	29.32	10.00	67.68	2.63
Eastern TX., C-325	15.67	128.85	28.65	11.82	64.95	2.80
Southwestern AR., C-327	14.38	125.79	28.32	10.08	65.48	2.93
Western TN., C-329	13.68	119.36	30.43	9.57	68.06	2.70

ror term) in all traits in Experiment 2. This interaction precluded a meaningful combination of data from the rangewide *P. taeda* in Experiments 1 and 2 in a single analysis.

The Experiment 2 site in Louisiana is much more fertile and uniform than the Experiment 1 site at Millard-Derby. The average height of the Experiment 2 planting was 31.8 feet at age 10, 14.3 feet taller than Experiment 1 at the same age. Evidently, differences in needle traits among samples are expressed better on the poorer site. Genetic potential seems to be expressed only under stress. Similar results have been recorted (Mergen *et al.* 1965, Knauf and Bilan 1974).

Shortleaf pine

In *P. echinata* the major difference detected by the present analyses is between populations of extreme northern and southern origin. The analysis of the rangewide Millard-Derby *P. taeda* and *P. echinata* shows five *P. echinata* samples tightly clustered with the most northern sample, New Jersey, and the most southern, southeastern Louisiana, diverging in opposite directions *(Fig. 3)*.

Analysis of the Harrison Experimental Forest *P. echinata* samples (Experiment 3), a rangewide collection derived from seed collections independent of those at Millard-Derby, also shows primarily latitude-related variation (with northern samples at one extreme and others in a random pattern) *(Fig. 5)*. The Pennsylvania sample, however, is outstanding. It diverges sharply from the other northern samples, indeed from all the other samples. This supports previous observations made in the parent stand at time of seed collection and in the nursery that hybridization with *P. rigida* Mill. appeared likely in the Franklin County, Pennsylvania, seed source. Hybridization between the two species has been reported (Austin 1928, Smouse and Saylor 1973).

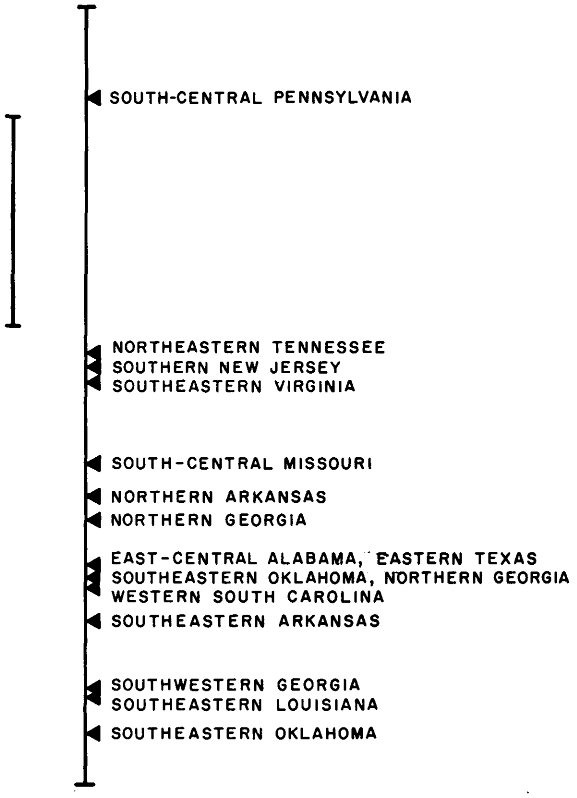

Figure 5. — Discriminate analysis of Harrison Experimental Forest rangewide *P. echinata* samples. Vertical bar indicates least significant difference on first discriminate axis.

In sum, the measurements of needle morphology demonstrated climate-related adaptation in both species and showed a strong discontinuity in the Pennsylvania population of shortleaf pine. Needle morphology, however, showed no differences between shortleaf populations east and west of the Mississippi River and only small differences between eastern and western loblolly pine. In spite of all the variations previously reported within the two species, they are still morphologically distinct, even west of the Mississippi River, If introgression did occur, it must have been many generations in the past and must have been followed by heavy gene flow towards one or both of the parent species.

Summary

Foliage samples collected from *Pinus taeda* L. and *P. echinata* Mill. provenance tests in Mississippi and Louisiana were analyzed to determine the extent and pattern of variation in needle morphology within and between the two species. The two species were distinctly separate from one another with no overlap between them. Within species variation was latitude-related in shortleaf pine and a contrast of coastal versus continental populations in loblolly pine. A distinct discontinuity from the general pattern of variation was found in *P. echinata* of Pennsylvania origin.

Many economically important variations have been found between eastern and western sources of each species, and introgression has been hypothesized. But in this study, no evidence of intermediacy was found. If introgression did occur, it must have been many generations in the past and must have been followed by heavy gene flow towards one or both of the parent species.

Key words: Genecology, Introgression, Needle traits.

Zusammenfassung

Nadeluntersuchungen in Provenienzversuchen von *Pinus taeda* L. und *P. echinata* Mill. erbrachten z. T. starke morphologische Unterschiede zwischen den beiden Arten, wobei es keine Überlagerungen gab. Dagegen waren Unterschiede innerhalb der Arten festzustellen, die bei *P. taeda* auf eine Abgrenzung zwischen Küstenformen und Inlandsformen hindeuten. Bei *Pinus echinata* wurden Unterschiede in der Nadelform zwischen Provenienzen verschiedener Breitengrade gefunden.

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