from these tests are being used to investigate some theoretical questions of mating and environmental design of progeny tests and of sampling the base population; these studies are in progress at the Oxford Forestry Institute and among other things, indicate that some caution is required in interpretation of the data when the population is represented by such small number of parents.

The practically significant conclusions from analyses of selected economically important traits in these *P. patula* tests in the eighth year are: —

1. Heritabilities are high for wood density, stem volume and stem straightness;
2. Specific combining ability, maternal and reciprocal effects are not of practical significance;
3. Genotype-locality interaction may be sufficiently pronounced in volume production traits to be used whereas it is absent in wood density;
4. Genotype-year interaction is not important;
5. Data from trees selectively rather than systematically thinned from progeny tests do not give significantly biased estimates of genetic parameters;
6. Sub-blocking with a lattice design does not give any useful improvement in ranking of families but appreciably smaller tests could be used to get the same precision in assessing some traits in some localities if the characteristics of the test site could be predicted;
7. The polycross mating design with half-sib families being produced by controlled pollination with a 20-pollen mix is an efficient method for determining *gca* and ranking families;
8. Both height and basal area are closely genetically correlated with stem volume within and between second, fifth and eighth years;
9. There is no genetic correlation of practical significance between wood density and stem volume;
10. In the nursery, seedlings with few long cotyledons and greater height develop into large trees with high density by the eighth year;
11. Trees with few branches and superior height in the second year develop into trees with high wood density and large volume by the eighth year;

It is emphasized that these interpretations of the data are only up to the eighth year. This is half a pulpwood and one quarter of a sawlog rotation. Franklin (1979) recognized 3 phases in stand development for four North American conifer species, juvenile-genotypic, mature-genotypic and co-dominance-suppression based on trends in additive genetic variance. He found that genetic correlations were high within and low between phases and he cautioned against juvenile selection in those populations. With the close spacing and rapid growth rate of *P. patula*, these tests in the eighth year can be said to be in the mature-genotypic phase. Genetic correlations between and within the first two phases are high and useable. It will be interesting to see whether they remain high for the fifteenth year traits which is the age at which the next assessment is scheduled.

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Influence of Selection for Volume Growth on the Genetic Variability of Southwestern Ponderosa Pine

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Summary

Genetic variability between 18 select and 50 non-select neighbour trees in three ponderosa pine (*Pinus ponderosa* Dougl. ex Laws. var. *scopulorum* Engelm.) geographical sources in the Southwest was characterised by isozyme analysis using starch gel electrophoresis. Twenty six isozyme loci in 15 enzymes were assayed. Measures of genetic diversity showed that the select and neighbour trees did not differ in isozyme variability. Contingency chi-square analysis did not detect any differences in allozyme frequencies between the select and neighbouring

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trees but there were differences among the three populations. Approximately 2% of the total variation is attributed to differences among geographic locations. Average heterozygosity was relatively high (H_e = 0.128), yet comparable to other isozyme studies of ponderosa pine, indicating a high level of genetic variation. Selection of ponderosa pine for superior growth characteristics appears to have little or no effect on genetic diversity at the isozyme level.

Key words: *Pinus ponderosa*, isozyme variation, electrophoresis, tree improvement.

Introduction

Historically, differences among individual trees within a stand have been used as a basis for selecting superior genotypes for specific improvement objectives in forestry breeding programs. There is evidence that forest tree species have a high level of genetic variation among individuals within a population (Hamrick and Godt, 1989). Still, there remains concern that selection for traits such as volume growth will result in reduced genetic diversity.

In most tree improvement program, trees with the desired traits (e. g. volume growth) are selected from natural stands. Genetic gain is achieved if the progeny from these parents also exhibit these traits, that is if desired traits are heritable. However, it is important that these parents contain a high level of genetic variation outside of the traits for which selection is based. It is preferable that the genetic variation be representative of that in the local stands to avoid the narrowing of genetic base (Zobel and Talbert, 1984). Progeny tests are normally conducted to determine the level of genetic variability in traits of economic importance (U. S. D. A. Forest Service, 1978). Alternately, isozyme analysis can be used to estimate the genetic structure of the population and characterize genetic variability independent of the selected phenotype.

Ponderosa pine, *Pinus ponderosa* Dougl. ex. Laws., in the Northern Rocky Mountains, North Plateau and Pacific Southwest (Conkle and Critchfield, 1988) and in Montana (Woods et al., 1983) exhibit high level of quantitative variability as well as high genetic variability from isozyme analysis. In the Northern Rocky Mountains, 88% of the total isozyme variability is due to genetic differences among individuals within a ponderosa pine stand, and 12% is due to differences among stands (Conkle and Critchfield, 1988). Madsen and Blake (1977) found that 28.5% of the genetic variation for two-year height growth of ponderosa pine was attributed to differences among stands. Though extensive work has been done in other parts of the United States, there is essentially no knowledge about the genetic variation in stands of ponderosa pine in the Southwest. This paper describes the use of isozyme analysis to examine genetic variation in the select and contiguous, non-select ponderosa pine subpopulations, and in the whole population.

Materials and Methods

Plant Materials

Ponderosa pine cones of selected, putative superior trees and adjacent non-select trees were collected from three geographical sources located on the (1) North Kaibab Plateau of the Kaibab National Forest, Arizona, (2) Long Valley District of the Coconino National Forest, Arizona, and (3) Luna District of the Gila National Forest in New Mexico. Althogether cones from 18 superior trees and 50 neighbour trees were sampled (*Table 1*). The selected trees were identified by U. S. Forest Service Southwestern Region's Tree Improvement program personnel and were selected based on: (1) age at 4.5 feet above ground level, (2) height growth (ft/yr), (3) total height, (4) diameter of the bole at 4.5 feet above ground level, (5) total volume of wood (ft^3), (6) volume of growth per year (ft^3), (7) straightness of the bole, (8) size of branches, (9) branching angle, (10) mistletoe rating and (11) crown diameter (USDA, 1978). All individuals sampled in the present study were tagged and mapped, and information on the above characteristics recorded. Trees were sampled from locations with similar environmental features.

Electrophoresis procedures

Seven seeds from each tree were assayed for isozyme variants at 26 isozyme loci representing 15 enzymes (*Table 2*). Seeds were soaked in 3% hydrogen peroxide for 30 min and then in water for 24 hrs. Soaked seeds were germinated at room temperature on moist filter paper. When the radicle had extended 3mm to 5 mm, the megagametophyte was dissected from the seed and crushed in 2 drops of 0.5M phosphate buffer pH 7.5. The homogenates was absorbed onto Whatman No. 3 filter paper wicks and placed separately in a 12% starch gel for electrophoresis.

Two separate gel buffer systems were used (*Table 3*). Electrophoretic technique and stain preparation were modified from Conkle et al. (1982), and Jech and Wheeler (1984). After the gels have moved about 8 cm, they were sliced horizontally into 1 mm thick gel slices, and then transferred to individual staining trays. The stains were

Table 1. — Site description and number of trees sampled for 3 locations.

				Age	# of trees	
Location	Longitude	Latitude	Elevation(m)	Distribution	Select	Adjacent
Luna, New Mexico	33°55.4'N	108°46.2'W	2340	79-86	4	11
North Kaibab, Arizona	36°29.9'N	112°19'W	2447	102-136	6	8
Long Valley, Arizona	34°28.4'N	111°24.4'W	2158	54-87	8	31

Table 2. — Enzymes, enzyme commission number (E. C. number), buffer systems, number of loci, and migration distances for alleles found at individual locus.

Enzyme Abbreviation	E.C. number	Buffer[a] System	Locus[b]	Allele[c] 1	2	3	4	Source
Acid Phosphatase (Acp)	3.1.3.2	M-C	1	100	71			
Aconitase (Aco)	4.2.1.3	M-C	1	100	110	90	100n[d]	
Aldolase (Ald)	4.1.2.13	M-C	1	100				Woods et al. 1983
			2	100	133	67		
Glucose-6-phosphate dehydrogenase (G6pd)	1.1.1.49	M-C	1	100	86	114		O'Malley et al. 1979
Glutamic-Oxaloacetic Transaminase (Got)	2.6.1.1	L-B	1	100	85			O'Malley et al. 1979
			2	100				
			3	100	85	8	169	
Isocitric dehydrogenase (Idh)	1.1.1.42	M-C	1	100	82			O'Malley et al. 1979
Leucine aminopeptidase (Lap)	3.4.11.1	L-B	1	100	96	100n[d]		O'Malley et al. 1979
			2	100	95	105		
Malate dehydrogenase (Mdh)	1.1.1.37	M-C	1	100	127			O'Malley et al. 1979
			3	100	128	214		
			4	100	33	167		
Malic enzyme (Me)	1.1.1.40	M-C	1	100				Woods et al. 1983
			2	100	129	200	100n[d]	
Menadione reductase (Mnr)	1.6.99.2	L-B	1	100				
Phosphoglucomutase (Pgm)	2.7.5.1	L-B	1	100	106	108	97	O'Malley et al. 1979
Phosphoglucose isomerase (Pgi)	5.3.1.9	M-C	1	100	103	97		O'Malley et al. 1979
			2	100	116	84		
6-Phosphogluconate dehydrogenase (6Pgd)	1.1.1.44	M-C	1	100	107			O'Malley et al. 1979
			2	100	108	92		
Shikimate dehydrogenase (Skd)	1.1.1.25	M-C	1	100	115			Linhart et al. 1989
			2	100	78			
Uridine Diphosphoglucose Pyrophosphorylase (Udp)	2.7.7.9	M-C	2	100	136	143		
			3	100				

[a]) Buffer system used.
M—C = Morpholine Citrate buffer
L—B = Lithium Borate buffer
[b]) Number of loci identified.
[c]) Number of alleles identified in each locus and their migration distances.
[d]) n = null allele

prepared fresh on the day of the run. The staining solutions were poured over the sliced gels and the gels were incubated in the dark. When the bands on the gels were well resolved, the solutions was removed and the gels were fixed by soaking them in 40% ethanol for 12 hours.

Enzyme names were abbreviated and designated by capital letters. All enzymes assayed in this study migrated anodally. An isozyme loci was numbered sequentially from the anode (+) to cathode (—) if more than one locus ocurred per enzyme. Within a locus, the variant allozymes were numbered and designated a relative mobility value (R_m) by comparing the migration distance of an allele to the most common allele, arbitrarily designated as 100. If no activity was observed, the allele was designated 100n (n = null). R_m was calculated as:

$$R_m = \frac{\text{migration distance of variant (mm)}}{\text{migration distance of standard (mm)}} \times 100 \quad (1)$$

Following staining and fixing, data were collected regarding the banding patterns in each sample. Subsequently allelic designations and genotypes for each tree were assigned. *Table 2* presents the enzyme abbreviations, enzyme commission codes (E. C. #), gel buffer system, the

Table 3. — Composition of gel and electrode buffers used for starch gel electrophoresis and electrophoretic conditions.

Name	Electrode Buffer	Gel Buffer	Current
Morpholine-Citrate (M-C)[a] pH 6.1	0.04M Citric acid (anhydrous) pH to 6.1 with N-(3-aminopropyl)-morpholine Refrigerate	1:19 electrode buffer to water	50mA
Lithium-Borate (L-B)[b] pH 8.1/8.5	0.06M Lithium 0.3M Boric acid pH 8.1 Store at room temperature	0.03M Tris 0.005M Citric acid (anhydrous) 1% electrode buffer, 99% gel buffer pH 8.5 Store at room temperature	45mA

[a]) Clayton and Tretiak, 1972
[b]) Ridgway et al., 1970

number of loci per enzyme and the migration distances of each allozyme.

Data Analysis

Allozyme frequency data were used to calculate, on a population basis, single locus measures of genetic diversity. Several different statistics were calculated, including percent polymorphic loci (P), average number of alleles per locus, expected heterozygosity (H_e) (Nei, 1975), observed heterozygosity (H_0) and genetic distance.

A locus was considered polymorphic when at least two allozymes occur, and the frequency of the least common allele was greater than 1.0%. P was calculated as:

$$P = \frac{\text{number of polymorphic loci}}{\text{total number of loci}} \quad (2)$$

Expected heterozygosity was calculated as:

$$(3) \qquad H_e = 1 - \sum_{i=1}^{k} x_i^2$$

where, x_i is the frequency of the ith allele summed over k alleles. Observed heterozygosity was calculated as:

$$H_o = \frac{\Sigma \text{ \% heterozygous loci/individual}}{\text{total number of individuals}} \quad (4)$$

H_0 gives the actual heterozygosity observed in the population. Genetic distance was calculated as:

$$D = -\ln (I) \quad (5)$$

where,

$$(6) \qquad I = \frac{J_{xy}}{\sqrt{J_{xx} J_{yy}}}$$

and I = normalized identity of an isozyme locus (Nei, 1975), J_{xx} = probability that 2 alleles chosen at random from population x are identical, J_{xy} = probability that two alleles are identical when one allele is chosen from population x and the other is chosen from population y, J_{yy} = probability that two alleles chosen at random from population y are identical and l = number of loci. If two populations have the same alleles in the same frequency, I = 1; when two populations have no alleles in common, I = 0.

Genetic distance was calculated for pairwise combinations of geographic locations including data from both the polymorphic and monomorphic loci (Nei, 1972). Genetic distances were estimated between populations for all individuals within the population and for just the selected individuals within the population.

Contingency chi-square analysis (Workman and Niswander, 1970) was performed for all alleles at each polymorphic locus to detect differences among geographic populations, and between select and adjacent subpopulations in a single geographic population. The chi-square values were summed over all loci to give an overall comparison of subpopulations. Contingency chi-square was calculated as:

$$(7) \qquad \chi^2 = 2N \left(\sum_{i=1}^{n} \frac{\sigma^2_{p_i}}{\bar{p}_i} \right)$$

where, N = total number of samples, k = number of alleles at a locus, $\bar{p}_i$ = weighted mean alloyme frequency across i populations, n = number of populations and σ^2_{pi} = variance of allozyme frequency across i populations.

Nei's F-Statistic (F_{st}), as modified by Weir and Cockerham (1984) for small, unequal sample size, was calculated based on differences among populations, among select subpopulations, and between select and adjacent subpopulations. Nei's F_{st} was calculated as:

$$(8) \; F_{st} = \frac{\Sigma \sigma^2_{p_i}}{\Sigma (\bar{p}_i (1 - \bar{p}_i))}$$

"Genestat" a statistical software (Black and Krafsur, 1985), was used to calculate the contingency chi-square

and F-statistics. It also calculated the expected Hardy-Weinberg proportion using the allele frequency data, and indicated whether the population examined was in Hardy-Weinberg proportion or not.

An average density cluster analysis was used to group populations based on genetic distance among populations. The cluster analysis was performed using SAS, version 5.0 (SAS, 1985). All statistical analysis were conducted at the $\alpha = 0.01$ level of significance.

Results

Genetic Variability Within a Population

Twenty six loci coding for 15 enzymes were resolved, of which 21 loci were polymorphic in at least one of the populations (*Table 2*). The 5 monomorphic loci were *Ald-1, Got-2, Me-1, Mnr,* and *Udp-3*. The observed allele frequencies did not differ significantly from Hardy-Weinberg expectations in any populations (data not shown).

Considerable genetic heterogeneity existed among populations (*Table 4*), as indicated by the range of polymorphic loci (P = 54% to 81%), and the range of expected heterozygosity (H_e = 0.123 to 0.136). The average percentage polymorphic loci over all populations was 66%. The average number of alleles/locus, including monomorphic loci, varied in accordance with percent polymorphic loci. Trees in the non-selected subpopulations had higher values for percent polymorphic loci and average number of alleles/locus. This discrepancy may be due to uneven sample size between the select and non-selected subpopulations. Average number of alleles/locus is known to be dependent upon sample size (Nei, 1975). In the overall population, 21 of the 26 loci were polymorphic (*Table 2*), yet within the select subpopulations, only 17 loci were polymorphic.

Average observed heterozygosity over all loci were calculated for each geographic population, and ranged from 0.115 to 0.162 (*Table 4*). Observed heterozygosities were slightly higher than expected heterozygosities, with the exception of the Luna population. However, the standard error for the Luna population indicated that there was no difference between the 2 parameters. Long Valley had the highest expected heterozygosity while the North Kaibab had the highest observed heterozygosity. As Linhart et al. (1981) noted, ponderosa pine exists in clusters of small family units composed of heterozygous individuals, and thus the expected and observed heterozygosity may differ. Still, observed frequencies did not differ significantly ($P > 0.005$) from Hardy-Weinberg expectations in any populations. In several other studies on conifers (Yeh and Layton, 1979; O'Malley et al., 1979), expected and observed heterozygosities were essentially the same.

Analysis of Gene Diversity

The contingency chi-square analysis indicated that there were significant differences in allozyme frequencies among geographic populations, particularly in *Aco, Got-1* and *Lap-1*, indicating that at least one of the populations differs from the rest (*Table 5*). Generally, there were no significant differences in allele frequencies between the select and adjacent non-selected subpopulations, with the exception of *Mdh-1* and *6pgd-2* in Long Valley. There was no overall trend in the deviations and this variation is likely due to chance. Interestingly, there were no detected differences in allozyme frequencies among the three selected subpopulations, even though there were differences among the three whole populations.

F-statistics (*Table 5*) and genetic distance (*Table 6*) analyses indicated that relatively little differentiation has occurred among the three studied ponderosa pine populations. F_{st} values range from —0.003 to 0.02. On average 1.4% of the total gene diversity was due to differences among populations, and 98.6% resided within a population. The F_{st} value for the 3 geographic populations is very similar to the F_{st} value in the study by Woods et al. (1983) (F_{st} = 0.0145). The F_{st} value for the select subpopulation was negative, indicating no differentiation among the three select subpopulation. These results correspond with the results from the chi-square analysis.

Genetic distance among pairwise populations ranged from 0.009 for Luna-North Kaibab, 0.006 for Luna-Long Valley, and 0.005 for North Kaibab-Long Valley (*Table 6*). Genetic distance values among pairwise select subpopulations ranged from 0.020 for Luna-North Kaibab, 0.013 for Luna-Long Valley, and 0.010 for North Kaibab-Long Valley.

Cluster analysis of the genetic distances showed that the select subpopulations are not genetically different

Table 4. — Descriptive isozyme data: Percent polymorphic loci (P), average number of alleles/locus, expected heterozygosity (H_e), and observed heterozygosity (H_o).

Location	Group	P	Average # of alleles	H_e	H_o
Luna New Mexico	Select	38	1.38	0.133± 0.038	0.115± 0.054
	Adjacent	46	1.54	0.107± 0.029	0.115± 0.015
	Total	54	1.73	0.123± 0.032	0.115± 0.017
North Kaibab Arizona	Select	42	1.62	0.119± 0.032	0.154± 0.014
	Adjacent	62	1.73	0.127± 0.029	0.168± 0.018
	Total	62	1.92	0.126± 0.030	0.162± 0.012
Long Valley Arizona	Select	54	1.77	0.139± 0.031	0.149± 0.015
	Adjacent	77	2.31	0.128± 0.030	0.139± 0.010
	Total	81	2.38	0.136± 0.030	0.142± 0.008
				Mean H_e = 0.128	Mean H_o = 0.139

Table 5. — Contingency chi-square table among populations, among subpopulations in a population and among select subpopulations within the populations.

Isozyme locus	Among Locations	Among groups within locations			Among select groups
		Luna	N.Kaibab	Long Valley	
Acp	0.62	0.55	0.78	0.55	0.43
Aco	14.21*	2.84	1.46	4.98	0.39
Ald-2	3.57	3.15	0.78	0.53	0.17
G6pd	1.85	0.60	2.09	0.89	0.48
Got-1	1.96**	-	0.02	0.53	0.12
Got-3	2.30	6.35	2.88	2.99	0.46
Idh	1.51	-	-	0.26	-
Lap-1	11.54*	1.21	-	0.26	-
Lap-2	0.77	2.84	0.78	0.56	0.47
Mdh-1	0.75	-	-	3.93*	0.48
Mdh-3	5.20	0.78	0.78	0.31	0.53
Mdh-4	1.51	-	-	0.53	-
Me-2	10.80	5.10	2.26	6.36	0.15
Pgm	4.63	0.38	2.91	2.45	0.61
Pgi-1	5.62	-	1.62	5.10	0.62
Pgi-2	3.06	-	-	0.56	0.53
6Pgd-1	5.19	0.38	0.12	0.26	0.36
6Pgd-2	8.15	0.55	5.46	6.26*	0.15
Skd-1	0.95	0.17	0.78	0.73	0.86
Skd-2	1.01	-	0.78	0.46	-
Udp-2	3.92	1.34	0.73	0.07	0.84
F_{ST} (± s.e.)	0.014 ±0.002	-0.003 ±0.006	0.020 ±0.010	0.008 ±0.004	-0.005 ±0.006

*) indicates significance at $\alpha \leq 0.05$
**) indicates significance at $\alpha \leq 0.01$

from each other (*Fig. 1*). On the other hand, cluster analysis on the 3 whole populations indicated that Luna and Long Valley populations are more closely related to each other than to the North Kaibab population (*Fig. 1*).

Discussion

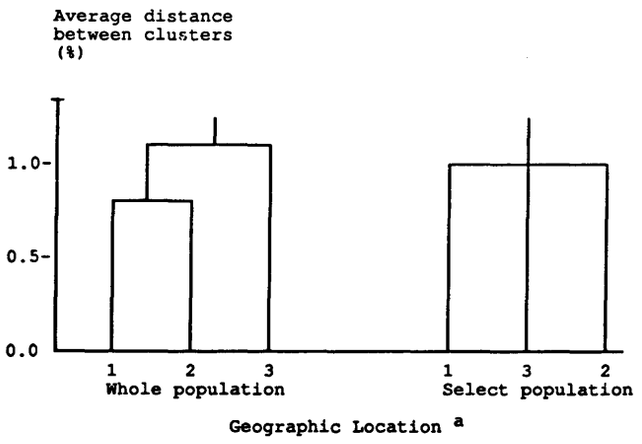
The primary conclusion of this study is that selected and adjacent non-selected ponderosa pines are similar in isozyme variation. These trees contain an appreciable amount of genetic variation, mainly within a population; little differentiation has occurred between geographic populations.

In this study, the sample number of 18 select trees and 50 adjacent trees are too small to be treated as populations. This lack cannot be improved. However, the data collected yielded some information about genetic structure of the select and adjacent trees. Throughout this paper, the trees in the three geographical sources are described as three populations, and the select and adjacent trees within a geographical source are described as subpopulations to facilitate discussions.

Hamrick et al. (1979) reported the average expected heterozygosity for 20 coniferous species as 0.207. The average expected heterozygosity in this study was only 0.128. This concurs with other studies on ponderosa pine based on 20 loci or more (O'Malley et al., 1979; Allendorf et al., 1982; Woods et al., 1983; Conkle and Critchfield, 1988). The differences in estimates of heterozygosity can be attributed to the number and choice of loci studied (Guries and Ledig, 1982). The distribution of single locus heterozygosities in this study was very similar to those reported by Woods et al. (1983). Level of expected heterozygosities varied greatly among loci within a population. Me-2 locus is a very variable locus in ponderosa pine,

Table 6. — Genetic distances among populations of *Pinus ponderosa* estimated for all sampled individuals in the population, and only the selected individuals within the population, based on 26 isozyme loci.

	N. Kaibab,AZ	Long Valley, AZ
Total population		
Luna, NM	0.00946	0.00572
N.Kaibab, AZ		0.00472
Select population		
Luna, NM	0.02335	0.01310
N.Kaibab, AZ		0.01039



a) 1 — Luna population
2 — Long Valley population
3 — North Kaibab population

Figure 1. — Average-linkage cluster analysis of genetic distances among 3 whole populations and 3 select populations from the Southwestern United States.

followed by *Udp-2* and *Got-3* (*Fig. 2*). The genetic distance estimates are similar to values in studies by Woods et al. (1983), Conkle and Critchfield (1988) and Hamrick et al. (1989).

The contingency chi-square analysis indicated that the populations differ in allele frequencies. Age class differences did exist among the geographic locations and differences in the genetic structure of various age classes can influence allozyme frequencies (Hartl, 1988). Yet Linhart et al. (1979) has shown that genetic differentiation among age classes is not pronounced in ponderosa pine. Thus, it is unlikely that age class differences account for the allozyme variation detected in the present study. Spatial differences account more for allozyme variation detected.

The cluster analysis and F_{st} are in agreement with the contingency chi-square analysis. There are differences among the three geographic populations. Alternatively, there were no differences among the selected subpopulations, and the select and adjacent trees did not differ significantly in isozyme variation. Subjective selection of morphologically similar trees appears to result in selection of trees with isozymes similar to their adjacent neighbours and thus similar among select trees.

It is interesting to note that Luna and Long Valley are clustered together prior to the North Kaibab population though Luna and North Kaibab populations are geographically closer together. The North Kaibab plateau is isolated from the other two populations which are within the continuous range of Southwestern ponderosa pine. The northern part of the Colorado plateau is separated by the Grand Canyon. This geographic barrier could account for the clustering results and slightly higher observed heterozygosity and F_{st} in the North Kaibab population compared to the other populations. Isolation of the North Kaibab population could have resulted in differentiation among populations, given the changes of pollen from outside source are more limited. The absence of ponderosa pines to the north and west of North Kaibab

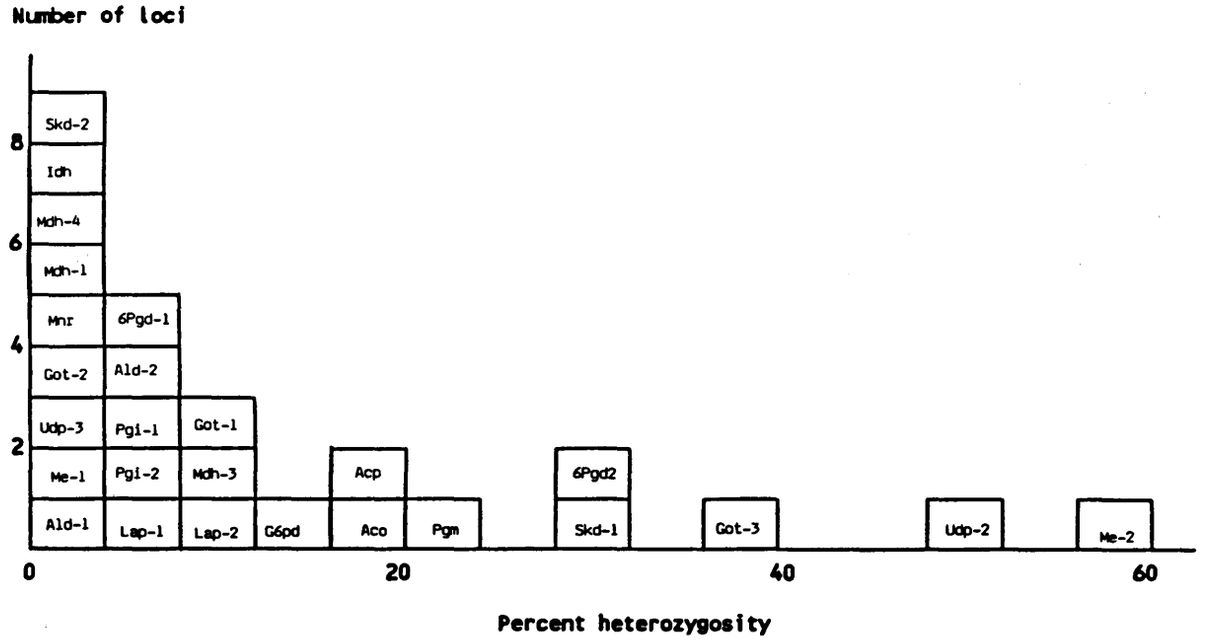


Figure 2. — Distribution of single locus heterozygosity, over all stands combined, for alleles determining electrophoretic variants in ponderosa pine from the Southwestern United States.

plus the prevailing winds that mainly blow from the west to the east may also cause the North Kaibab trees to function somewhat as an island population. It may be that the populations came from the same ancestors but after isolation, the North Kaibab population began to diverge slowly from the other 2 populations. If the three populations studied indeed represented 2 separate populations, we may need to develop separate tree improvement programs to fully exploit their potentials. Further sampling in all populations is needed to verify this hypothesis.

A small percentage of genetic diversity appeared to be due to differences between populations. Still, by choosing parent trees from a single population, all but 1.4% of the genetic variation in the population can generally be sampled. In achieving genetic gain and conserving a reasonably large superior genetic base, a trade-off usually occurs. This study indicated that there is minimum trade-off as the superior trees maintain a diverse genetic base. Phenotypic selection with natural regeneration should result in tree improvement without a significant loss in genetic diversity.

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Micropropagation of Ailanthus malabarica DC. Using Juvenile and Mature Tree Tissues

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Summary

Multiple buds were induced from nodes of mature trees (NM) as well as from cotyledonary nodes (NC) of the seedlings of *Ailanthus malabarica* DC. The number of buds induced from NM was highest on Murashige and Skoog's (MS) medium supplemented with 50 mg · l^{-1} activated charcoal, 175.29 mM sucrose and 133.2 μM BA while in case of NC, the number induced was highest on MS medium supplemented with 175.29 mM sucrose and 88.8 μM BA. Buds induced from NM failed to elongate and could not be rooted. Buds obtained from NC could be elongated to form microshoots on MS medium supplemented with 22.2 μM BA and 2.32 μM KN. The elongated microshoots were harvested at the end of each subculture. They were rooted *in vitro* through a sequential treatment involving an initial 15-day treatment on MS medium supplemented with 58.43 mM sucrose and 26.85 μM NAA followed by transfer of the microshoots to MS medium

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