GENETIC DIVERSITY OF MARGINAL VS. CENTRAL POPULATIONS OF PITCH PINE AND JACK PINE[1]

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ABSTRACT

Pitch pine (*Pinus rigida* Mill.) and jack pine (*P. banksiana* Lamb.) were examined electrophoretically to compare the extent and distribution of genetic variability between marginal and central populations. Seeds were collected from three central pitch pine (MA, PA, NJ), three marginal pitch pine (VT, PQ), two central jack pine (ON) and two marginal jack pine (NY, NH) populations. Starch-gel electrophoresis was used to identify and confirm alleles at 33 loci in pitch pine and 29 loci in jack pine. Pitch pine was found to have a relatively high degree of genetic variability, with 63% polymorphic loci and 22.8% expected heterozygosity, while jack pine was found to have a lower degree of genetic variability, with 44.1% polymorphic loci and 14.8% expected heterozygosity. Marginal populations of pitch pine had fewer polymorphic loci and lower expected heterozygosity than central populations. Genetic distance measures and chi-square tests of heterogeneity, combined with examination of gene frequency differences, showed that differences between marginal and central populations were most heavily influenced by the two smallest, most isolated, marginal populations. We propose that genetic drift has caused the differentiation in pitch pine. In jack pine, there was no difference in level of genetic variability between marginal and central populations, despite the fact that one of the marginal populations was extremely small in size (N = 75) and extremely isolated from other populations. Genetic distance measures showed this population to be genetically different from the other populations studied, and it is believed that genetic drift, combined with selection against inbred individuals, has caused the differentiation. For both species, marginal populations are genetically distinct from central populations, and may therefore be considered valuable sources of genetic diversity.

INTRODUCTION

Genetic drift, selection, and migration are three factors responsible for determining the patterns of genetic variation after it has arisen from mutation and recombination. While migration tends to keep populations genetically similar, drift and selection tend to promote population differentiation. Geographically marginal, disjunct populations might be expected to be genetically distinct from central populations because drift and/or selection will act in these populations without the moderating effect of migration. Selection is expected to be particularly strong in marginal

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populations because they exist under extreme environmental conditions for the species. Selection is normally thought to decrease variation by eliminating unfavorable phenotypes, and this was believed to be the case in a rangewide study of lodgepole pine (Pinus contorta Dougl.), which showed a definite trend towards decreased genetic variability in marginal populations (Yeh and Layton 1979). However, it is also possible that selection may favor heterozygotes, and therefore increase variation in stressed populations. This was believed to be the case for a balsam fir (Abies balsamea (L.) Mill.) population that showed a significantly higher proportion of progeny from outcrossing than other populations (Neale and Adams 1985). Similarly, Douglas-fir (Pseudotsuga menziessi Mirb.) was found to have a high degree of heterozygosity, and Yeh and O'Malley (1980) suggested this was due to the wide range of climates and sites occupied by this species. Genetic drift is also expected to be a strong force in marginal populations because they are usually small and may be inbred. By comparing large marginal populations to small marginal populations, all isolated and subjected to similar environmental pressures, one can judge the relative importance of drift and selection in shaping the genetic structure of marginal populations.

The Champlain Valley of Vermont is estimated to have populations of 27 tree species at the margins of their ranges (DeHayes and Bassett 1980). The valley has many unusual species for this latitude because it has a moderate climate compared to surrounding areas, due in part to its low elevation and to its proximity to Lake Champlain. Marginal populations of both pitch pine (Pinus rigida Mill.) and jack pine (P. banksiana Lamb.) occur in this area. While pitch pine ranges across the southeastern United States, jack pine is a northern species occurring over much of Canada. The purpose of this study was to examine the extent and distribution of genetic variation in central and marginal populations of these two species of pine by comparing isozyme variability detected by starch-gel electrophoresis. If marginal populations are genetically unique, they can be valuable as sources of genetic diversity vital to the survival of species under changing environmental conditions. Rapid development of the Champlain Valley is quickly eliminating many of these unique ecosystems. For example, many of the pitch pine trees from which seeds were collected for this study have already been cleared to allow construction of a shopping mall. Therefore, it is important to recognize any unique populations existing in this area as quickly as possible so they may be protected.

MATERIALS AND METHODS

Seeds were collected from trees representing six pitch pine populations, three of which are central and three marginal, and four jack pine populations, two of which are central and two marginal (Table 1). For both species, the marginal populations sampled are also disjunct from the continuous range, thereby minimizing the probability of pollen flow between marginal and central populations. Three of the marginal populations are very small in size, number of individuals, and stand area, while two are fairly large. All marginal populations are located in northern New England or adjacent areas, suggesting they have been subjected to similar environmental stresses.

Haploid megagametophytes were excised from germinants and examined by starch-gel electrophoresis to quantify and identify genetic variability at 33 pitch pine loci and 29 jack pine loci. Sampling followed Morris and Spieth (1978), who recommended sampling one female gametophyte per tree to maximize the efficiency of estimating allelic frequencies in a population. Approximately 60 independent gametes per locus per population were sampled in order to have a 95% probability of detecting an allele with a frequency of 5%. It is possible to sample 60 genes either by assaying one megagametophyte from 60 different individuals, or by assaying seven megagametophytes from each of 30 different individuals and determining both their maternal and paternal gametic contributions. The latter method requires more laboratory effort, but is advantageous when seed is available only from a limited number of individuals, or when one wishes to measure observed heterozygosity. Individual tree genotypes were determined for two populations, one of jack pine and one of pitch pine, to confirm alleles and to sample an adequate number of genes. Electrophoretic methods followed those described by Jech and Wheeler (1984).

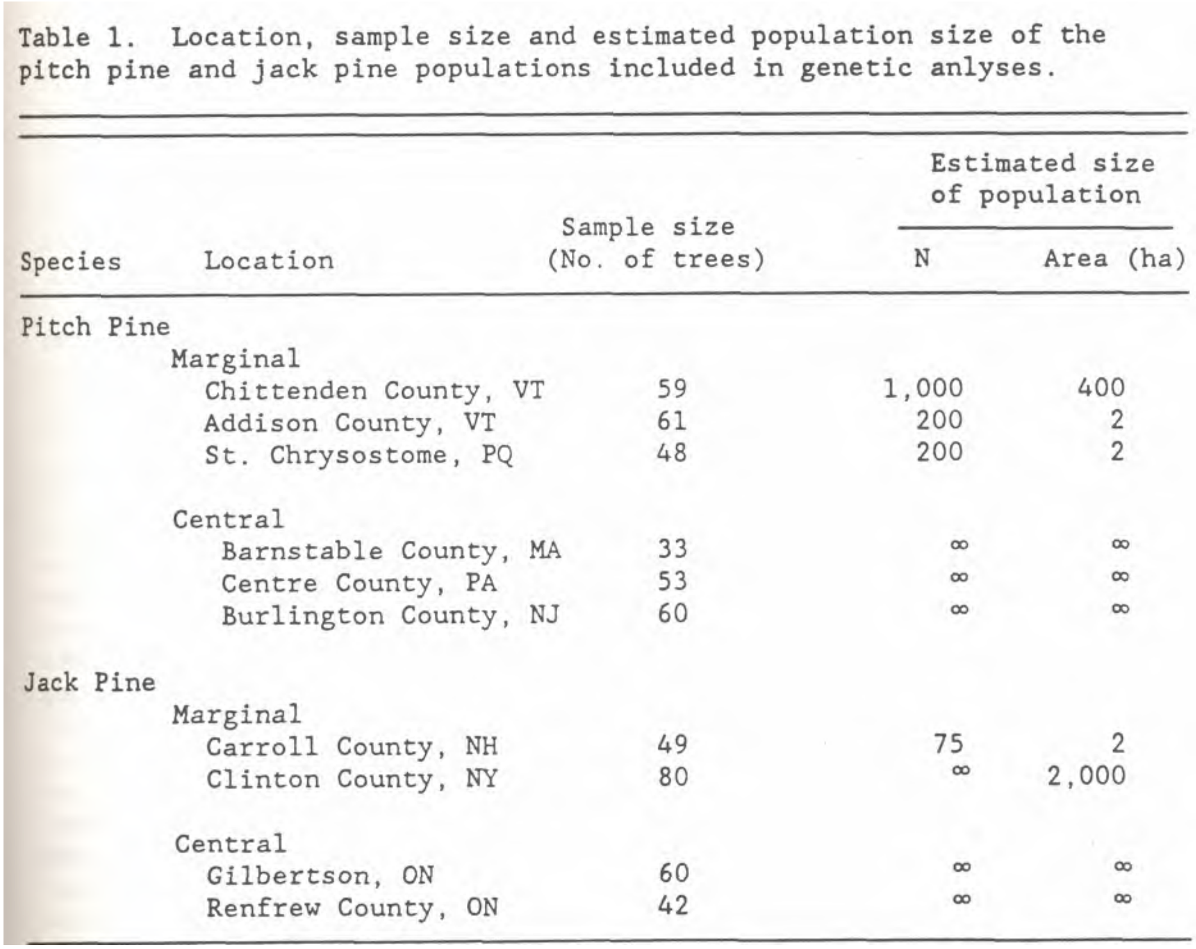

Table 1. **Location, sample size and estimated population size of the pitch pine and jack pine populations included in genetic anlyses.**

Species	Location	Sample size (No. of trees)	Estimated size of population N	Estimated size of population Area (ha)
Pitch Pine				
	Marginal			
	Chittenden County, VT	59	1,000	400
	Addison County, VT	61	200	2
	St. Chrysostome, PQ	48	200	2
	Central			
	Barnstable County, MA	33	∞	∞
	Centre County, PA	53	∞	∞
	Burlington County, NJ	60	∞	∞
Jack Pine				
	Marginal			
	Carroll County, NH	49	75	2
	Clinton County, NY	80	∞	2,000
	Central			
	Gilbertson, ON	60	∞	∞
	Renfrew County, ON	42	∞	∞

Measures of electrophoretic variability included: (1) proportion of polymorphic loci, (2) effective number of alleles per locus, and (3) expected heterozygosity assuming Hardy-Weinberg equilibrium (Nei 1975). A t-test was used to test for significant differences in the proportion of polymorphic loci between marginal and central populations. Analyses of variance were used to test for significant differences in effective number of alleles per locus and expected heterozygosity among populations, and between marginal and central populations. Total variation was partitioned into components due to locus, population, and population X locus interaction; the population component of variation was further subdivided to examine the contribution of population type (marginal versus central) and population within type. Nei's (1975) genetic distance was calculated between pairs of populations and used to generate a phenogram based on unweighted pair group matrix analysis (UPGMA). Chi-square tests of heterogeneity were used to determine the significance of differences in allelic frequencies between populations for each locus examined (Workman and Niswander 1970).

RESULTS AND DISCUSSION

Species Comparisons

Pitch and jack pines differ in level of genic diversity, but are similar in distribution of genetic variation. The average expected heterozygosity of the six pitch pine populations is 22.8%, while the average expected heterozygosity of the four jack pine populations is 14.8%. Pitch pine also exhibits genetic variation at about 64% of the loci assayed, compared to only 44% for jack pine. Despite substantial differences in degree of genic diversity between species, the distribution of genetic variation between and within populations is similar. For both species, 93% to 94% of the electrophoretically detectable genetic variation is distributed within populations. The concentration of genetic diversity within populations is partly a function of inherent differences in polymorphic expression among loci examined, as well as substantial variation among individuals within populations. Similar patterns of genetic variation have been reported for several other conifer species (Yeh and Layton 1979, Dancik and Yeh 1982, Curies and Ledig 1982, Wheeler and Curies 1982).

Although explanations for differences in the degree of genetic variation among distantly related species must be interpreted with caution, it is conceivable that differences between jack and pitch pine in level of genetic diversity may be related to the geographic distribution of the two species. The predominantly northern and longitudinal distribution of jack pine may have resulted in a drastic reduction in population numbers and a concomitant loss of genetic variation as the Wisconsin glaciation advanced through much of the species range. In contrast, the largely southern and latitudinal distribution of pitch pine probably resulted in relatively little reduction in population numbers and genetic diversity as a result of glaciation.

The genetic diversity parameters reported in this study of jack pine are similar to those derived from electrophoretic assessments of genetic variation in Ontario (Danzman and Buchert 1983) and Alberta (Dancik and Yeh 1983) populations of jack pine. However, this is not the case for pitch pine. In comparison to our heterozygosity estimate of 22.8% for pitch pine, Curies and Ledig (1982) reported an average expected heterozygosity of 14.6% in a range-wide assessment of genic diversity at 21 isozyme loci that included nine central and two marginal populations. The relatively low expected heterozygosity they reported is particularly surprising because our estimate was derived from a smaller portion of the range and a large proportion of potentially inbred marginal populations. The discrepancy between the studies does not appear to be a function of the number of loci examined. If one considers only the 17 loci that were common to the two studies, our estimate would still exceed theirs by a substantial margin. Presumably, differences in electrophoretic procedures or materials, such as gel and tray buffers or stain ingredients, are responsible for the discrepancy.

Comparison between central and marginal populations

Pitch pine. The average expected heterozygosity and proportion of polymorphic loci for marginal populations of pitch pine are significantly lower than for central populations, but no difference was found between marginal and central populations in the effective number of alleles per locus (Table 2). The relatively low genetic variation estimates for marginal populations of pitch pine can be attributed primarily to the small and isolated Addison County, VT and St. Chrysostome, PQ populations, which have expected heterozygosities 4% to 7% lower than those for the relatively large marginal population in Chittenden County, VT and all three central populations. The three marginal populations are located at the northern interior limit of the pitch pine range and might be expected to be subjected to extreme, but similar, environmental pressures. As a result, differences in the extent of genetic diversity among the marginal populations do not appear to be a result of directional selection for characteristics associated with the unique northern location of the stands. In fact, it appears that inbreeding associated with small population size (Table 1) and isolation has had a greater effect on the extent of genetic variability than either marginal location per se or extreme selection pressures.

Chi-square tests of heterogeneity revealed significant differences in gene frequencies among the six populations for 75% of the 33 loci examined. Inspection of the data revealed that gene frequencies were generally similar for the three central populations, but were frequently quite different among the three marginal populations and between most pairs of marginal and central populations. It was not uncommon for alleles that occurred at a consistently low frequency (2 to 10%) in central populations to fail to be detected in one or more marginal populations. For instance, an allele at the ME locus occurred at frequencies of 4, 9, and 12% in the three central populations, but was not detected in two of the three marginal populations. Similar patterns were evident at several other loci, including PGI-2, BEST, GR-1, NADHDH, 6PGD-1, and DIA-1. More important, however, were cases where marginal populations differed from each other and from central populations in the frequencies of alleles that were relatively common and consistent in central populations, or where unique alleles occurred with high frequency in a marginal population. An example of the latter situation was evident at the LAP-2 locus: an allele with a frequency of 0 to 2% in the three central populations occurred with a frequency of 17% in the small, isolated Addison County, VT population. Frequencies of alleles at several loci were consistent among central populations, but varied considerably and randomly among marginal populations (Table 3). Reduced genetic variation and the apparent random distribution of gene frequencies at many loci in the marginal populations suggest that genetic drift, either in the form of founder effects or an abrupt bottleneck in the northern part of the range, has been instrumental in shaping the nature and distribution of genetic variation in these northern interior disjunct populations of pitch pine. Although it is difficult to know whether founder events or a population bottleneck is responsible, post-hypsithermal climatic cooling has been suggested as a mechanism responsible for the vast reduction in the distribution of pitch pine in northern New England and Canada (Johnson 1980).

Table 2. Estimates of genetic variability for central and marginal populations of pitch pine and jack pine.

Species	Population	No. loci	Effective no. alleles/locus	% Polymorphic loci	% Expected heterozygosity
Pitch pine					
	Marginal				
	Chit., VT	32	1.471	62.5	23.7
	Add., VT	33	1.400	39.4	18.8
	St. Chrys., PQ	33	1.343	66.7	20.2
	Average		1.405	56.2**[1]	20.9**
	Central				
	Barn., MA	31	1.400	80.6	25.1
	Burl., NJ	33	1.476	72.7	25.0
	Centre, PA	33	1.410	60.6	23.7
	Average		1.429	71.3**	24.6**
Jack pine					
	Marginal				
	Carr., NH	30	1.319	46.4	17.5
	Clinton, NY	30	1.238	42.9	13.8
	Average		1.279	44.7	15.7
	Central				
	Gilb., ON	29	1.291	44.8	14.7
	Renfrew, ON	29	1.214	42.3	13.1
	Average		1.253	43.6	13.9

[1] Means in a column followed by ** are significantly different at p 0.01.

Table 3. Frequency of the most common allele at several loci that were significantly different in marginal populations compared to central populations of pitch pine.

	Marginal Populations			Central Populations		
Locus	Chit. VT	Add. VT	PQ	MA	NJ	PA
LAP-1	0.707	0.383	0.938	0.848	0.788	0.820
B-EST	0.508	0.383	0.652	0.606	0.635	0.459
6PGD-2	0.491	0.724	0.667	0.688	0.750	0.656
G6PD	0.542	0.459	0.604	-	0.885	0.900
MDH-3	0.288	0.246	0.174	0.409	0.538	0.393
MDH-4	0.627	0.803	0.438	0.803	0.692	0.656

Nei's genetic distance (1975), which is a pairwise comparison of allelic substitutions between populations, revealed little genetic differentiation among central populations. In contrast, marginal populations differed considerably from each other and the central populations. The average genetic distance among central populations is 0.010 (range of 0.007 to 0.014), while the average genetic distances among marginal populations and between marginal and central populations are 0.021 (range of 0.017 to 0.30) and 0.024 (range of 0.016 to 0.033), respectively. That is, marginal populations differ from central populations at about 2.4% of their loci. Genetic distances among marginal populations and between marginal and central populations are considerably greater than those reported among central populations of most conifer species (Yeh and O'Malley 1980, Dancik and Yeh 1982, Curies and Ledig 1982, Wheeler and Curies 1982, Boyle and Morgenstern 1984) and are testimony to the genetic uniqueness and biological value of these marginal populations of pitch pine. A phenogram based on UPGMA clustering of Nei's genetic distance estimates illustrates the genetic relationship among marginal and central populations of pitch pine (Figure 1). It is clear that the marginal populations are quite different from each other and from the central populations. The isolated population from St. Chrysostome, PQ is most distinct and very distantly related to all other populations examined. Although relatively low in genic diversity and probably somewhat inbred, these northern marginal populations of pitch pine are clearly genetically distinct and may represent a unique source of genetic diversity for the species.

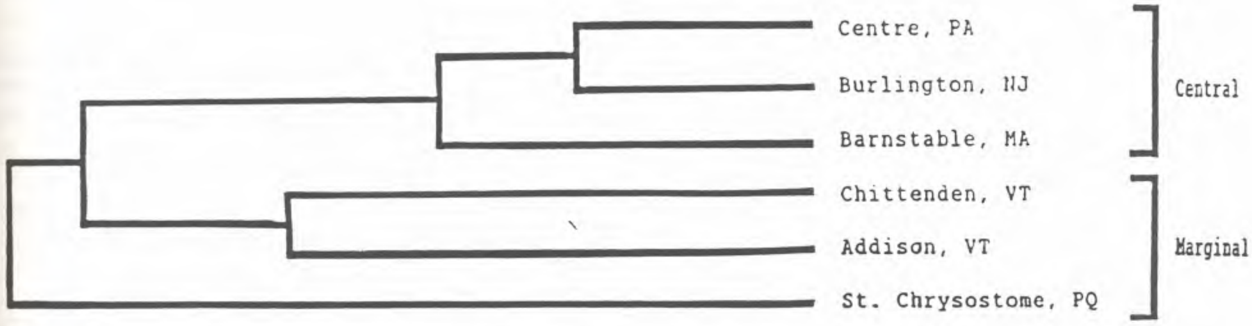

Figure 1. Phenogram of pitch pine populations based on UPGMA clustering of Nei's genetic distance measures.

Jack pine. No significant differences were found among jack pine populations in proportion of polymorphic loci, expected heterozygosity or effective number of alleles per locus (Table 2), even though the smallest and most isolated population of any studied was the New Hampshire population of jack pine. This population consists of about 75 individuals confined to a ledge on Mount Chocorua, with the nearest known population being small, isolated, and approximately 18 km away. Moreover, there are only four known naturally occurring populations of jack pine in the entire state, with none in the neighboring state of Vermont (Baldwin 1979). Despite its small size and almost complete isolation, this population has the largest proportion of polymorphic loci, the greatest number of effective alleles per locus, and the highest expected heterozygosity of all jack pine populations examined. In fact, the average expected heterozygosity of the New Hampshire population is 3 to 4% higher than the other populations. However, one would also expect this population to be the most inbred and, therefore, least genetically variable of the four populations examined. Assuming at least moderate levels of inbreeding in this population, it would appear that selection has eliminated most individuals resulting from consanguineous matings, and as a result, has maintained population heterozygosity at levels that are typical of outcrossed populations of jack pine. This is obviously in contrast to pitch pine, in which small isolated marginal populations exhibited reduced genetic variability, presumably as a result of inbreeding. Perhaps jack pine possesses a higher genetic load than pitch pine, resulting in more rapid elimination of individuals that are homozygous for deleterious alleles as a result of inbreeding.

Chi-square tests of heterogeneity indicated that 41% of the 29 loci examined exhibited significant differences in allelic frequencies among populations. Examination of the data reveals that gene frequencies are similar among the two central populations and the large marginal population in New York, but the small marginal population in New Hampshire is quite distinct. There is a tendency for alleles that occur at relatively low frequencies in the central and New York populations to occur at relatively high frequencies in the small, isolated New Hampshire population (Table 4). As a result, the New Hampshire population of jack pine represents a storehouse of relatively uncommon alleles and a valuable source of genetic diversity for the species.

Table 4. Frequencies of alleles that differ considerably between the marginal New Hampshire population and the other jack pine populations examined.

		Marginal populations		Central populations	
Locus	Allele no.	NH	NY	Gilb., ON	Renfrew, ON
AAT-2	2	0.467	0.000	0.133	0.228
GDH	2	0.213	0.083	0.017	0.017
MPI	2	0.275	0.098	0.041	0.083
6PGD-2	2	0.381	0.000	0.000	0.053
MDH-2	2	0.128	0.053	0.017	0.018
ACO	2	0.525	0.158	0.304	0.345

The genetic distance measures also show the New Hampshire population to be genetically different from all other populations examined. The average genetic distance between the New Hampshire population and all other populations is 0.030 (range from 0.027 to 0.035), while the average distance between all other populations is 0.006 (range from 0.005 to 0.007). It is clear that the marginal New Hampshire population is genetically distinct from the other jack pine populations examined and, in fact, is among the most genetically distant populations reported within any conifer species. The genetic uniqueness of this population could conceivably be due to directional selection in an extreme environment, or to genetic drift. However, both explanations would be expected to result in reduced genetic variability relative to other populations. Although drift would not necessarily result in reduced genetic variability if inbred individuals are eliminated as previously described, it is difficult to conceive of a situation where directional selection would not result in reduced genetic variability. Furthermore, the Wisconsin glaciation resulted in a major reduction in population size for many boreal species, and jack pine is known to have existed in refugial populations that were probably influenced by genetic drift (Critchfield 1984). It has been hypothesized that transitory races of jack pine emerged during glacial times (Critchfield 1984) and the New Hampshire population of jack pine may represent a remnant of one such transitory race.

CONCLUSIONS

Genetic drift appears to be more important than selection in determining the distribution of electrophoretically detectable genetic variation in marginal populations of pitch and jack pines. While small, isolated marginal populations of both species are genetically distinct from central populations, the differences seem to be random, rather than the result of directional selection. In pitch pine, marginal populations exhibit reduced genetic variability, and are as different from each other as they are from central populations, implying that the reduction in variability has occurred in a random fashion. In jack pine, marginal populations do not exhibit the reduced variability expected if directional selection were occurring, and given the biogeography of this species, it is probable that extreme isolation and genetic drift have resulted in population differentiation. Marginal populations of pitch and jack pine appear to be a valuable source of genetic diversity for the species. Because there is considerable genetic variation among the marginal populations of pitch pine examined, conservation of these populations would contribute to the maintenance of genetic variability for the species. In jack pine, the genetic uniqueness and high variability of the Mt. Chocorua, New Hampshire population are testimony to its biological value and to the necessity for its protection.

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