VARIATION PATTERNS IN NATURAL STANDS OF LOBLOLLY PINE

by

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Very few studies have been made to determine the natural variation of different species of the genus Pious. But by sampling natural populations of some pine species it has become increasingly evident that the varietal limits of the species are very broad.

A very complete study of natural variation in lodgepole pine has recently been completed by Critchfield (1957). Based on low principal morphological characteristics (resin canal number, leaf width, cone *density,* and cone angle) he was able to point out that not only were four subspecies present, but these populations were connected by very pronounced geographic gradients. Although the morphological characteristics in general were unique in their variation patterns, they all had in common an elevational component of varying importance. Latitude, on the other hand, did not show a clear-cut association with any single morphological characteristic. Since elevational gradients, unlike latitudinal gradients, are closely reflected by differences in the moisture climate in much of western North America, Critchfield interpreted the gradual changes on morpholopy to be the result of natural selection of trees adapted to xeric and mesic environments.

Rudolf et al. (1957) studied the natural variation of Jack pine in Minnesota and found molar variations in characteristics of the cones, stem and crown form, branching habit, and disease and insect resistance, Evidence was found to support the hypothesis that the closed or "serotinous" cone character shows a clinal change from predom inantly closed-cone types in the northeast to open-cone types if the southern port of the range.

Coulter pine (P. couleri) has been studied by Zobel (1952) and Fielding[2]. Zobel found that despite the varied ecological and geographical conditions which prevailed in

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[1] Data for this paper are taken from a Ph. D. dissertation submitted to North Carolina State College by the author.

[2] Unpublished notes„

nine isolated populations, most of the characteristics which were examined showed a remarkable similarity. Fielding did not find any differences in seed coat color between different populations, but he did find great differences in cone shapes between two locations. Fielding (1953) also studied the variation in Monterey pine (P.radiata); a species which only embraces three small forests along the coast of California and a grove of trees on Guadalupe Island. Distinct differences between area in cone and seed size, seed color, and needles per fascicle were present.

In sand pine (P. clausa) two races are present; one has closed cones, the other open cones (Little and Dorman 1952). However, no other morphological differences were found to exist between the two forms. In the other southern pines evidently no serious attempt has been made to investigate such morphological characteristics in natural populations.

Only one phase of variation has been intensively studied; the variation in specific gravity and tracheid length. Mitchell and Wheeler (1959) determined specific gravity variation for the major southern pines, and concluded that loblolly pine specific gravity showed a good relationship with the warm-season rainfall. They found that this resulted in a broadly diagonal effect of increasing specific gravity from the north-western to the southeastern part of Mississippi. Zobel and McElwee (1958) analyzed wood samples from a large number of trees from the eastern part of the loblolly range; their data indicated that a trend was present for lower specific gravity in the westward direction. Kramer (1957) found large variation among trees in tracheid length. This was confirmed by zobel et al. (1960) who also discovered that relatively large and statistically significant differences were present between different physiographic areas for this characteristic. A rather gradual increase in tracheid length was apparent in the north-south direction. The geographic distribution of trees with certain gravity characteristics was still more striking; the lowest specific gravities are found at the north and northwest extremes (Delaware and Tennessee), the piedmont has intermediate values, *and the* coastal plain of Georgia and the Carolinas belongs to the high density group.

Material and Methods

Collections were made in the fall of 1958 through the cooperation of thirteen different organizations. The location of the eighteen areas which were included in this study, the names of the cooperators, and some physiographical and climatological data are listed in Table 1 . Figure 1 shows the distribution of the plots within the natural range of loblolly pine.

Table 1. Location and Description of Sampling Areas

Area No.	Location	Latitude North	Longitude West	Collector
1	Bastrop, Tex.	30	97	C. L. Brown, Texas Forest Service
2	Rusk, Tex.	32	95	C. L. Brown, Texas Forest Service
3	Hornbeck, La.	32	93	T. E. Campbell, A. J. Hodges Ind.
4.	Walker, La.	31	91	G. P. Finger, Gaylord Cont. Corp.
5	Crossett, Ark.	33	92	H. C. Grigsby, South. For.Exp.Sta.
6	Woodville, Miss.	32	91	B.W. Henry, South.For.Exp.Sta.
7	Tishomingo, Miss.	35	88	J. H. Hill, Hiwassee Land Company
8	Cullman, Ala.	34	87	J. H. Hill, Hiwassee Land Company
9	Opelika, Ala.	33	86	G. I. Garin, Alabama Polyt. Inst.
10	Griffin, Ga.	33	84	J. C. Barber, Southeastern For. Exp.Sta.
11	Bainbridge, Ga.	31	84	F.C. Cech, International Paper Comp.
12	Ocala, Fla.	29	82	T. O. Perry, Univ. of Florida
13	Georgetown, S.C.	33	79	P.J. Otterbach, International Paper Comp
14	Johnston, S.C.	34	82	D.E. Cole, Cair Woodlands Corp.
15	Jacksonville, N.C.	35	78	Author
16	Wadesboro, N.C.	35	81	Author
17	Accomac, Va.	38	76	R. L. Marler, Virginia Div. For.
18	Chilesburg, Va.	38	78	R. L. Marler, Virginia Div. For.

Table 1. Location and Description of Sampling Areas - Continued.

	Physiographic location	Av. Monthly Temp. °F[a] January	July	Frostfree[a] Season, Dys.	Annual prec.[a] inches
1	Piedmont	50	85	270	34
2	Piedmont	48	82	248	44
3	Coastal plain	49	82	222	52
4	Coastal plain	52	81	269	59
5	Piedmont	47	82	228	49
6	Coastal plain	51	82	245	56
7	Piedmont	43	80	218	55
8	Piedmont	43	79	199	55
9	Transition zone	49	80	237	53
10.	Piedmont	47	81	240	45
11	Transition zone	52	82	264	53
12	Coastal plain	58	81	286	52
13	Coastal plain	50	80	272	48
14	Piedmont	47	81	246	45
15	Coastal plain	48	79	237	51
16	Piedmont	45	79	214	46
17	Coastal plain	38	78	220	45
18	Piedmont	38	77	200	41

[a] Harold W. Hocker. Climatological summaries for selected stations in and near the southern pine region, 1921O195O. Southeastern For. Exp. Sta. Paper No. 56.

Figure 1. Loblolly Pine Range and Sample Plot Locations

Approximately twenty sound and mature cones and five twigs were collected from fifteen different trees within each area. This material was kept separate by individual trees and shipped to the writer who immediately extracted the seed and placed the twigs in cold storage.

270 trees were sampled and more than twenty thousand observations on seed, cone, and needle characteristics were obtained from this material, Only five morphological characteristics were obtained from this material. Only five morphological characteristics which showed a regional variation pattern will be discussed in some detail. These are cone weight, seed form, seed coat thickness, number of cotyledons and stomatal frequency (stomates per linear unit).

The data were analyzed according to the nested sampling procedures (Snedecor 1956). With a large number of determinations and several levels of organization (sub-samples) the analysis of variance may become very time-consuming. The Department of Experimental Statistics at North Carolina State College developed a nested analysis program for their IBM 650 computer which was used to analyze the data in this study, The F - test was used to determine if significant differences were present among trees or locations.

Since several areas are involved in a population study the problem becomes one of multiple comparisons. A multiple range and multiple F - test designed by Duncan (1955) was used to illustrate differences present among populations.

Results

Tables 2 and 3 indicate that although a considerable amount of variation is present within trees the among tree variation is highly significant. The proportion of the total variation which is accounted for by individual trees varies from 21 percent fear number of cotyledons to 63 percent for cone weight. Individual tree variation will be the basis for a large proportion of the improvement which tree breeders hope to obtain by selection of trees with desirable characteristics. The tree breeder should be encouraged by the tremendous tree to tree variation in loblolly pine, which tends to conceal differences which may be present among populations.

Of the thirteen morphological characteristics which showed highly significant differences among areas in the analysis of variance of natural populations, the majority did not show regional trends (e.g. wing length, seed length, cone length, needle length, and frequency of serrations on the needle margin), Random distribution of means may, of course, be interpreted as resulting from local environmental effects on the expression of characters.

Table 2. Analysis of Variance

Variable	Source of Variation	df	Mean Square
Seed Form	Areas	17	.043996 ***
	Trees in areas	252	.009829 ***
	Determinations	1060	.001658
Seed Coat Thickness	Areas	17	.00011944 ***
	Trees in areas	252	.00001043 ***
	Determinations	1080	.0000084
Cone Weight	Areas	17	918.95 ***
	Trees in areas	252	160.89 ***
	Determinations	1080	8.12
No. Cotyledons	Areas	17	9.09 ***
	Trees in areas	252	1.55 ***
	Determinations	1080	.61
Stomatal Frequency	Areas	17	18.93 ***
	Trees in areas	252	4.86 ***
	Determinations	1080	.94

Table 3. Components of Variance in Percent

Component	Seed Form	Seed Coat Thickness	Cone Weight
Area	12	34	20
Tree	44	46	63
Determ.	44	20	17
Total	100	100	100

	No. Cotyledons	Stomatal Frequency
Area	11	10
Tree	21	41
Determ.	66	49
Total	100	100

However, the possibility that genetic factors are involved cannot be disregarded. If initial population sizes were very small, Wright (1943; 1946) has pointed out that local populations may develop which are genetically distinct. "Genetic drift" may thus be a factor in differentiation of morphological characteristics in loblolly pine. Since most of the characteristics measured in the present study have no apparent selective value, although they may be linked to some physiological character, it is not probable that strong selective forces have acted on them. However, seed form, seed coat thickness, cone weight, cotyledon numbers, and stomatal frequency did show some regional trends, and it is possible that some of these characters have a selective value.

<u>Seed Form</u>. Seed form, expressed as seed width in percent of seed length, gives strong evidence of a regional pattern. Figure 2 shows that plot 6 to 3 have narrow seeds, plots 7 and 10 are intermediate, and the plots from 2 to 16 have a more rounded type of seeds.

This relationship is illustrated graphically in Figure 3. All the plots from the northern and eastern portion of the species range have rounded seeds; while all those from the southern and western part of the range, with the exception of plot number 2, have narrow seeds, Plot number 2 is in some disagreement with the regular pattern, but it should be kept in mind that this deviation may not be significant. From Figure 2 it is clear that plot 2 (Rusk) is not significantly different from the main group with narrow seeds even though it is barely different, at the one percent level, from the plot with the most narrow seeds (plot 6) . On the other hand, around Rusk, Sonderegger (<u>P. taeda</u> x P. palustris) are very common and may account for the rounded seed and the large cones present in this area.

There is no indication of a sharp break in the distribution; rather there is a trend toward more rounded seed in the northeast direction. The number of plots is, however, too small to warrant a conclusion of clinal distribution. Even though it is difficult to imagine that seed form can have any adaptive value it is possible that this characteristic is associated by genetic linkage with some other characteristic, perhaps a physiological property. It is also feasible that seed form is a secondary effect of a gene or genes controlling some other morphological characteristic.

<u>Seed Coat Thickness</u>. Figure 4 shows that there is no indication of any discontinuity in the distribution of seed coat thickness. However, it is evident that the four plots which belong to the group with extremely thin seed coats are from the western part of the range while the ten plots with thick seed coats are from the more eastern part. This fact is illustrated in Figure 5. The number of plots is too small to prove that there is a continuous increase in seed coat thickness in the west- east direction, but the indication of a clinal pattern is quite strong.

p :	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
R_p :	4.15	4.33	4.45	4.54	4.61	4.66	4.72	4.75	4.79	4.82	4.86	4.88	4.91	4.93	4.95	4.97	4.99

Area #																	
6	5	1	4	12	11	3	7	10	2	9	14	15	18	17	8	13	16
Mean																	
65.40	66.48	66.61	67.03	67.53	68.41	68.47	69.31	69.88	70.28	70.80	70.99	71.11	71.12	71.81	71.84	73.50	73.60

Figure 2. "Duncan Test"; Seed Form, Expressed as Seed Width in Percent of Seed Length, Related to Physiographic Location.

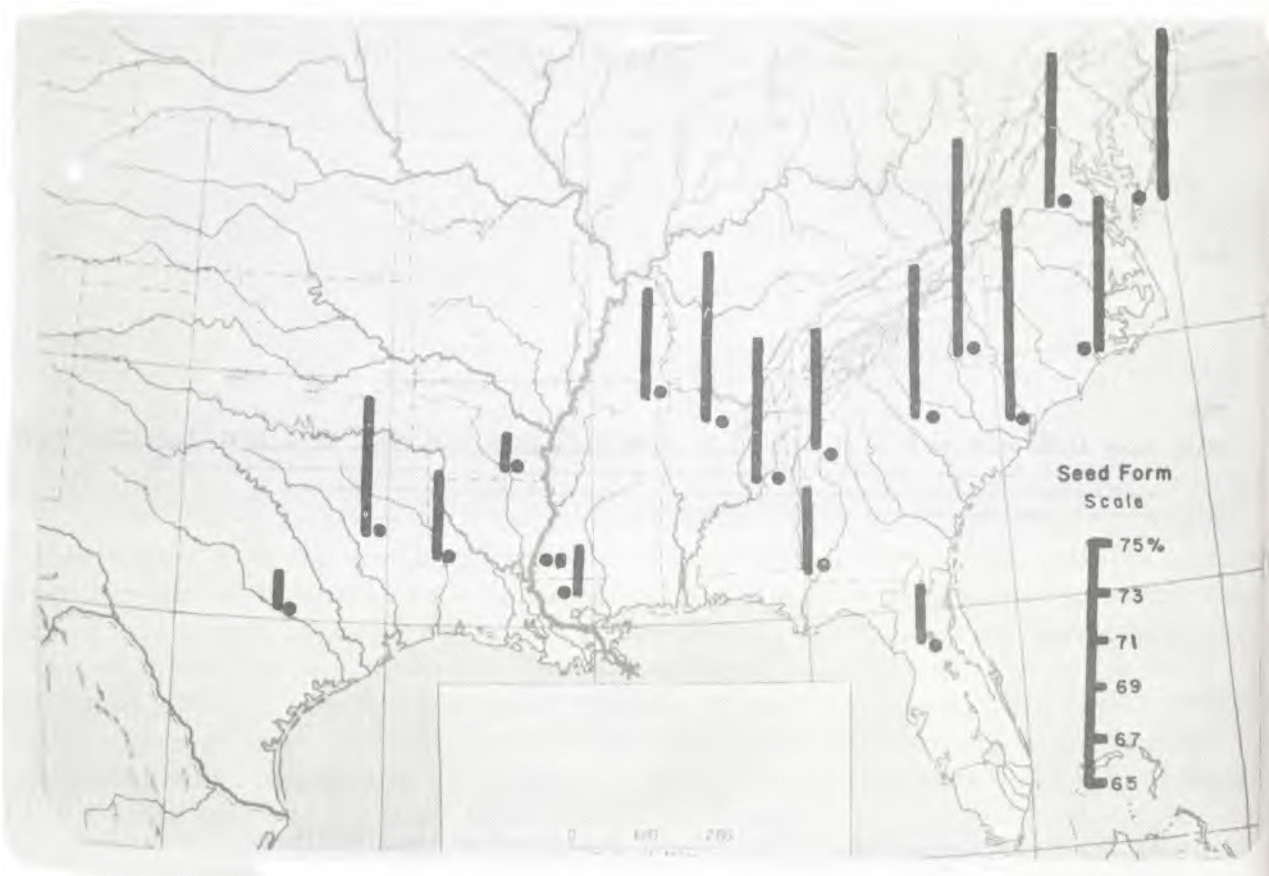

Figure 3. Means of Seed Forms, Expressed as Seed Width in Percent of Seed Length, for Regional Plots

P	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
R_p	1	.00135	.00141	.00144	.00147	.00149	.00151	.00154	.00155	.00156	.00157	.00158	.00159	.00159	.00160	.00161	.00161	.00162

Area #																	
5	2	1	6	3	4	18	7	16	14	8	10	9	11	17	12	13	15

Mean																	
.0076	.01127	.01144	.01172	.01264	.01299	.01301	.01307	.01341	.01353	.01361	.01387	.01393	.01431	.01449	.01463	.01480	.01493

Figure 4. "Duncan Test"; Seed Coat Thickness, In Fraction of an Inch, Related to Physiographic Location

Figure 5. Means of Seed Coat Thickness for Regional Plots

Further examination of the distribution of means reveals another interesting fact; all the coastal plots have higher mean values than the inland plots. The eastern part of the range is sampled in a manner to show such relationships; each Coastal Plain plot has a corresponding plot on the Piedmont. When averages for five coastal areas are examined we find that the range of values runs from .0143 to .0149 inch, while the corresponding Piedmont values range from .0130 to .0139. Thus, there is no overlap in the distribution, and the differences between plots within the same physiographic region are negligible. It is rather tempting to conclude that two distinct types are present for this characteristic; however, such a conclusion has to be tested against other information available. From collections in North Carolina we know that there is no abrupt reduction in seed coat thickness from the coast to the western extreme of the species range.

If we define the ecotype as a race whose characteristics adopt it to a distinct habitat, there may be some objections to the use of seed coat thickness as an indicator of distinct Coastal Plain and Piedmont ecotypes. One objection is the apparently continuous or clinal distribution of the means. Since an ecological gradient is present it would, however, be expected that the corresponding means are distributed along a gradient. This means that the ecotype concept must be modified according to the scheme proposed by Huxley (1939) who suggested that an intergroup cline may be present.

In addition to this modification of the ecotype concept it would be highly desirable to show that the characteristic in question has some selective value. Since seed coat thickness distribution is highly correlated with the precipitation - evaporation ratio as developed by Transeau (1905), one working hypothesis may be that thinner seed coats allow for more rapid water uptake.

Kozlowski and Gentile (1959) found that white pine seeds with punctured seed coats had higher moisture contents than intect seeds; but the writer has not been successful in finding any reference on the effect of different thicknesses of seed coat on water absorption. A simple test is the laboratory with three lots of seeds; one with extremely thick coats, one with average, and one with *very* thin seed coats did not reveal any differences in ability to absorb water. After one, two, four, and eight days of soaking the moisture content on the seeds remained about constant at 30 percent, and no differences were present among seed lots. This test is by no means conclusive; when germination starts more rapid water uptake will follow and it is possible that seed coat thickness may be of importance in this part of the process.

Cone Weight. Small cones are found on trees located in the extreme southwest and southeast of the species range. The large cones are found in the central part of the species range, and the intermediates in the northeastern part. Cone size is to some degree dependent upon the environment; Ehrenbert et al. (1955) found that grafts of Scotch pine cultivated in a favorable climate produced larger and heavier cones than the mother trees. In this study, however, the largest cones were found in areas with different climatic conditions, and both Coastal Plan and Piedmont sites were capable of producing large cones.

Figure 6 indicates that only two plots, numbers 2 and 6 are in disagreement with the trend towards smaller cones in the south and west directions. Since the Duncan test revealed that plot number 6 (Woodville) is different from the other western plots, and even different from plot 4 which is located only fifty miles away, it is probable that local environmental condition are important in determining the size of cones. It is also feasible that hybridization between loblolly pine and longleaf pine has resulted in local hybrid swarms. The introversion of shortleaf pine into loblolly could possibly explain why the cones are so small at the western extreme of the species range. In Texas[1] there is a considerable amount of natural hybridization between loblolly and shortleaf due to some overlap in time of pollen flight. Although the cooperators in this area were selecting "typical" loblolly pines for their samples it is entirely possible that some trees were contaminated with blocks of genes from shortleaf pine.

Number of Cotyledons. The Duncan test indicates that two groups with different cotyledon numbers are present. The break between these two populations is not sharp; the lower plots in the high group are not significantly different from the higher plots in the low group. The plots representing the extreme northeast, southeast, and west have fewer cotyledons than the plots from the central portion of the species range. It is also possible that all trees of the extreme south produce seeds with few cotyledons; the four southernmost locations studied have low cotyledon numbers.

Stomatal Frequency. Except for two intermediate plots there are two groups present; one small group with low stomatal frequency and one large group with high frequency. The group with low frequency is made up of the plots west of the Mississippi River with one exception, plot number 9 from the central poetion of the species range. That plot 9 is included in this group is definitely due to error in sampling. When the Opelika cone samples arrived there were no needles included and a separate collection had to be made for twigs. These twigs were evidently not collected from the top of mature trees; they were quite delicate and the needles were extremely short and light green. The writer is confident that plot 9 may be disregarded in further discussions of needle characteristics.

There is an increase in stomatal frequency in the west- east direction (Figure 7). This finding may be compared with data obtained by Mergen (1958) from a slash pine seed source test.

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[1] Personal communication with Dr. B. J. Zobel

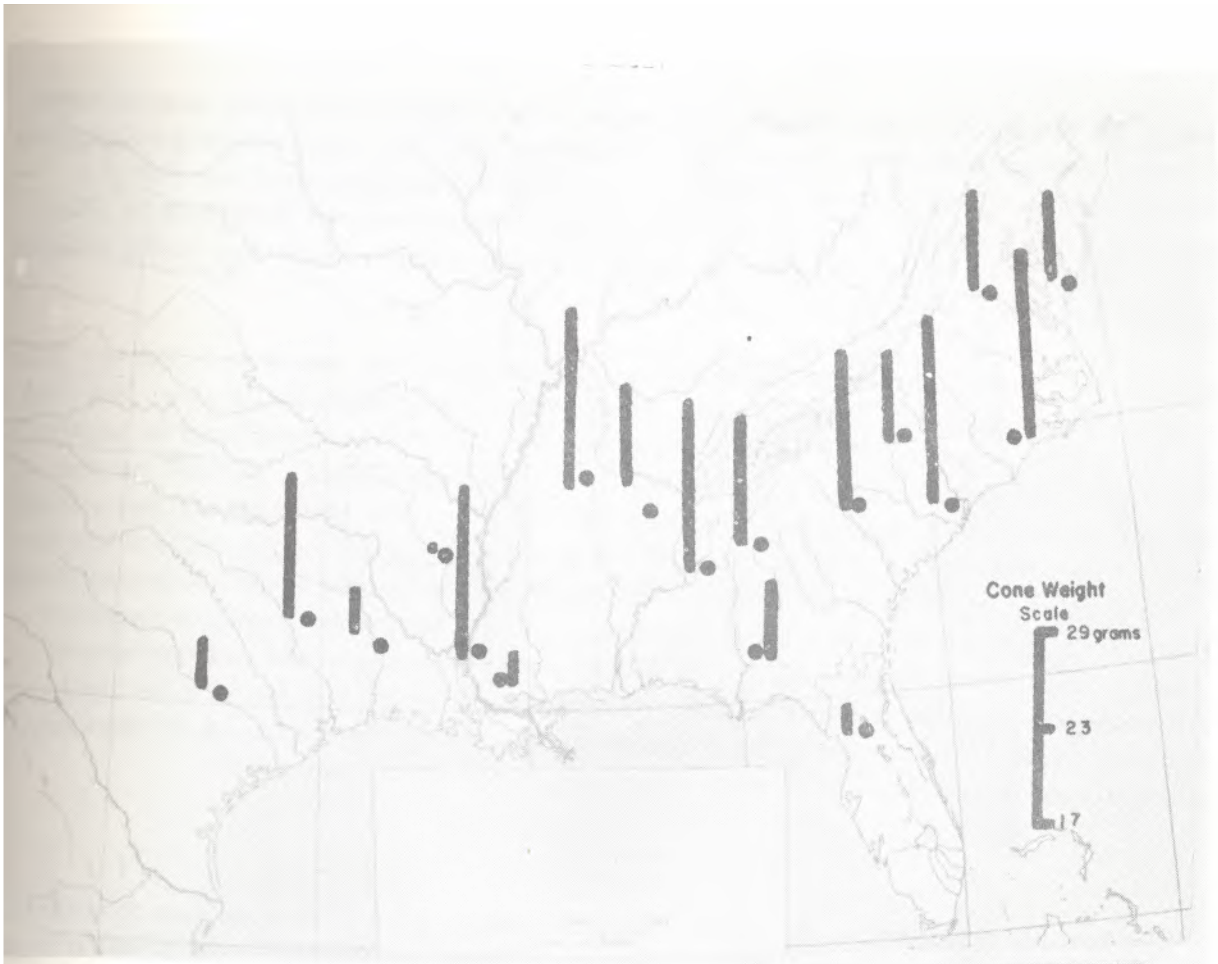

Figure 6. Means of Cone Weights for Regional Plots

When he plotted the average value for the number of stomata per millimeter over latitude, longitude, average number of days without killing frost, and the warm season precipitation, he found that the only distribution pattern that indicated a definite trend was that of a comparison with longitude. When stomatal frequency was plotted over longitude, a clinal variation pattern was revealed, with the sources from the eastern longitudes having a higher number of stomates than those from the western sources.

Thus, the general pattern of lower stomatal frequency in the west than in the east holds true for both slash pine and loblolly pine. The question is whether loblolly pine, like slash pine, has a clinal pattern. If we assume that this characteristic has developed in response to an environmental factor such a percipitation we would also expect a gradual increase in stomatal frequency with a gradual increase in precipitation . We would expect that a fewer number of stomates may be of some adaptive value under draughty conditions, and can test this hypothesis by correlating the stomatal frequency with a drought index. A drought index was constructed for each plot by using the precipitation temperature ratio as suggested by Lang (1920). When the ratio of may to August precipitation over the average summer temperature was correlated with stomatal frequency, the r-value was found to be highly significant (0.666). Therefore, there is some evidence that the number of stomates may have developed as an adaptive dine to a gradual change in moisture supply.

Summary

In this study of variation in natural stands of loblolly pine a large number of morphological characteristics were investigated. In one respect all these variables behaved alike; a large proportion of the total variation is accounted for the tree to tree variation.

Of the thirteen characteristics which showed highly significant differences among areas the majority did not show any regional trend. "Genetic drift" may thus be a factor in differentiation of morphological characteristics in loblolly pine. Seed farm, seed coat thickness, cone weight, cotyledon number, and stomata frequency gave good evidence of regional variation patterns.

The most outstanding character which varies in a clinal pattern is seed coat thickness. There is a strong increase in seed coat thickness from the western to the eastern part of the range. Similarly, all the coastal plots have higher mean values than the corresponding island plots. Thus, there is a high correlation between seed coat thickness and the precipitation evaporation ratio.

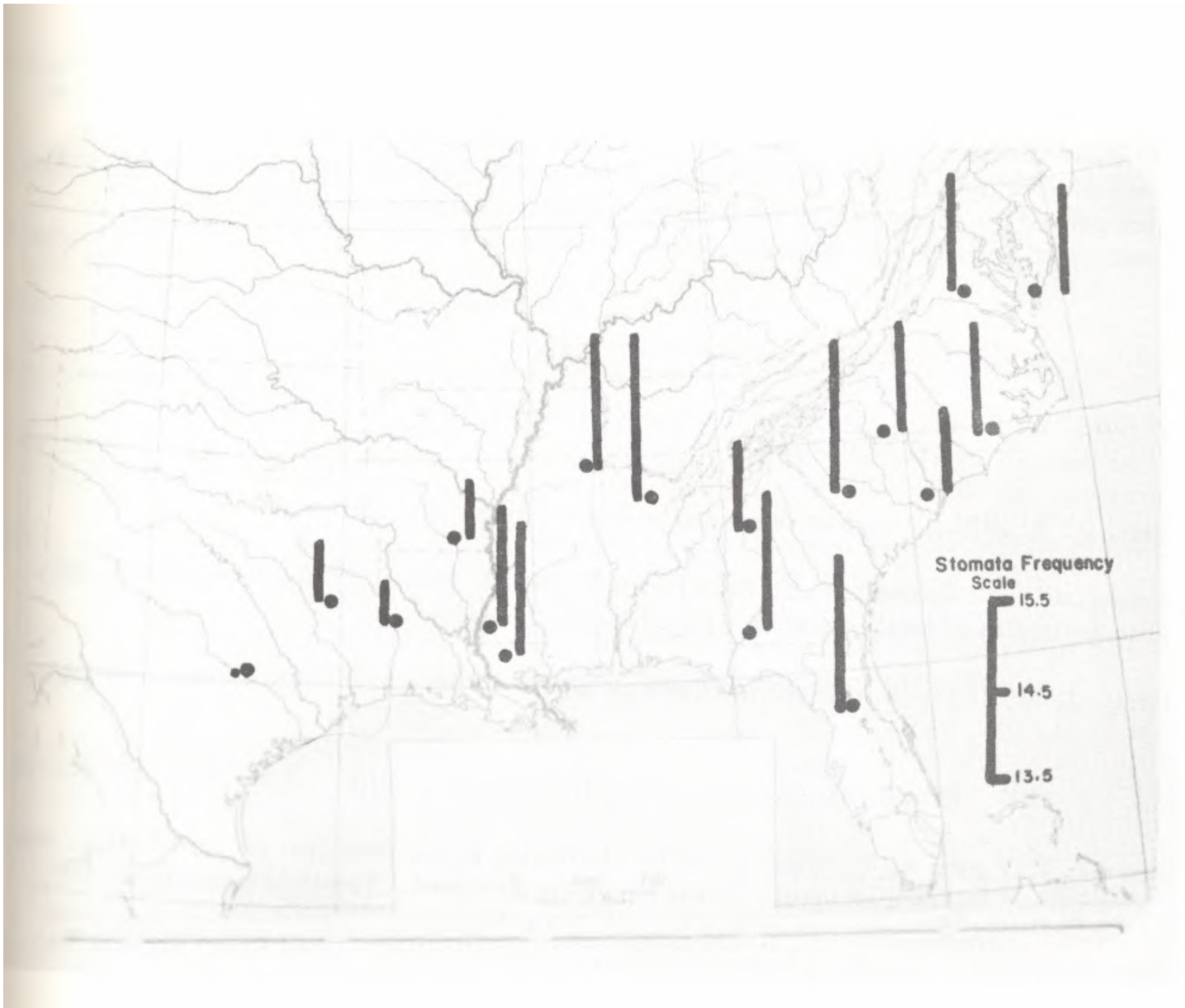

Figure 7. Means of Stomatal Frequency for Regional Plots (Plot 9, Opelika was deleted due to reasons described on page 39.)

A similar clinal distribution pattern was found for stomatal frequency. The other three characteristics, seed form, cone weight, and cotyledon number, exhibited different patterns of distribution and indicate that the different morphological characteristics have a more or less unique variation pattern. Cotyledon number, for example, was high in the central and northern part of the range, while the seedlings originating from the extreme west, south, southeast, and northeast had relatively few cotyledons.

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