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GENETIC DIVERSITY AND POPULATION STRUCTURE IN PITCH PINE (*PINUS RIGIDA* MILL.)

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Electrophoretic studies of protein polymorphisms in plants have focused upon herbaceous species, primarily inbreeding annuals, in efforts to characterize the levels and patterns of genic variation within and between populations (Clegg and Allard, 1972; Gottlieb, 1973, 1975; Levin, 1975, 1978; Levy and Levin, 1975; Schaal, 1975; Roose and Gottlieb, 1976; Brown et al., 1978; and others). These studies have indicated that predominantly outbreeding species maintain higher levels of intrapopulation variation than predominantly inbreeding species, while inbreeders exhibit a greater degree of population differentiation than outbreeders (Brown, 1979; Hamrick et al., 1979). This relationship is by no means perfect as Levin (1978) points out, because of differences in ecological requirements, breeding systems, dispersal mechanisms, evolutionary history, and other factors which affect the genetic system (Grant, 1958, 1971; Brown, 1979; Hamrick et al., 1979). Whether long-lived perennials such as forest trees conform to the general pattern is still an open question.

Allozyme studies of forest tree species have suggested that levels of genic variation are exceptionally high in natural populations (Tigerstedt, 1973; Rudin et al., 1974; Lundkvist and Rudin, 1977; Yang et al., 1977; Hamrick, 1979; Hamrick et al., 1979; Lundkvist, 1979), that certain populations appear to be moderately inbred (Rudin et al., 1974; Mejnartowicz and Bergmann, 1975; Phillips and Brown, 1977), and that populations have become differentiated over relatively short distances (Sakai and Park, 1971; Mitton et al., 1977). However, many inferences have been drawn from only one or a few loci, or only from loci known to be highly polymorphic. Valid estimates of mating system parameters may be obtained by examining only a few loci, but for estimates of heterozygosity, genic diversity, and the extent of differentiation, a large number of loci is preferred (Lewontin, 1974; Nei, 1975).

As part of a continuing study of the genetics and ecology of pitch pine (*Pinus rigida* Mill.), we have surveyed 21 enzymatic loci in 11 populations across the species range. Pitch pine occurs from coastal Maine and southern Quebec to northern Georgia and from the Atlantic Coast to central Ohio, but almost always on relatively infertile sites. In spite of a history of overexploitation, it appears to have retained appreciable variation (Ledig and Fryer, 1974). Pitch pine demonstrates clinal patterns of variation in cone serotiny (Ledig and Fryer, 1972), wood properties (Ledig et al., 1975), and seedling growth (Ledig et al., 1976), as well as seed and needle characters (Ledig, unpubl.). It is uncertain whether these clinal patterns are the result of gene flow among pockets of differential fitness or reflect a continuous gradient in selection pressures (Endler, 1977; Givnish, 1981).

The objectives of the present study were to determine the levels of genic diversity characteristic of pitch pine, and to examine the organization of genic variability within the species and the patterning of genic differentiation between populations. Among other comparisons, we contrasted marginal vs. central populations and the

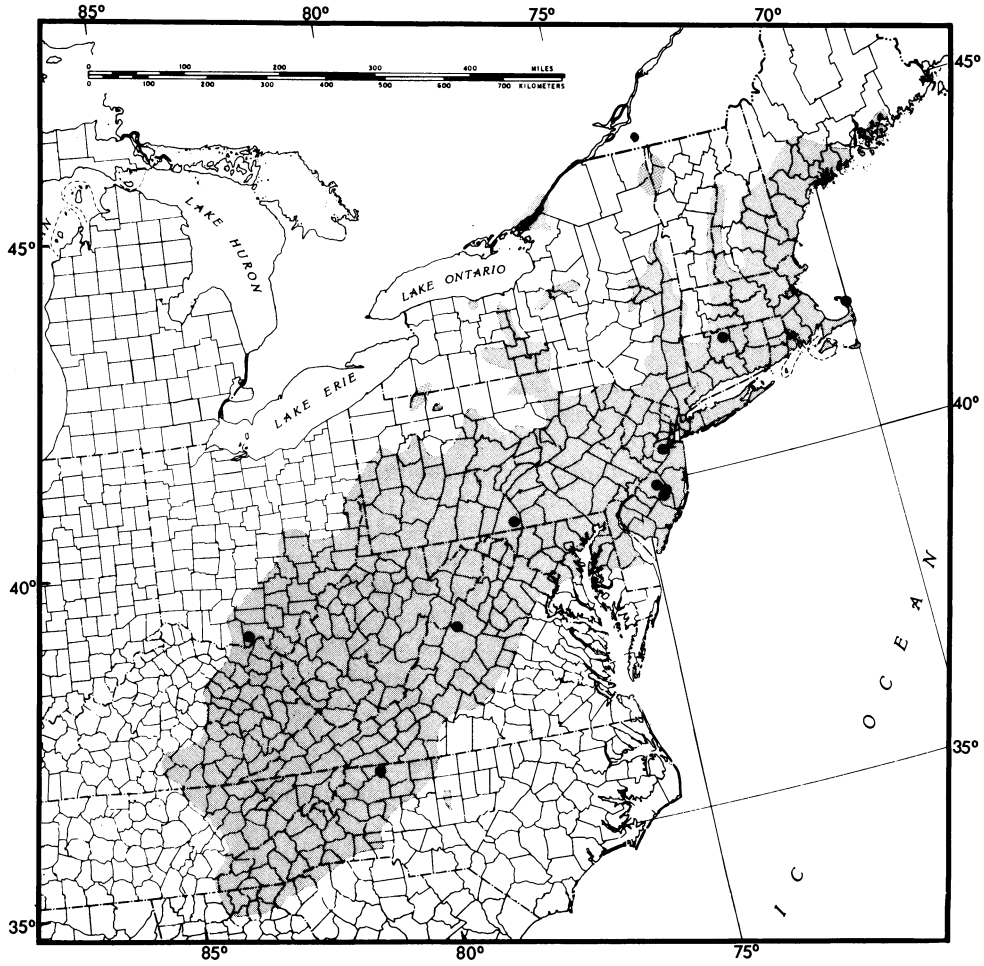

FIG. 1. Range map of pitch pine showing the locations of seed collections (●).

New Jersey Pine Plains, a dwarf forest, with populations from the surrounding tall forest.

MATERIALS AND METHODS

Populations

Cones were collected from 11 natural populations of pitch pine during the autumn of 1976 (Fig. 1 and Appendix 1). A plot center was arbitrarily established and every tree bearing cones was sampled around the center, extending the plot to concentric circles until approximately 60 trees were included. Actual numbers varied from 39 to 122. Geographically peripheral populations were sampled at St. Chrysostôme, Quebec, located at the extreme northern edge of the range, and Shawnee State Forest, Ohio, near the western edge. St. Chrysostôme is a true marginal outlier of ca 400 ha extent approximately 50 km north of the next northernmost stands of pitch pine in the Champlain Valley of New York and Vermont. It is most likely a relict rather than an advance colony, a remnant of the hypsithermal period 6,000 years BP (Davis, 1976). Subsequently, a cooling climate resulted in the retreat of the species boundary southward. Pitch pine at St. Chrysostôme is at an edaphic extreme; i.e., a shallow (0–0.45 m) soil on a sandstone cap

(Grandtner, 1961), where it is able to survive with little competition from other species and replace itself in spite of an inhospitable climate. Stands on the Shawnee State Forest are merely peripheral populations.

Other stands sampled were well within the inclusive range boundary of pitch pine. Lebanon State Forest, New Jersey, in the middle of the New Jersey Pine Barrens, is representative of central populations. The Pine Barrens once occupied 5,830 square kilometers of the New Jersey coastal plain (McCormick and Forman, 1979). Encroachment by urban development has reduced the core in recent decades, but there are still at least 2,600 square kilometers of essentially wildland within the Barrens. Within the central core there are two sizeable areas (ca 2,400 ha each) of dwarf forest known as the Pine Plains, represented by the East and West Plains in our collection. The dwarf forest appears to be an evolutionary response to wildfires, which were more frequent and intense in the Plains than in the surrounding Barrens (Lutz, 1934). Pitch pine on the Plains differs from that on the Barrens by virtue of earlier reproductive maturity, shorter stature, and predominance of serotinous cones (Ledig et al., 1976; Good and Good, 1979; Ledig and Little, 1979). Helmetta is an outlying pocket of coastal plain sediments, supporting typical Pine Barren vegetation but separated by about 16 km from the contiguous Barrens.

The Marconi Station, Massachusetts, and Bradley Field, Connecticut, populations are located on coastal plain or outwash plain sediments. West of Maryland, pitch pine is displaced from the coastal plain. It occurs on ridges and dry slopes in the Appalachian Mountains and its foothills, represented by collections at Big Run, Virginia, Blue Ridge, North Carolina, and Big Pine Flat Ridge, Pennsylvania.

Starch Gel Electrophoresis.

Individuals were genotyped using 6–8 megagametophytes per tree. Methods of analysis for malate dehydrogenase (*Mdh*), isocitrate dehydrogenase (*Idh*), phosphoglucoisomerase (*Pgi*), 6-phosphogluconate dehydrogenase (*6-Pgd*), phosphoglucomutase (*Pgm*), leucine aminopeptidase (*Lap*), glutamate oxalacetate transaminase (*Got*), and aconitase (*Aco*) were previously reported (Guries and Ledig, 1978). Methods for alcohol dehydrogenase (*Adh*), acid phosphatase (*Acp*), aldolase (*Ald*), fumarase (*Fum*), glucose-6-phosphate dehydrogenase (*G-6-pd*), and glutamate dehydrogenase (*Gdh*) were similar to those reported by Brewer (1970), Shaw and Prasad (1970) and Nichols and Ruddle (1973). All gels were 12% Electrostarch, Lot #307 (Otto Hiller, Madison, Wisconsin).

Inheritance of the allozymes has been determined for the polymorphic loci studied (Guries and Ledig, 1978; Guries et al., 1978; Guries, unpubl.). We used 60 or more haploid megagametophytes to determine the inheritance of variants; segregation ratios were 1:1 as expected for codominant alleles at heterozygous loci. Such segregation in haploid products of meiosis has been confirmed in a number of conifers by relating allozyme segregation in megagametophytes to the inheritance of allozymes in progeny obtained from control crossings (Lundkvist, 1975, 1977; Rudin, 1975, 1977; Simonsen and Wellendorf, 1975). Several additional allozymes were observed on gels stained for *Got, Adh,* and *Acp* but their inheritance has not been determined and they were excluded from the analysis.

Where several zones of activity were observed for a single enzyme, hyphenated numerals following the enzyme abbreviation were used; the most anodal zone was designated by the lowest numeral. Within a zone of activity, the most common allele was arbitrarily designated *100,* and faster and slower migrating variants received designations according to their relative mobility with respect to the common allele. For example, the designation *90* indicates a variant which migrates to a position 10% closer to the origin than the common allele. Following convention, loci were designated polymorphic if the most common allele had a frequency of less than 95% in at least one population.

RESULTS

Gene Diversity

Allele frequencies for the 21 loci studied are presented in Appendix 2. Two of the loci studied, *Adh* and *Gdh,* were essentially monomorphic; only one or two variants were detected for these loci and these only in the central New Jersey populations. All populations shared the same common allele at each locus with the exception of the *Mdh-2* locus at St. Chrysostôme. There was, however, appreciable variation among populations in allele frequencies at several loci.

Sixteen loci (76.2%) were polymorphic (95% criterion) in at least one population, and most such loci segregated for two or three alleles. Some loci, such as *Mdh-2, 6-Pgd-1* and *Aco* segregated for four or more alleles in some populations. The mean number of alleles per locus was 3.10 when averaged over all loci, including monomorphic loci. The two Pine Plains samples had the greatest number of alleles per locus, and St. Chrysostôme, the marginal outlier, had the smallest number of alleles per locus. However, the average number of alleles per locus has been criticized as a diversity criterion because it is strongly dependent upon sample size (Nei, 1975; Avise, 1977). The criticism appears justified because a significant correlation exists between mean number of alleles per locus and sample size ($r = 0.72; P < .05$), and our Pine Plains samples were the largest.

The expected proportion of heterozygotes (H_e) was calculated as

$$H_e = 1 - \sum_{i=1}^{k} x_i^2$$

where x_i is the frequency of the ith allele, summed over k alleles. Expected and observed proportions of heterozygotes averaged over all loci are 0.146 and 0.138, respectively (Table 1). Observed heterozygosities were equal to or slightly less than those expected under random mating, but observed frequencies did not differ significantly ($P > .05$) from Hardy-Weinberg expectations in any population.

TABLE 1. *Sample sizes* (N), *observed* (H_o) *and expected* (H_e) *heterozygosities, and the standard error (SE) for the expected heterozygosity for 21 loci in pitch pine.*

Population	N	H_o	H_e	SE (H_e)
St. Chrysostôme, QP	55	.117	.120	.036
Bradley Field, CT	46	.133	.152	.036
Marconi Stn., MA	62	.171	.173	.040
Big Pine Flat Ridge, PA	39	.104	.118	.032
Shawnee State Forest, OH	41	.120	.122	.033
Helmetta, NJ	61	.163	.167	.041
West Plains, NJ	118	.140	.146	.033
East Plains, NJ	122	.148	.155	.036
Lebanon Lakes, NJ	69	.148	.155	.033
Big Run, VA	39	.138	.154	.036
Blue Ridge, VA	42	.138	.143	.039

The marginal populations located at St. Chrysostôme, Quebec, and Shawnee State Forest, Ohio, and the centrally located populations at Big Pine Flat Ridge, Pennsylvania, and Bradley Field, Connecticut, exhibited less than average levels of heterozygosity (Table 1). The most heterozygous populations were located on the coastal plain and New England seaboard, extending from Massachusetts to New Jersey.

A commonly used measure of genetic variation is H_T, total gene diversity (Nei, 1973, 1975) given as

$$H_T = 1 - \sum_{i=1}^{k} \bar{x}_i^2$$

where $\bar{x}_i$ is the mean frequency of the ith of k alleles. H_T is a measure of the mean heterozygosity expected under random mating. Total gene diversity may be partitioned into average gene diversities within (H_S) and between (D_{ST}) populations where

$$H_T = H_S + D_{ST}.$$

Nei (1973, 1975) also defines an absolute measure of gene differentiation, $\tilde{D}_m$, independent of gene diversities within subpopulations, which serves as a measure of the minimum net codon differences between populations. The estimate of $\tilde{D}_m$ is (Nei, 1975)

$$\tilde{D}_m = sD_{ST}/(s - 1)$$

where s is the number of subpopulations sampled. Both D_{ST} and $\bar{D}_m$ are absolute measures of gene differentiation, but $\bar{D}_m$ excludes comparisons of populations with themselves and is therefore appropriate for comparing gene differentiation in different organisms. $\bar{D}_m$ is also useful for computing the interpopulational gene diversity relative to the intrapopulational gene diversity given by Nei (1975) as

$$R_{ST} = \bar{D}_m/H_S.$$

Gene diversity statistics for 21 loci in pitch pine are presented in Table 2. Averaging over all loci, the total gene diversity (H_T) in pitch pine is 0.152. For any one locus, the largest proportion of this diversity is attributable to within population diversity (H_S) which ranged from 0.45 for *Aco* to 0.003 for *Adh* with a mean value of 0.147. R_{ST} averaged over all loci is 0.026 indicating that there is approximately 3% as much variation between populations as there is within populations (Table 2).

Analysis of Population Genetic Structure

The organization of genetic variation in pitch pine populations was examined using the F-statistics developed by Wright (1951, 1965, 1969; Kirby, 1975; Nei, 1977). This method was originally devised to examine structuring in hierarchical populations utilizing the correlation between uniting gametes within and among subpopulations and for the population as a whole. These statistics have as a common focal point the fixation index, F, which represents the deviation from Hardy-Weinberg proportions due to the combined effects of finite population size, selection, inbreeding, and other forces shaping the genetic makeup of the population.

The estimate of F, corrected for finite population size (Kirby, 1975), is

$$F = 1 - H/2pq\left(1 + \frac{1}{2N - 1}\right)$$

where H is the observed number of heterozygotes and $2pq\left(1 + \frac{1}{2N - 1}\right)$ is the expected number. The mean fixation index over all subpopulations is $\hat{F}_{IS}$ (Kirby, 1975) and represents the average deviation of the population's genotypic proportions from Hardy-Weinberg equilibrium.

The extent of heterogeneity among subpopulations is estimated by F_{ST}, the correlation between random gametes within subpopulations relative to those of the total population. For multiple alleles at a locus, the estimate of F_{ST} is

$$F_{ST} = \sigma_p^2/\bar{p}\bar{q}$$

where σ_p^2 is the weighted sum of squared deviations of the subpopulation gene frequencies from the mean gene frequency and $\bar{p}$ and $\bar{q}$ are weighted mean gene frequencies. Nei (1975) has shown that his coefficient of gene differentiation, G_{ST}, is equivalent to F_{ST} and is computationally easier. Differences between estimates of F_{ST} and G_{ST} are negligible.

The overall fixation index, F_{IT}, represents the correlation between uniting gametes relative to the total population and was estimated indirectly as

$$F_{IT} = F_{IS} + (1 - F_{IS})F_{ST}.$$

Estimates of $\hat{F}_{IS}$, F_{ST} and F_{IT} are given in Table 3 for 21 enzyme loci. Mean values of $\hat{F}_{IS}$ are at or near zero for all loci, indicating that the observed genotypic arrays are approximately those expected at Hardy-Weinberg equilibrium. Treating the entire species as the unit of random mating, estimates of the total fixation index, F_{IT}, are only slightly larger on average than F_{IS} for the loci surveyed. While a large increase in heterozygote deficiency might be expected to accompany this hierarchical step, it was in fact only about 2%.

For 18 of the 21 loci, a slight deficiency of heterozygotes was indicated by positive values of F_{IT}. To test whether these values represented significant deviations from panmixia, we calculated one-tailed Chi-square values according to the formula of Li and Horvitz (1953):

$$\chi^2 = F^2N(k - 1)$$

for

$$k(k - 1)/2$$

TABLE 2. *Estimates of gene diversity parameters for 21 pitch pine loci.*

Locus	H_T	H_S	D_{ST}	$\bar{D}_m$	R_{ST}
Mdh-1	.0507	.0500	.0007	.0009	.0174
Mdh-2	.4439	.4233	.0206	.0226	.0534
Idh	.2007	.1869	.0138	.0152	.0812
Fum	.0098	.0097	.0001	.0001	.0073
Pgm-1	.0478	.0475	.0003	.0004	.0075
Pgm-2	.0440	.0431	.0009	.0010	.0223
Pgi-1	.0183	.0181	.0002	.0002	.0133
Pgi-2	.0544	.0537	.0007	.0007	.0140
6-Pgd-1	.3890	.3741	.0148	.0163	.0436
6-Pgd-2	.3916	.3809	.0107	.0118	.0309
G-6-pd	.1573	.1542	.0030	.0033	.0214
Lap-1	.1924	.1883	.0042	.0046	.0243
Lap-2	.3194	.3139	.0055	.0060	.0192
Got-1	.1342	.1316	.0026	.0029	.0218
Got-2	.1151	.1136	.0014	.0016	.0138
Acp	.0705	.0695	.0009	.0010	.0150
Aco	.4852	.4650	.0201	.0222	.0477
Gdh	.0044	.0043	.0001	.0001	.0257
Adh	.0028	.0028	.0000	.0000	.0029
Ald-1	.0159	.0157	.0002	.0002	.0130
Ald-2	.0465	.0442	.0023	.0025	.0571
Mean	.1521	.1472	.0049	.0054	.0263

TABLE 3. *Estimates of* $\hat{F}_{IS}$, F_{IT}, F_{ST}, *and* G_{ST} *for 21 loci in pitch pine.*

Locus	$\hat{F}_{IS}$	F_{IT}	F_{ST}	G_{ST}
Mdh-1	0.010	0.024	0.016	0.016
Mdh-2	0.130	0.168	0.050	0.046
Idh	−0.001	0.078	0.069	0.069
Fum	0.000	0.007	0.007	0.007
Pgm-1	0.037	0.045	0.007	0.007
Pgm-2	−0.002	0.019	0.022	0.020
Pgi-1	−0.101	−0.088	0.012	0.012
Pgi-2	0.000	0.014	0.015	0.013
6-Pgd-1	−0.050	−0.003	0.038	0.038
6-Pgd-2	0.014	0.047	0.029	0.027
G-6-pd	0.031	0.053	0.019	0.019
Lap-1	0.014	0.037	0.020	0.022
Lap-2	0.027	0.049	0.022	0.017
Got-1	0.005	0.026	0.019	0.019
Got-2	0.033	0.046	0.012	0.012
Acp	−0.039	−0.021	0.014	0.013
Aco	0.073	0.117	0.044	0.042
Gdh	0.000	0.023	0.023	0.023
Adh	0.000	0.003	0.003	0.003
Ald-1	0.002	0.015	0.010	0.012
Ald-2	0.001	0.048	0.061	0.049
Mean	0.009	0.034	0.024	0.023

degrees of freedom where F is the estimate of F_{IT} for a population sample, N, with k alleles. Values were not significant for any locus.

The extent of genic differentiation among populations as measured by F_{ST} is in agreement with the diversity analysis. F_{ST} values range from 0.003 for *Adh* to 0.069 for *Idh* with a mean over all loci of 0.024. Chi-square values for gene frequency heterogeneity reported earlier (Guries and Ledig, 1981) were significant ($P < .005$) for 15 of the 16 polymorphic loci, indicating that population differences do exist for the 11 populations studied. However, the extent of differentiation appears remarkably small in view of the fact that the sample transect extends over 1,000 km in both the north-south and east-west directions.

Genetic Distance

The concept of genetic distance was developed to utilize electrophoretic data as a measure of the accumulated number of detectable gene substitutions per locus (Nei, 1972). Estimates for the genetic identity function, I, and genetic distance, D, are given in Table 4. Values of I range from 0.999 between several central populations to 0.980 between St. Chrysostôme, Quebec and the population at Helmetta, New Jersey. The average identity between 55 pairs of populations is 0.995. A positive correlation ($r = 0.26$) exists between the genetic and geographic distances but it is only marginally significant ($.1 > P > .05$), and explains less than 7% of the variation in gene frequency differences among populations.

DISCUSSION

The most important conclusions to be drawn from these analyses are that pitch pine contains an appreciable amount of genic variation, but this variation is distributed in a manner which suggests that little differentiation has occurred among populations. The existence of high levels of genic variation in plants in general is becoming well documented, although data for forest tree species are still limited (Hamrick et al., 1979). Among vascular plants, woody species appear to be the

TABLE 4. *Estimates of genetic identity* I *(below diagonal) and genetic distance,* D *(above diagonal) based upon data from 21 loci.*

Population	ST CH	BF	MS	BPFR	SSF	HM	WP	EP	LSF	BR	BLR
ST CH		.016	.014	.015	.015	.020	.013	.014	.014	.013	.016
BF	.984		.004	.002	.002	.003	.001	.002	.001	.002	.001
MS	.986	.996		.005	.008	.005	.006	.005	.005	.005	.003
BPFR	.985	.998	.995		.005	.006	.004	.003	.003	.004	.001
SSF	.985	.998	.990	.995		.007	.004	.008	.005	.001	.006
HM	.980	.997	.995	.994	.993		.002	.004	.001	.002	.004
WP	.987	.999	.994	.996	.996	.998		.002	.001	.001	.003
EP	.986	.998	.995	.997	.992	.996	.998		.001	.003	.001
LSF	.986	.999	.995	.997	.995	.999	.999	.999		.001	.002
BR	.987	.998	.995	.996	.999	.998	.999	.997	.999		.003
BLR	.984	.999	.997	.999	.994	.996	.997	.999	.998	.997	

most polymorphic with a mean polymorphic index (PI = expected proportion of heterozygotes at equilibrum) of 0.354 based on nine species (Hamrick, 1979). The expected heterozygote frequency in pitch pine was less than half this amount (0.146). We attribute this difference in estimates of heterozygosity primarily to the number and choice of loci examined. Estimates for other woody species summarized by Hamrick (1979), were based on a mean of 6.8 loci usually included because they were known to be polymorphic. Recent estimates of observed average heterozygosity based upon 20 or more loci are listed in Table 5 for several woody species. These estimates range from 0.123 for ponderosa pine to 0.160 for lodgepole pine. We conclude that variation in degree of heterozygosity exists among woody plant species, perhaps as a response to the considerable temporal and spatial heterogeneity experienced by long-lived, widely distributed perennials. However, it also seems clear that some earlier estimates based upon a small number of very polymorphic loci have led to inflated estimates of heterozygosity in woody plants.

In all 11 populations, the expected heterozygosity exceeded the observed heterozygosity by 0–2% (Table 1). While the bulk of our data supports the conclusion that little population substructuring or differentiation has occurred, the consistent though nonsignificant deficiency of heterozygotes may be due to one or more of several factors. Brown (1979) has reviewed this so-called heterozygosity paradox wherein outbreeders show a deficit of heterozygotes while inbreeders show a surplus of heterozygotes, relative to expectations. Although we cannot exclude such possible explanations as dominant alleles at modifer loci, and negative heterosis (Brown, 1979), it seems more likely that the pooling of individuals from several breeding groups (Wahlund effect) is responsible. Our collection procedure (described earlier) sometimes required sampling all trees within a radius of 50 m. At

TABLE 5. *Estimates of observed heterozygosity* (H_o) *for recent studies of genic variation in forest trees employing 20 or more loci.*

Species	Number of populations	Number of loci	H_o	Reference
Pinus rigida	11	21	0.138	Present study
P. ponderosa	10	23	0.123	O'Malley et al., 1979
P. contorta	9	25	0.161	Yeh and Layton, 1979
Pseudotsuga menziesii	11	21	0.155	Yeh and O'Malley, 1980
Picea sitchensis[1]	10	24	0.150	Yeh and El-Kassaby, 1979

[1] Heterozygosity estimated from gene frequencies without genotyping mother trees.

TABLE 6. *F-statistics reported for several plant species.*

Species	Number of loci	F_{IS}	F_{ST}	F_{IT}	Reference
Polygonum pensylvanicum	3	0.67	0.12	0.71	Kubetin and Schaal, 1979
Liatris cylindracea	15	0.41	0.07	0.43	Schaal, 1975
Sarracenia purpurea	4–5	−0.10	0.23	—	Schwaegerle and Schaal, 1979
Phlox cuspidata	5	0.67	0.41	0.80	Levin, 1978
Phlox roemariana	4	0.42	0.21	0.54	Levin, 1978
Oenothera laciniata	5	0.13	0.24	0.37	Ellstrand and Levin, 1980
Oenothera grandis	6	0.04	0.09	0.19	Ellstrand and Levin, 1980
Desmodium nudiflorum	5	0.04	0.17	0.20[1]	Schaal and Smith, 1980
Pinus rigida	21	0.01	0.02	0.03	Present study

[1] F_{IT} estimated indirectly from data as $F_{IT} = F_{IS} + (1 - F_{IS})F_{ST}$.

the extremes, trees as far apart as 100 m were considered as part of a single population. While we have no estimate of neighborhood size in pitch pine, the slight deficiencies of heterozygotes noted could be explained on the basis of matings among closely adjacent individuals within a stand.

An alternative explanation documented for *Eucalyptus obliqua* (Brown et al., 1975) involves partial self-pollination. Although pitch pine, like most conifers, exhibits pronounced inbreeding depression on selfing (Wright, 1976), a modest amount of inbreeding could bias estimates of F upwards. The examination of embryo genotypes from mother trees heterozygous for a rare allele can provide estimates of self-pollination, as such trees are expected to yield a proportion of embryos which are homozygous for such alleles if selfing occurs (Müller, 1976). Using this procedure, we estimate that self-pollinations account for about 5% of the fertilizations which occur in natural populations (Guries and Ledig, unpubl.). However, both the Wahlund effect, and a small amount of inbreeding each generation, probably contribute to the slight deficiency of heterozygotes noted in these populations.

The organization of gene diversity in pitch pine is quite comparable to that observed for other conifers. Approximately 97% of the genic variation in pitch pine resides within stands, as compared with 88% for ponderosa pine in the northern Rocky Mountains (O'Malley et al., 1979), 96% for lodgepole pine in British Columbia (Yeh and Layton, 1979), and 97% for Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco) in coastal British Columbia (Yeh and O'Malley, 1980).

For purposes of comparison among a number of organisms, $\bar{D}_m$ is a better measure of gene differences than D_{ST} (Nei, 1975). Values of $\bar{D}_m$ for most organisms range from 1–3%, but values as large as 8% have been observed for certain insects (Varvio-Aho, 1979) and plants ($\bar{D}_m$ = 0.085 for *Phlox roemariana* calculated from data of Levin, 1978). The value of 1% observed for pitch pine lies at the low end of the differentiation spectrum relative to most other organisms.

The F-statistics and genetic distance analyses confirm that relatively little genetic differentiation has occurred in pitch pine. Table 6 lists summary F-statistics for several plant species. It is clear that populations of pitch pine come closer to approximating single panmictic units than do most other species. The high values of F_{IS} observed for knotweed (*Polygonum pensylvanicum* L.), phlox species, and cylindric blazing star (*Liatris cylindracea* Michx.) are probably due to consanguineous mating. Despite some mechanisms which promote outcrossing, mating among relatives appears to be quite common in most herbs because of limited seed dispersal and the tendency of insect pollinators to travel between adjacent flowers (Levin and Kerster, 1968). Crossing among neighbors may be equivalent to crossing among relatives, resulting in heterozygote deficiencies. F_{IS} values are much lower in pitcher plant (*Sarracenia purpurea* L.) and

tick-trefoil (*Desmodium nudiflorum* [L.] DC) than in knotweed, phlox, or blazing star, perhaps as a result of differences in population size, gene flow, or other factors which have affected the ecological history of these species (Schwaegerle and Schaal, 1979; Schaal and Smith, 1980).

Pitch pine is a fugitive species, often colonizing areas after wildfire, so that stands may originate from a few parents which happen to be within dispersal distances of the site. Even when it succeeds itself, it follows fire, which may spare a relatively few scattered trees that eventually regenerate the site. Exceptions are those areas where seed is stored in serotinous cones so that even individuals killed by fire contribute to the next generation. But in most areas, fire creates a bottleneck, and bottlenecks should lead to the loss of alleles, reducing heterozygosity. However, the same mechanism would also promote differentiation among populations, a feature not characteristic of pitch pine. The similarity in allele frequencies among stands suggests either effective gene flow, probably mediated by long-distance pollen dispersal, or range-wide similarity in selection pressures for the loci we scored. We favor the former explanation, but cannot exclude the latter. Significant correlations exist between allele frequencies of some genes and climatic variables (Guries and Ledig, 1981). Such correlations do not provide conclusive evidence for the operation of natural selection, but they do parallel phenotypic patterns of clinal variation noted for several metric traits considered important for growth and survival (Ledig and Fryer, 1972; Ledig et al.,1975, 1976). The clines may reflect historical events associated with past migration from glacial refugia in the southeastern United States. Many allozymes may be largely neutral, and the simplest explanation for both low levels of genic diversity and lack of differentiation could be that pitch pine was greatly reduced in numbers during glacial epochs and there has not been sufficient time for selection to sort out the remaining allozyme variants.

Populations at, or approaching, the margin of the pitch pine range, particularly St. Chrysostôme, appeared to have the lowest levels of heterozygosity, although the differences between central and peripheral populations are not great relative to the standard errors. Data for other plant and animal species (Ayala et al., 1972; Avise and Selander, 1972; Saura et al., 1973; Gorman et al., 1975; Rick et al., 1977; Yeh and Layton, 1979) suggest that marginal populations exhibit reduced variability, perhaps due to more intense selection in marginal environments, genetic drift, or greater inbreeding in small populations. Most such interpretations rephrase the conclusions of Carson (1955, 1959), Mayr (1963), and Dobzhansky (1970) based upon studies of chromosomal polymorphisms in *Drosophila* spp. However, patterns of genic variation in *Drosophila* have not paralleled necessarily the cytogenetic observations (Prakash, 1973). Without adequate documentation of changing environmental conditions over space and time, it is difficult at best to separate populations which are ecologically (but not necessarily geographically) marginal from those which are merely geographically peripheral. In a study of ecologically marginal and central populations of foxtail barley (*Hordeum jubatum* L.) Schumaker and Babbel (1980) concluded that central populations were more variable than marginal ones. Foxtail barley appears to respond to changes in habitat with corresponding changes in genotypic arrays within populations. By contrast, pitch pine is phenotypically variable (Ledig and Fryer, 1974) and may adapt to changes in microhabitat via phenotypic plasticity rather than by changes in gene frequency.

Two of the 11 populations examined (East Plains and West Plains, New Jersey) were included because they represented forests of dwarf trees predominantly 1–2 m in height, completely surrounded by the tall forest of the New Jersey Pine Barrens. Environmental hypotheses advanced to account for the dwarf stature of the Pine Barrens and later abandoned include im-

poverished soils, hard pan, frequent and extreme fires, and toxic levels of soluble aluminum (Andresen, 1959; McCormick and Buell, 1968). Recent results of "common garden" experiments indicate that the dwarfism exhibited by these Pine Plains populations has a genetic basis (Good and Good, 1975; Ledig et al., 1976). Pine Plains trees are genetically distinct from those in the Barrens in other characteristics as well. The Pine Plains populations have a much higher frequency for the serotinous cone trait than Barrens populations (Ledig and Fryer, 1972) and individuals mature earlier (Ledig and Little, 1979). However, there has been little change at enzymatic loci, as evidenced by the high genetic identity values between the Pine Plains populations and Pine Barrens populations in New Jersey.

The similarity between populations of pitch pine for allozyme loci contrasts sharply with phenotypic differences in form, growth, wood properties, and the morphology of seed, cone, and needles observed in situ (Ledig and Fryer, 1974) and genetic differences in growth, phenology, and cone production revealed in common garden studies (Ledig et al., 1976; Ledig and Little, 1979). The distribution of variation for morphological characteristics was not the same as that for allozymes; for growth, almost all variation was among populations and variation within populations was nil (Ledig et al., 1976). On the other hand, pitch pine proved relatively uniform both among and within populations for physiological characteristics such as photosynthetic and respiratory response to temperature and light (Ledig and Clark, 1977; Ledig et al., 1977). In another well-studied example, the slender wild oat (*Avena barbata* Brot.), no single class of loci gave a complete picture of the organization of genic variability (Allard et al., 1978; Kahler et al., 1980). In Indian amaranths (*Amaranthus* sp.) morphological variation is striking while enzymes are nearly monomorphic (Jain et al., 1980). These comparisons emphasize that morphological and metrical traits are a set of characteristics distinct from allozymes which may be more directly exposed to environmental selection and, therefore, more likely to differentiate populations. An examination of both types of loci is necessary to understand the genetic structure of a species.

Summary

Genic diversity and the organization of genetic variability in pitch pine were examined in 11 populations across the species range. Pitch pine is genetically variable; 76.2% of the loci studied were polymorphic and the average individual heterozygosity was 14.6%, but this is less than that exhibited by several other woody species. The contrast may reflect differences among species in their evolutionary history, or may be an artifact relating to the choice of enzyme systems analyzed.

Only a small percent of the observed genic diversity in pitch pine appears to be interpopulation, the remainder is due to differences between individuals within populations, in agreement with results for other tree species. Analysis of F-statistics indicates that populations of pitch pine simulate panmixis with an $\bar{F}_{IS}$ of 0.009. Populations are only weakly differentiated, and genetic and geographic distance are only weakly correlated.

Central populations seem to be more variable than those near the species border. An isolated population at the northern extreme of the species range has a heterozygosity of 11.7%, compared to the species average of 13.8% and an average of 15.0% for central populations from New Jersey.

The dwarf forest populations of the New Jersey Pine Plains are essentially identical in genic constitution to tall forest of the New Jersey Pine Barrens, at least for the allozyme loci we sampled. Whatever factors are responsible for the dwarf stature of these populations, they have not resulted in detectable changes in allozyme frequencies among populations. In general, results of allozyme analysis are not in agreement with previously reported patterns for morphological and growth traits, emphasizing the need to sample

several types of loci to adequately understand the genetic structure of populations.

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APPENDIX 1. *Collection sites and localities for 11 pitch pine populations in eastern U.S. and Canada.*

Name	Abbrev	Lat.	Long	Elev. (m)	Locality
St. Chrysostôme	ST CH	45°15′	73°30′	75	W side of Rocher Rd., 8 km NW of St. Chrysostôme, Quebec Prov., CANADA
Bradley Field	BF	41°45′	72°45′	50	.5 km N of Rte. 20, adj. to Bradley Field, Hartford Co., CT
Marconi Station	MS	42°00′	70°00′	5	N side of Beach Rd., 1 km S of Marconi Station Hqtrs., Cape Cod National Seashore, Barnstable Co., MA
Big Pine Flat Ridge	BPFR	40°00′	77°45′	150	adj. to Big Flat Fire Control Center, 2 km W of Rte. 233, Franklin Co., PA
Shawnee State Forest	SSF	38°30′	83°00′	250	adj. to Pigeon Roost Bridle Path, and F.S. Rd. 6, Scioto Co., OH
Helmetta	HM	40°30′	74°30′	95	S side of Helmetta Blvd., 1 km E of Rte. 535, Middlesex Co., NJ
West Plains	WP	40°00′	74°15′	45	S side of Rte. 72, 7 km N of Chatsworth, Burlington Co., NJ
East Plains	EP	40°00′	74°15′	45	N side of Rte. 532, 5 km E of Chatsworth, Burlington Co., NJ
Lebanon State Forest	LSF	40°00′	74°15′	45	E side of Butler Place Rd., 1 km W of Rte. 72, Burlington Co., NJ
Big Run	BR	38°30′	79°00′	760	N side of Rte. 730, 6 km W of Ottobine, George Washington National Forest, Rockingham Co., VA
Blue Ridge	BLR	36°30′	81°45′	915	W side of Blue Ridge Parkway near Big Pine Creek, Alleghany Co., NC

APPENDIX 2. *Gene frequency data (21 loci) from 11 populations of pitch pine.*

Locus/ allele		St. Chryso-stôme, Quebec	Bradley Field, CT	Marconi Stn., MA	Big Pine Flat Ridge, PA	Shawnee State Forest, OH	Helmetta, NJ	W. Plains, NJ	E. Plains, NJ	Lebanon Lakes, NJ	Big Run, VA	Blue Ridge, NC
Mdh-1	*1.25*	—	—	.008	—	—	—	.030	.004	.022	—	—
	1.00	.982	.978	.935	.974	.976	.959	.962	.996	.964	1.00	1.00
	.71	.018	.022	.056	.026	.024	.041	.008	—	.014	—	—
Mdh-2	*1.95*	—	.022	.024	—	.024	—	.013	.008	.007	—	.048
	1.00	.473	.717	.484	.731	.610	.721	.742	.775	.775	.641	.702
	.80	.527	.250	.444	.256	.366	.270	.242	.205	.217	.333	.250
	.50	—	.011	.048	.013	—	.008	.004	.012	—	.026	—
Idh	*1.13*	.364	.054	.121	.077	.037	.041	.072	.160	.087	.103	.083
	1.00	.636	.946	.879	.923	.963	.959	.928	.840	.913	.897	.917
Fum	*1.21*	—	.011	.008	—	—	.016	.004	—	.007	—	—
	1.00	1.00	.989	.992	1.00	1.00	.984	.996	1.00	.986	1.00	1.00
	.79	—	—	—	—	—	—	—	—	.007	—	—
Pgm-1	*1.06*	.009	.054	.032	.013	.024	.008	.038	.025	.022	.013	.012
	1.00	.991	.946	.968	.987	.976	.992	.962	.975	.978	.987	.988
Pgm-2	*1.10*	—	—	—	—	—	—	—	.012	.022	—	—
	1.00	1.00	.989	1.00	.962	.951	.934	.987	.984	.956	.974	1.00
	.87	—	.011	—	.038	.049	.066	.013	.004	.022	.026	—
Pgi-1	*1.06*	—	—	.032	.013	.024	—	.008	.004	—	.026	.012
	1.00	1.00	1.00	.968	.987	.976	1.00	.992	.996	1.00	.974	.988
Pgi-2	*1.11*	—	.011	.016	.026	—	.016	.008	.004	—	.013	—
	1.00	1.00	.978	.968	.936	1.00	.968	.975	.947	.986	.962	1.00
	.86	—	.011	.016	.038	—	.016	.017	.049	.014	.026	—
6-Pgd-1	*1.06*	—	—	—	—	—	.066	.081	.020	.022	.013	—
	1.00	.964	.707	.758	.808	.622	.623	.716	.803	.768	.641	.798
	.94	—	.011	—	—	—	—	—	.004	—	.026	.012
	.91	.036	.283	.246	.192	.378	.262	.195	.172	.210	.321	.190
	.85	—	—	—	—	—	.049	.008	—	—	—	—
6-Pgd-2	*1.07*	.009	.022	—	.026	—	.025	.013	.012	.022	.026	.036
	1.00	.827	.783	.726	.859	.902	.598	.754	.689	.703	.731	.738
	.93	.161	.196	.274	.115	.098	.377	.233	.295	.275	.244	.226
	.86	—	—	—	—	—	—	—	.004	—	—	—
G-6-pd	*1.03*	—	.065	.113	.013	.073	.082	.072	.123	.094	.128	.119
	1.00	1.00	.935	.887	.987	.927	.918	.928	.872	.906	.872	.869
	.67	—	—	—	—	—	—	—	.004	—	—	.012
Lap-1	*1.00*	.955	.815	.887	.962	.902	.951	.915	.824	.899	.936	.881
	.95	.045	.043	.048	.038	.037	.016	.025	.086	.065	.013	.083
	null	—	.141	.065	—	.061	.033	.059	.090	.036	.051	.036
Lap-2	*1.02*	.036	.076	.113	.038	.012	.066	.081	.061	.072	.051	.048
	1.00	.764	.826	.750	.910	.927	.770	.775	.824	.775	.885	.893
	.96	.200	.098	.137	.051	.061	.164	.144	.115	.152	.064	.060
Got-1	*1.07*	—	.011	.097	.051	.037	.107	.064	.041	.058	.090	.024
	1.00	1.00	.957	.879	.949	.963	.893	.924	.947	.899	.872	.940
	.77	—	.032	.024	—	—	—	.012	.012	.043	.038	.036
Got-2	*1.95*	.055	.120	.032	.038	.061	.049	.076	.033	.080	.038	.107
	1.00	.945	.880	.968	.962	.939	.951	.924	.967	.920	.949	.893
	.05	—	—	—	—	—	—	—	—	—	.013	—
Acp	*1.15*	—	—	—	—	.012	—	—	—	—	—	—
	1.00	.982	.978	.944	.987	.988	.926	.962	.967	.942	.974	.988
	.85	.018	—	.048	—	—	.074	.038	.033	.058	.013	—
	null	—	.022	.008	.013	—	—	—	—	—	.013	.012

APPENDIX 2. *Continued.*

Locus/ allele		St. Chryso-stôme, Quebec	Bradley Field, CT	Marconi Stn., MA	Big Pine Flat Ridge, PA	Shawnee State Forest, OH	Helmetta, NJ	W Plains, NJ	E. Plains, NJ	Lebanon Lakes, NJ	Big Run, VA	Blue Ridge, NC
Aco	*1.08*	.155	.348	.468	.436	.195	.279	.242	.348	.283	.205	.369
	1.00	.836	.620	.484	.526	.756	.598	.737	.607	.688	.744	.488
	.87	.009	.033	.048	.038	.024	.123	.021	.045	.029	.051	.143
	.56	—	—	—	—	.024	—	—	—	—	—	—
Gdh	*1.25*	—	—	—	—	—	.025	—	—	—	—	—
	1.00	1.00	1.00	1.00	1.00	1.00	.995	1.00	1.00	1.00	1.00	1.00
Adh	*1.00*	1.00	1.00	1.00	1.00	1.00	1.00	.996	.996	1.00	1.00	1.00
	.89	—	—	—	—	—	—	.004	.004	—	—	—
Ald-1	*1.08*	.009	—	—	—	—	—	.017	.008	—	—	—
	1.00	.991	1.00	1.00	1.00	1.00	1.00	.983	.975	1.00	1.00	1.00
	.92	—	—	—	—	—	—	—	.017	—	—	—
Ald-2	*1.13*	—	—	—	—	—	—	—	.008	.014	—	—
	1.00	.873	.967	1.00	.974	.988	1.00	.996	.988	.971	.949	.988
	.67	.127	.033	—	.026	.012	—	.004	.004	.014	.013	.012
	null	—	—	—	—	—	—	—	—	—	.038	—