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Agreement Between Patterns of Morphological Variability and Isozyme Band Phenotypes in Pitch Pine*)

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Summary

Seventy-six isozyme bands could be consistently resolved in the peroxidase, acid phosphatase, cytochrome oxidase, and leucine aminopeptidase enzyme systems in extracts of pitch pine roots. Using band frequencies in seedlings from 15 populations, the populations were resolved into three geographic groups by multivariate techniques. Morphological characteristics, including seedling height, weight, and needle length, were also subjected to multivariate analyses. The grouping based on morphological variables was very similar to that based on the biochemical markers; only two populations shifted between groups. The results suggest that multivariate analysis of isozyme data may provide largely the same information on patterns of geographic variation obtained from morphological measurements in early tests, offering a rapid alternative to nursery or greenhouse experiments.

Key words: Multivariate analysis, *Pinus rigida*, electrophoresis, geographic variation.

Zusammenfassung

Sechszwanzig Isoenzymbänder der Peroxydase, Acid-Phosphatase, Cytochrom-Oxydase und Leucin-Amino-Peptidase konnten in Extrakten von *Pinus rigida*-Wurzeln bestimmt werden. Anhand der Bandhäufigkeiten von Sämlingen aus 15 Populationen wurden die Populationen mittels multivariater Verfahren in drei geographische Gruppen eingeteilt. Morphologische Merkmale, Sämlingshöhe, Gewicht und Nadellänge eingeschlossen, wurden ebenfalls multivariaten Analysen unterzogen. Die Gruppierung, die auf morphologischen Variablen basierte, war der, die auf biochemischen Merkmalen basierte, sehr ähnlich. Nur zwei Populationen wechselten zwischen Gruppen. Die Resultate legen nahe, daß multivariate Analysen von Isoenzymdaten im großen und ganzen die gleiche Information geographischer Variationsmuster beschaffen, wie morphologische Bonituren im Frühstadium und eine schnelle Alternative zu Baumschul- oder Gewächshausversuchen darstellen.

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Introduction

No one has demonstrated whether isozymes of forest trees can be used to define regions of similar adaptation, that is, whether they correlate with patterns of variation in characteristics of selective value. This paper compares macrogeographic patterns in isozyme band phenotypes of pitch pine (*Pinus rigida* MILL.) with those in morphological characteristics of seedlings grown in a uniform environment, and relates the patterns to climatic variation over the range of the species by multiple regression analysis and clustering techniques.

Numerous studies of forest trees have demonstrated the existence of genetic variation within and between populations for morphological, physiological, and biochemical traits (WRIGHT, 1976, FERET and BERGMANN 1976). Clinal patterns of isozyme variation have been detected with respect to latitude, longitude, or elevation in a few instances (BERGMANN 1975, 1978, YEH and O'MALLEY 1981). While these correlations could be interpreted as showing adaptation of isozymes to the environmental gradient, they do not preclude other explanations, such as drift followed by migration between pockets of differentiation. Isozyme differentiation appears in most cases to be distributed in localized mosaic patterns not clearly related to obvious environmental variation (e.g., MUHS 1974, GURIES and LEDIG 1982) and may reflect the presence of relatively small, discrete neighborhoods, isolated by distance and limited seed dispersal (SAKAI and PARK 1971, MITTON *et al.* 1977, YEH and LAYTON 1979). According to the "neutrality hypothesis" (KIMURA and OHTA 1971), most electrophoretically detected variants are not functionally different and are, therefore, selectively neutral. If the neutralists are correct, isozymes are of value primarily as gene markers and are of limited usefulness in studies of selection in natural populations.

Pitch pine is a species with substantial variability in growth and form throughout the eastern United States (LEDIG and FRYER 1974). Strong phenotypic variation in

cone and seed size, cone serotiny, and wood properties have been reported (LEDIG and FRYER 1974, LEDIG and LITTLE 1979, LEDIG *et al.* 1975). Provenance studies (LEDIG *et al.* 1976, GOOD and GOOD 1975) showed pronounced provenance differences for a number of seedling characteristics. Phenology and photosynthetic behavior varied between northern and southern populations (VAARTAJA 1959, LEDIG *et al.* 1977).

Despite the variability in growth and form, the geographic variation of allozymes at 21 loci in populations that covered much of the range of pitch pine did not exhibit strong trends (GURIES and LEDIG, 1981, 1982); correlations between NEI's (1972) genetic distance and geographic distance were poor. However, significant correlations between the frequencies of a number of allozymes and several climatic variables, particularly temperature (GURIES and LEDIG 1981) were suggestive of selection at certain loci. The present study suggests that isozyme phenotypes in pitch pine are correlated with characteristics generally considered to have adaptive value.

Materials and Methods

Seed was collected from 6 to 10 mother trees at 15 sites throughout the natural range of pitch pine (Figure 1, Table 1). Details were given in LEDIG *et al.* (1975) and LEDIG and FRYER (1972). Eight seedlings per mother tree from each population (minimum 48 seedlings per population) were raised in a heated glasshouse until 12 months old. When all plants were dormant, several morphological characteristics were measured and tissue samples were taken for isozyme analysis. The morphological characters were: total fresh weight, stem length, stem diameter at base of epicotyl, length of the longest root, needle length, and basal crook. Basal crook is an adaptation to fire (LITTLE and SOMES 1956). On the underside of the crook, dormant buds of the cotyledonary node are pressed into the soil, where they are insulated from fire. They sprout following death or injury to the top. Basal crook was measured as the complement of the angle between the vertical extension of the tap root and the main stem.

Root tip tissue proved the most satisfactory source for a clear resolution of a number of enzyme systems. Extraction procedures were modified from MACKO *et al.* (1967) using liquid nitrogen to enhance cell disruption. Electrophoresis of the extracts on polyacrylamide gel columns was used to resolve isozymes for the enzyme systems acid phosphatase (ACP), cytochrome oxidase (CYO), leucine aminopeptidase (LAP), and peroxidase (PER). Staining technique followed BREWBAKER *et al.* (1968) except for CYO which was resolved using the technique described by HARE (1970).

Following staining and fixing, gels with unsatisfactory resolution were discarded and the sample repeated in following runs. Each isozyme band was scored for position relative to the origin and to the tracking-dye band that indicated the migration front for the extract. These data were compiled into a zymogram for each enzyme for each seedling, where the actual distance migrated was converted to an Rf value. Rf is the distance from the origin to the center of the isozyme band (to 0.5 mm) divided by the distance from the origin to the center of the tracking-dye band. Visual interpretation of isozyme profiles was verified periodically by scanning the gels with a high resolution densitometer.

As with any chromatographic technique, Rf values of isozymes varied between samples. The limits of this variation were determined by replicated runs of the same extract of a seedling, and of different extracts of the same

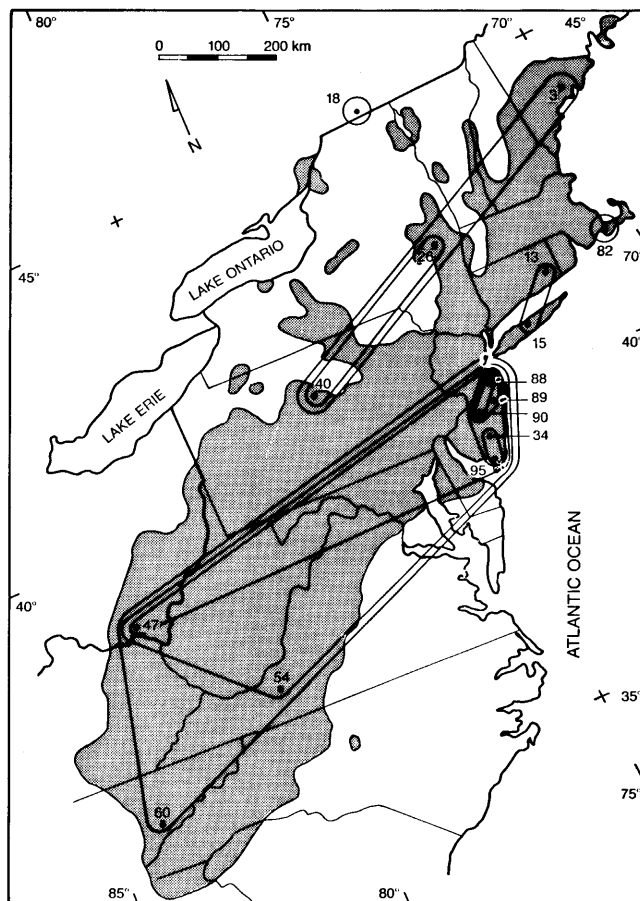


Figure 1. — Natural range of pitch pine (shaded), location of 15 sampled populations (refer to Table 1 for codes), and aggregation contours for cluster analysis of climatic variables.

seedling. The maximum variation for an isozyme over five electrophoretic runs was used as a limit to identify and classify the isozymes. To assign frequencies to each isozyme band, the total Rf values for all enzymes in all populations were plotted as histograms and the midpoints of the frequency peaks were taken as the mean Rf values. All occurrences within the limits around this mean were scored as bands with identical Rf.

Maximum variation of Rf values was ± 0.01 Rf units ($\pm 1\%$). This value was determined for PER at the highest mobilities (Rf 0.5 to 0.88) and was generally less for isozymes with mobility below Rf 0.50, which included most isozymes of PER and all isozymes of ACP, CYO and LAP. The maximum variation of ± 0.01 Rf units was then used to assign isozymes to Rf classes. In a few cases it was not possible to differentiate closely spaced isozymes with confidence, even with this technique. In such cases it was necessary to coelectrophorese two samples containing the uncategorized isozyme(s) to determine conclusively whether there were two closely spaced isozymes or only one. A total of 76 isozymes were identified in the four enzyme systems (35 in PER, 17 in ACP, 12 in LAP, and 12 in CYO).

Isozyme frequencies ranged from low (0 to 20%) to high (80 to 100%). Without family information or diploid-haploid comparisons, it was not possible to provide empirical demonstration of allelism for these isozymes. It is quite probable that some of these isozymes were not direct allelic products but reflected post-translational modification (WATT 1977). The inclusion of possible epigenetic variants was accepted on the basis that such variation is under

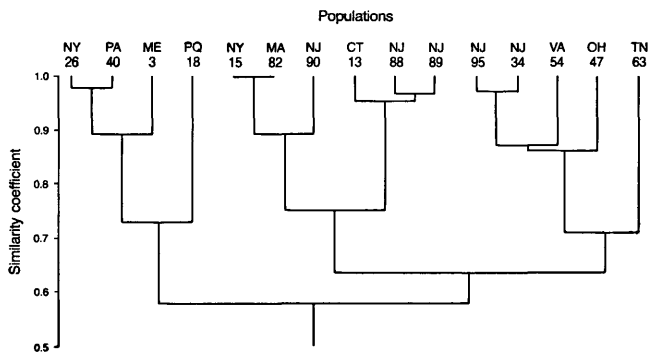


Figure 2. — Dendrogram for unweighted pair group cluster analysis of mean morphological characteristics for 15 pitch pine populations (refer to Table 1 for codes).

genetic control through the influence of regulatory gene systems (JOHNSON 1979). The use of isozyme bands rather than isozyme alleles has at least one advantage: it avoids the usual bias against those systems whose patterns of inheritance are notoriously difficult to unravel. In any case, the objective was to determine whether isozymes reflected geographic patterns of variation in morphology, and the mode of inheritance for neither the isozyme bands nor the morphological variables was crucial to the determination, although hindsight suggests that the conclusions would have been stronger if inheritance of the isozymes had been known. However, these analyses were conducted in 1971, before the use of female gametophytes had become standard practice.

Data Analysis

Population means of the morphological characters were analyzed for simple correlation with 100-seed weight and with scalars reflecting provenance origin (i.e., latitude, longitude, elevation, distance from the Atlantic Coast and distance from population 60). The scalar, distance from population 60, was used to describe spatial trends along the major northeast-southwest axis of the species distribution (Figure 1).

Correlation analysis was also performed with morphological characters and winter, spring, summer, and autumn mean daily mean temperature, mean monthly maximum temperature, mean monthly minimum temperature, mean monthly precipitation, and mean length of the frost-free season (17 variables) at the population origin, using U.S. Weather Bureau records. The relationship between the geographic scalars and climatic variables was investigated by correlation analysis and cluster analysis using the CLUST procedure (SAS 1979).

To differentiate between intercorrelated climatic and geographic variables, stepwise multiple regression analyses were performed using STEPWISE MAX R improvement technique (SAS 1979) which created models for morphological variables with maximum R^2 values using combined independent variables. Ranking of effects of variables on models was by stepwise multiple regression of the FORWARD selection procedure (SAS 1979).

Geographic variation in morphological characters was explored through the unweighted pair group clustering algorithm (SNEATH and SOKAL 1973). Hierarchical clusters were derived from a similarity matrix, using the distance coefficient described by SNEATH and SOKAL (1973). Distance (d) between populations j_1 and j_2 was defined as:

$$d_{j_1 j_2} = \left[\sum_{i=1}^n (\bar{x}_{i j_1} - \bar{x}_{i j_2})^2 \right]^{1/2},$$

where n = number of populations, \bar{x}_{ij} = mean of the i th character in the j th population divided by the maximum value of \bar{x}_i in all populations. Standardized genetic distance was computed as:

$$D_{j_1 j_2} = d_{j_1 j_2} / \max d,$$

where $\max d$ = maximum value of d in all pairwise population comparisons, ignoring self comparisons. Similarity coefficients are the complements of $d_{j_1 j_2}$ values:

$$S_{j_1 j_2} = 1 - D_{j_1 j_2}.$$

Table 1. — Pitch pine populations sampled in this study.

Code	Provenance	County and State or Province	Latitude (N)	Longitude (W)	Elevation (m)
3	Brunswick Naval Air Station	Sagadahoc, Maine	43°53'	69°55'	12
13	Pachaug State Forest	New London, Connecticut	41°35'	71°52'	82
15	Upton Station	Suffolk, New York	40°51'	72°52'	15
18	St. Chrysostome	Chateauguay, Quebec	45°06'	73°54'	69
26	Westmere	Albany, New York	42°42'	73°53'	91
34	Great Egg Harbor River	Atlantic, New Jersey	39°30'	74°46'	9
40	Sproul State Forest	Clinton, Pennsylvania	41°14'	77°35'	488
47	Shawnee State Forest	Scioto, Ohio	38°49'	83°10'	229
54	Jefferson National Forest	Pulaski, Virginia	37°02'	80°50'	677
60	Bates Mountain	Blount, Tennessee	35°43'	83°45'	518
82	Bourne	Barnstable, Massachusetts	41°46'	70°32'	61
88	Green Cove	Monmouth, New Jersey	40°15'	74°05'	30
89	Lakehurst	Ocean, New Jersey	40°01'	74°18'	18
90	Lebanon Lakes	Burlington, New Jersey	39°53'	74°34'	33
95	Clermont	Cape May, New Jersey	39°10'	74°46'	4

Similarity coefficients and geographic distance between population origins were analyzed for correlation using CORR procedure of SAS (1979). The results were visualized as dendrograms and aggregation contour maps.

Similar correlation, multiple regression, and cluster analyses were performed on isozyme frequencies in each population, using values 0.00 to 1.00 (0 to 100% frequency).

Results and Discussion

Climate

Correlation analysis of climatic variables and geographic scalars revealed no strong dependence between the two over the range of pitch pine. Only latitude was significantly correlated with a reasonable number of the climatic variables (9 of 17). Evidently, climate in the Atlantic Coast region is controlled by factors not fully expressed by simple linear models on the geographic variables examined here.

As would be expected, however, climate could be defined into broad zones. SAS (1979) CLUST procedure applied to the 17 climatic variables generated an optimal cluster grouping of three clusters (climatic zones) plus two individual populations (Figure 1). The broad zones of climate over the range of pitch pine consisted of a northern zone stretching from coastal Maine to northern Pennsylvania, a coastal, southern New England zone and a large southern zone extending from southern New Jersey to eastern Tennessee. The climates at the southern Quebec and Cape Cod provenance origins were strongly dissimilar to all other climates. There appeared to be an abrupt climatic shift between New Jersey and the neighboring states to the northeast.

Morphology

Of the various morphological variables, only mean height was significantly correlated ($r = 0.68$, $P < 0.01$) with 100-

seed weight, a relationship also reported by LEDIG *et al.* (1976). However, by standardized partial correlation LEDIG *et al.* (1976) showed that most of the variation in seedling height was related to temperature at the seed origin rather than seed weight, which is itself strongly correlated with latitude or temperature.

Six of the 15 possible correlations between morphological characters were significant, at a maximum value $r = 0.81$ ($P < 0.01$). As these were not logical correlations, in the sense of one property being the logical consequence of another, they did not suggest any serious redundancy in characters analyzed.

Table 3. — Similarity coefficients between pitch pine populations based on morphological characters.

	Populations														
	CT	NY	PQ	NY	NJ	PA	OH	VA	TN	HA	NJ	NJ	NJ	NJ	NJ
	13	15	18	26	34	40	47	54	60	82	88	89	90	95	
3	0.65	0.63	0.73	0.95	0.50	0.82	0.59	0.65	0.29	0.70	0.61	0.67	0.45	0.54	
13	_____	0.90	0.50	0.61	0.55	0.54	0.54	0.49	0.34	0.64	0.94	0.96	0.65	0.62	
15	_____	_____	0.43	0.67	0.59	0.55	0.70	0.54	0.56	1.00	0.74	0.90	0.90	0.73	
18	_____	_____	_____	0.65	0.69	0.80	0.54	0.83	0.32	0.53	0.63	0.65	0.34	0.57	
26	_____	_____	_____	_____	0.58	0.98	0.77	0.73	0.43	0.73	0.55	0.68	0.57	0.63	
34	_____	_____	_____	_____	_____	0.61	0.81	0.90	0.62	0.76	0.63	0.71	0.71	0.97	
40	_____	_____	_____	_____	_____	_____	0.67	0.77	0.36	0.65	0.52	0.59	0.44	0.57	
47	_____	_____	_____	_____	_____	_____	_____	0.84	0.83	0.71	0.52	0.72	0.81	0.93	
54	_____	_____	_____	_____	_____	_____	_____	_____	0.64	0.57	0.57	0.67	0.57	0.83	
60	_____	_____	_____	_____	_____	_____	_____	_____	_____	0.52	0.41	0.61	0.75	0.76	
82	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	0.74	0.87	0.86	0.75	
88	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	0.96	0.56	0.67	
89	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	0.76	0.83	
90	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	0.83	

Table 2. — Best significant regression models for seedling morphological characteristics of pitch pine as a function of climatic and geographic variables, based on minimum c [P] values.

Characteristics	Model ¹⁾	R ²
Fresh Weight	= 135.9 - 2.96 LAT - 0.01 ELEV - 0.04 WMN	0.8590
Height	= 95.6 - 2.86 LAT - 0.22 WMT + 0.14 SUMT + 0.13 SRF	0.8762
Diameter	= 3.6 + 0.03 WMT - 0.01 WMN - 0.01 ELEV	0.8305
Root Length	= 221.7 - 3.45 LONG + 0.77SRF + 0.22 WMX	0.6937
Needle Length	= 8.1 + 0.02 WMX	0.3368
Basal Crook	= 21.3 - 0.15 SUMN + 0.10 WMX	0.5106

¹⁾ Independent variables are listed in descending order of contribution to the model. Independent variables ranked in decreasing order of contribution to all regression models were WMX (26), LAT (20), WMT (19), WMN (17), ELEV (17), SRF (16), SUMN (10), LONG (10), and SUMT (8). ELEV = elevation; LAT = Latitude; LONG = longitude; SRF = spring mean monthly precipitation; SUMN = summer mean monthly minimum temperature; SUMT = summer mean daily mean temperature; SRF = summer mean monthly precipitation; WMN = winter mean monthly minimum temperature; WMT = winter mean daily mean temperature; and WMX = winter mean monthly maximum temperature.

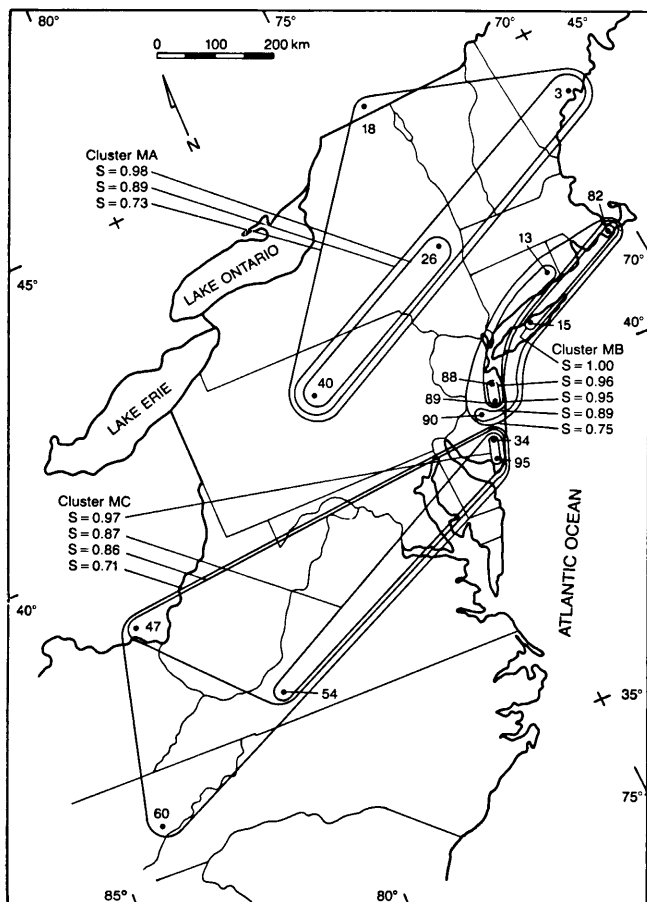


Figure 3. — Aggregation contours for similarity coefficients (S) between pitch pine populations based on morphological criteria.

Most morphological characters varied significantly with population origin, particularly latitude. Height was especially sensitive. Height, fresh weight, and diameter were correlated with a number of climatic variables, mainly temperature. These observations are similar to those made by LEDIG *et al.* (1976) for first and second year growth in nursery beds.

As there appeared to be a complex relationship between climatic and geographic variables, multiple regression analyses on selected models sought to determine the co-relationships of these variables and morphology. Because the degrees of freedom were limited (the number of populations was only 15), only independent variables with a significant correlation ($P < 0.05$) with at least one morphological character were examined, to restrict the number of independent variables to less than 10. MAX R procedure (SAS 1979) produced the multivariate model and signified the "best significant model" of all possible models, by minimum $c [P]$ value. FORWARD procedure listed the variables in order of their contribution to the model (Table 2).

Both climate and location of population had significant and independent relationships with morphology. All morphological traits were significantly correlated with at least one independent variable, although the relationships for needle length and basal crook were weak. Winter mean, maximum, and minimum temperatures explained more variation than other climatic variables, and the location variables, latitude, longitude, and elevation, were also associated with a large proportion of the morphological variation.

Similarity coefficients for all pairwise comparisons of populations (Table 3) bore little relationship to geographic distance between populations. Correlation between similarity coefficients and geographic distance ($r = 0.44$, $P > 0.05$) showed that simple spatial proximity could explain little of the relationship between populations.

The cluster analysis (Figures 2 and 3) permitted an interpretation of group rather than individual affinities. The optimal cluster as defined by the method of RATLIFF and PIEPER (1981) indicated three significantly different clusters ($P < 0.01$) described by the 0.70 phenon line, the similarity level which describes groups or "phenons" according to an arbitrary or objective decision (SNEATH and SOKAL 1973). These optimal clusters were: populations 3, 18, 26, 40 (Cluster MA); 13, 15, 82, 88, 89, 90 (Cluster MB); and 34, 47, 54, 60, 95 (Cluster MC).

Cluster MA grouped the northern populations, although population 40 was marginally closer to the New Jersey populations than to its nearest neighbour in MA. Cluster MB comprised a predominantly coastal subgroup extending from Cape Cod to central New Jersey. This region included the most extensive stands of the species, its apparent ecological center. Cluster MC grouped the three southwestern Appalachian populations, but in an apparent geographic anomaly, also included the two most southerly populations sampled in New Jersey (34 and 95). It may be of interest that these two populations had a greater similarity to pitch pine growing some 600–700 km distant than to adjacent populations in New Jersey. The affinities of population 40 with the northern group and 34 and 95 with the southern group may appear to be a spatial anomaly, but

Table 4. — Simple correlation coefficients of isozyme frequencies with geographic variables. Isozymes with no significant correlations (at $P < 0.05$) have been omitted from the table.

Isozyme	Latitude	Longitude	Elevation	Distance from	
				population 60	coast
CYO-4	0.13	0.22	0.32	-0.12	0.53*
CYO-6	0.14	-0.46	-0.33	0.37	-0.54*
CYO-7	-0.43	0.37	0.62*	-0.42	0.32
LAP-1	0.55*	-0.19	-0.15	0.33	0.11
LAP-9	0.53*	-0.35	-0.27	0.43	-0.07
ACO-2	0.74**	-0.58*	-0.38	0.68**	-0.31
ACP-6	-0.37	0.59*	0.67**	-0.55*	0.65**
ACP-7	-0.71**	0.21	0.05	-0.41	-0.17
ACP-8	-0.54*	0.75**	0.37	-0.69**	0.65**
ACP-9	-0.13	0.54*	0.66**	-0.44	0.80**
ACP-10	0.53*	-0.37	-0.19	0.44	-0.17
ACP-11	0.47	-0.40	-0.58*	0.47	-0.32
PER-2	-0.25	0.59*	0.39	-0.49	0.71**
PER-3	0.55*	-0.30	-0.21	0.40	-0.01
PER-4	-0.57*	0.35	0.56*	-0.47	0.17
PER-5	-0.11	0.50	0.42	-0.38	0.63*
PER-6	0.25	-0.44	-0.45	0.42	-0.52*
PER-9	-0.82**	0.76**	0.50	-0.82**	0.52*
PER-11	0.70**	-0.41	-0.29	0.55*	-0.11
PER-14	0.46	-0.53*	-0.47	0.53*	-0.51
PER-15	0.53*	-0.30	-0.22	0.39	-0.08
PER-17	-0.25	0.53*	0.35	-0.44	0.42
PER-18	0.58*	-0.39	-0.37	0.47	-0.17
PER-26	-0.42	0.28	0.52*	-0.38	0.18
PER-27	-0.33	-0.56*	-0.62*	0.52*	-0.62*
PER-28	0.55*	-0.74**	-0.57*	0.72**	-0.66**

* Significant ($0.05 < P < 0.01$)

** Highly significant ($P < 0.01$)

is readily explained by reference to the climatic zones defined by cluster analysis (Figure 1). Population 40 shared a common climatic zone with 3 and 26, as did 34 and 95 with 47, 54, and 60.

The relationship between climatic and morphological variation both in regression analysis of individual characters and in multivariate cluster analysis suggests a significant adaptive component in the observed variation.

Isozyme Band Phenotypes

Of the 76 isozyme bands tested for simple correlation, 25 had significant relationships with at least one geographic variable that expressed population origin (Table 4). However, for the CYO and LAP systems, the numbers of significant correlations were less than might be expected by chance.

Forty of the isozymes had significant correlations with at least one of the 17 climatic variables, although for CYO

Table 5. — Best significant regression models for isozymes as a function of climatic and geographic variables based on minimum c [P] values.

Isozymes	Model ¹	R ²
CYO-4	= 3.52 - 0.023 SURF + 0.002 WMX - 0.009 WMT + 0.009 SUMT - 0.087 LAT	0.9388
CYO-7	= 3.92 - 0.014 SRF + 0.002 WMN - 0.014 SUMT - 0.007 WMX	0.7382
LAP-1	= - 0.17 + 0.004 SRF - 0.001 WMN - 0.005 SURF + 0.015 SUMT - 0.003 SUMN - 0.004 WMX - 0.025 LAT - 0.019 LONG + 0.001 ELEV	0.9904
LAP-5	= 1.35 - 0.004 WMT + 0.007 SUMN - 0.011 SUMT + 0.003 WMX + 0.003 SURF - 0.003 SRF	0.9446
LAP-6	= 0.61 - 0.002 SUMT	0.3884
LAP-8	= 0.05 - 0.001 WMT	0.3706
LAP-9	= 0.17 + 0.019 LAT	0.2830
LAP-10	= 1.68 - 0.006 SUMT	0.3150
ACP-1	= 0.30 + 0.003 WMX	0.3501
ACP-2	= - 1.48 + 0.051 LAT	0.5433
ACP-3	= 2.86 - 0.015 SUMN + 0.014 WMT - 0.001 ELEV - 0.011 SURF	0.8289
ACP-6	= 0.72 + 0.0003 ELEV	0.4505
ACP-7	= 1.18 + 0.001 WMT - 0.00004 ELEV - 0.0008 SUMT	0.9335
ACP-8	= - 2.56 + 0.038 LONG - 0.0003 ELEV	0.6387
ACP-9	= - 0.71 + 0.020 LONG - 0.005 SURF	0.7129
ACP-11	= 0.93 - 0.00006 ELEV - 0.0006 SUMN + 0.0002 WMN + 0.002 LONG	0.6774
ACP-16	= 1.99 - 0.002 WMN - 0.048 LAT	0.6751
PER-2	= - 8.64 + 0.051 LONG - 0.002 WMX + 0.129 LAT + 0.007 WMT	0.8414
PER-3	= - 0.25 - 0.004 SUMN + 0.009 SRF	0.5195
PER-4	= 1.05 + 0.010 SRF + 0.0005 ELEV - 0.016 LONG	0.8834
PER-5	= - 1.53 + 0.027 LONG - 0.001 WMN	0.4688
PER-6	= 0.47 - 0.007 LONG + 0.002 SURF	0.4040
PER-8	= 1.01 + 0.008 SURF - 0.0008 SUMT + 0.0003 WMX + 0.0004 SRF	0.8288
PER-9	= 1.03 + 0.008 WMX - 0.014 SURF + 0.002 WMN	0.8088
PER-11	= 0.817 - 0.002 WMT	0.4929
PER-12	= - 0.54 - 0.002 WMN + 0.004 SURF	0.5856
PER-14	= - 5.47 + 0.003 ELEV + 0.0138 LAT + 0.009 SURF + 0.002 WMN	0.7153
PER-15	= 0.29 - 0.003 WMT	0.3078
PER-17	= 3.14 + 0.012 LONG - 0.007 WMT + 0.002 WMN - 0.067 LAT - 0.002 ELEV - 0.001 SUMN	0.8512
PER-18	= - 0.96 - 0.006 WMT + 0.006 SUMT	0.5535
PER-26	= 0.31 - 0.001 ELEV	0.2696
PER-27	= 0.66 - 0.004 ELEV	0.3893
PER-28	= 2.24 - 0.018 LONG	0.5483

¹) Independent variables are listed in descending order of contribution to the model. Independent variables, ranked in decreasing order of contribution to all regression models, were WMT (93), ELEV (92), LONG (78), SUMT (77), WMX (68), SURF (67), LAT (63), WMN (62), SRF (50), SUMN (49). See Table 2 for abbreviations

Table 6. — Similarity coefficients between pitch pine populations based on isozyme frequencies.

	Populations													
	CT 13	NY 15	PQ 18	NY 26	NJ 34	PA 40	OH 47	VA 54	TN 60	MA 82	NJ 88	NJ 89	NJ 90	NJ 95
3	0.42	0.31	0.54	0.84	0.63	0.60	0.54	0.58	0.57	0.65	0.34	0.39	0.58	0.47
13	_____	0.50	0.32	0.49	0.41	0.53	0.53	0.58	0.45	0.78	0.60	0.40	0.60	0.35
15	_____	_____	0.59	0.46	0.50	0.43	0.13	0.25	0.20	0.38	0.57	0.81	0.82	0.21
18	_____	_____	_____	0.64	0.57	0.35	0.00	0.27	0.20	0.37	0.39	0.53	0.61	0.01
26	_____	_____	_____	_____	0.58	0.48	0.53	0.65	0.69	0.62	0.40	0.50	0.60	0.46
34	_____	_____	_____	_____	_____	0.56	0.42	0.38	0.40	0.51	0.46	0.55	0.65	0.33
40	_____	_____	_____	_____	_____	_____	0.53	0.39	0.41	0.88	0.62	0.61	0.63	0.55
47	_____	_____	_____	_____	_____	_____	_____	0.79	0.80	0.60	0.40	0.18	0.36	0.56
54	_____	_____	_____	_____	_____	_____	_____	_____	1.00	0.59	0.44	0.25	0.49	0.64
60	_____	_____	_____	_____	_____	_____	_____	_____	_____	0.59	0.48	0.26	0.48	0.72
82	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	0.70	0.54	0.60	0.75
88	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	0.77	0.75	0.56
89	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	0.82	0.41
90	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	0.41

the number did not exceed that which might be expected by chance. In a ranking of the five climatic variables most strongly correlated with isozyme frequencies, winter mean daily mean temperature was clearly the most influential, followed by summer mean monthly minimum temperature, winter mean monthly maximum temperature, and winter mean monthly minimum temperature (FRYER 1982). The strong association of temperature with isozyme frequencies and with morphological characters is paralleled by the relationships between wood specific gravity and tracheid length and mean annual temperature in another study which included the same provenances (LEDIG *et al.* 1975).

For the multiple regression analyses, models were restricted to a maximum of 10 independent variables. Because of the large number of isozymes, the 33 chosen for investigation were those which had at least one highly significant correlation at P < 0.01 or more than one significant correlation at P < 0.05 with climatic variables. The best significant models generated are in Table 5.

The predominant factor associated with isozyme variation in these models appeared to be the various expressions of winter temperature, although other climatic and geographic variables made substantial contributions to regression. GURIES and LEDIG (1981) examined the frequency of the most common allele of 11 polymorphic loci in pitch pine and found that six of the alleles were significantly cor-

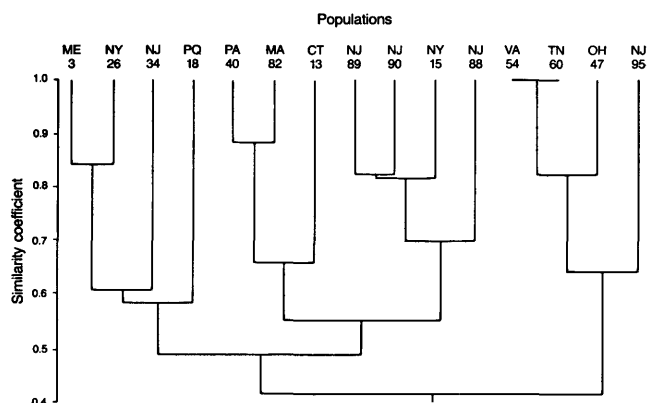


Figure 4. — Dendrogram for unweighted pair group cluster analysis of isozyme band frequencies for 15 pitch pine populations (refer to Table 1 for codes).

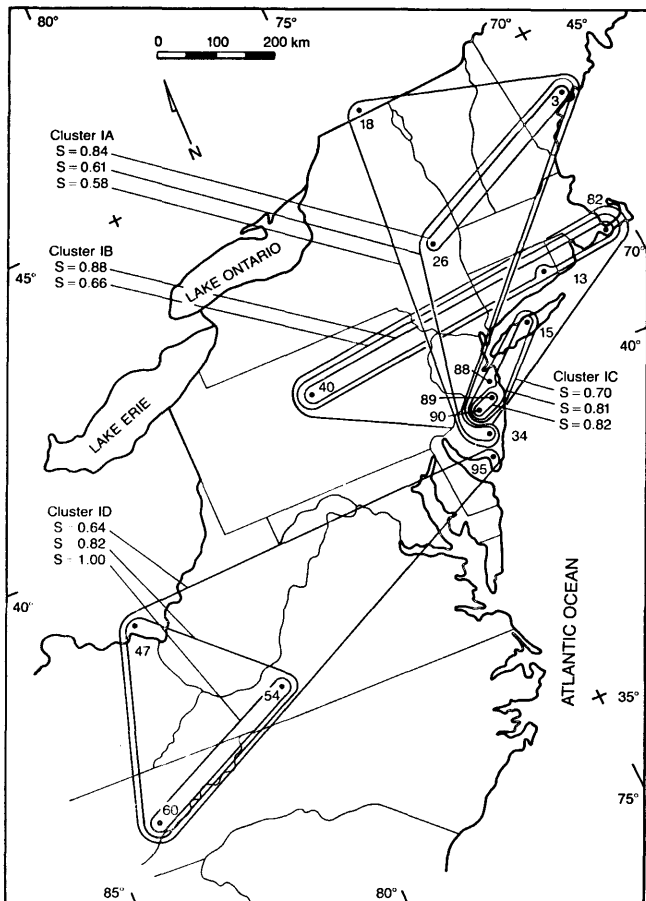


Figure 5. — Aggregation contours for similarity coefficients (S) between pitch pine populations based on isozyme band frequencies.

related with climatic variables, and four of the six involved winter temperature or snowfall.

Similarity coefficients for all pairwise population comparisons for isozymes (Table 6) had little relationship to geographic distance ($r = -0.28$, $P < 0.05$), as was the case for the morphological variables. Genetic distance (NEI 1972) as determined by GURIES and LEDIG (1982) was also poorly correlated with geographic distance ($r = 0.26$).

The dendrogram produced by cluster analysis (Figure 4) was divided into 3 clusters (Figure 5) at the 0.32 phenon line, but these were not significantly different at $P < 0.01$ by the method of RATLIFF and PIEPER (1981) and must be considered as arbitrary. These divisions grouped populations 3, 18, 26, 34 (cluster IA); 13, 15, 40, 82, 88, 89, 90 (IB,C); and 47, 54, 60, 95 (ID). Cluster IA clearly conforms to a northern grouping of pitch pine. Cluster IB,C groups the southern New England, Long Island and central New Jersey and Pennsylvania populations. Cluster ID was made up of the southwestern populations and also included the southernmost New Jersey population.

Conclusions

Clusters defined by morphology and those defined by isozyme frequencies were in substantial agreement. Only two populations, 40 and 34, shifted clusters depending on type of trait, morphological or biochemical. This is one of the first examples in tree species to demonstrate that isozymes reflect patterns of affinity similar to those determined by growth in early tests. Isozyme analysis, particularly using

the large number of systems and loci available in the haploid female gametophyte of conifers, could provide an exceptionally quick means of defining patterns of variation prior to establishment of breeding zones for applied tree improvement.

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Adjusting for Inter-Row Competition in a Jack Pine Provenance Trial

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Summary

Results from field trials in forest genetics can be severely biased if trees of different genetic identity are allowed to compete with each other following crown closure. Two methods of reducing such bias by adjusting observed values to expected values in large genotypically homogenous stands are given in this paper. One method uses empirical regression models of tree growth and its dependence on competition to calculate adjustments to observations. The second method derives the influence of size of neighbour trees on the size of a subject tree by solving a linear first-order auto-normal scheme of a two-dimensional Markov process. Both a maximum likelihood solution and an iterative solution of ordinary least-squares estimates are presented. The two estimates were identical. The effects of tree mortality on competition and tree growth were addressed for each method. Data of tree height and diameter collected at age 12 and 20 years in a range-wide *Pinus banksiana* (LAMB.) provenance trial in Ontario with 10-tree single-row plots showed that inter-row competition significantly affected provenance mean tree diameters at both ages. Tree heights were little affected by differential competition in the five provenances analyzed. A local Petawawa provenance which ranked among the best in tree height was exposed to substantially less competition than would otherwise be the case in a single provenance stand without mortality. Diameters in this provenance were adjusted downwards by 8% at age 12 years and 11% at age 20. Growth rates of diameters per unit stem size were not significantly affected by competition.

Key words: *Pinus banksiana*, Provenance, Intraspecific Competition Modeling, Growth.

Zusammenfassung

Wenn verschiedene Genotypen miteinander konkurrieren, können die Ergebnisse forstgenetischer Freilandversuche mit schwerwiegenden systematischen Fehlern behaftet sein. Die vorliegende Arbeit stellt zwei Methoden dar, solche systematischen Fehler durch Angleichung der beobachteten Werte an die in großen, genotypisch homogenen Beständen zu erwartenden Werte zu reduzieren. Empirische Regressionsmodelle des Zuwachs in Abhängigkeit von Konkurrenz werden in der ersten Methode verwendet, um die Angleichung der beobachteten Werte zu berechnen. In der zweiten Methode wird der Einfluß der Nachbarstämme auf die Größe des Versuchsstammes durch das Lösen eines linearen „autonormalen“ Schemas erster Ordnung der zwei-dimensionalen Markov-Gleichung hergeleitet. Sowohl eine „maximum likelihood“-Lösung als auch eine iterative Lösung

mit Schätzungen der kleinsten Quadrate werden vorgestellt. Mittels der zwei geschilderten Methoden wird der Einfluß des Stammausfalls auf Konkurrenz und Wachstum angesprochen. Die im Alter von 12 und 20 Jahren erhobenen Höhen- und Durchmesserwerte eines das Verbreitungsgebiet von *Pinus banksiana* (LAMB.) umfassenden Provenienzversuches in Ontario, dessen Parzellen in Einzelreihen von jeweils 10 Stämmen angelegt wurden, wiesen in beiden Altersgruppen einen signifikanten Einfluß von Konkurrenz zwischen Reihen auf die Mitteldurchmesser der Provenienzen auf. Die Baumhöhe wurde in den 5 untersuchten Provenienzen von Konkurrenz nur wenig beeinflusst. Eine örtliche Provenienz, Petawawa, die eine der besten Höhenwuchsleistungen aufwies, wurde in diesem Versuch weitaus weniger Konkurrenz ausgesetzt, als sonst in einem Bestand aus nur einer Provenienz. Der Durchmesser dieser Provenienz wurde für das Alter 12 Jahre um 8% und für das Alter 20 Jahre um 11% nach unten korrigiert. Das Durchmesserzuwachsprozent wurde nicht signifikant durch Konkurrenz beeinflusst.

Introduction

The use of small plot units in forest genetic field trials has increased the effect of competition on tree growth. Methods to assess and adjust for competition are required to obtain accurate estimates of genetic parameters. Only few attempts have been made to introduce competition correction measures in forest genetics. A range-wide jack pine (*Pinus banksiana* LAMB.) provenance (seed source) trial arranged in single row plots offered an opportunity to quantify the effect of inter-row competition and to develop procedures of adjustment. Two methods of quantification and adjustment are introduced. One method (Approach I) relates tree growth to competitive status which enables the calculation of adjusted periodic growth. The second method (Approach II) uses size of neighbouring trees (both within plots and in neighbouring plots) as covariates in a linear analysis of provenance tree heights and diameters. In the latter case we introduce a simple solution to the iterative process of estimating competition effects from a least-squares approximation. Tree mortality is taken into account in both methods.

Review of literature

Separation of genetic and non-genetic variation is the primary objective of quantitative genetic studies (FALCONER, 1981). Hence, minimizing or stratifying environmental variation is a priority in the choice of experimental design. Environmental heterogeneity in forest genetic experiments is unavoidable. Trees are larger than most agricultural crops, and occupy considerable space on variable forest sites. In addition, genetics trials are conducted for many years to several decades. Much attention has therefore been

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