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Amount and Distribution of Isozyme Variation in Ponderosa pine from Eastern Montana

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Amount and Distribution of Isozyme Variation in Ponderosa pine from Eastern Montana

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Summary

Pinus ponderosa seeds from 50 trees in each of six small isolated stands located within a nine kilometer radius of Colstrip, Montana, were examined at 23 isozyme loci. Megagametophyte and embryo tissue from each seed were screened separately. Measures of genetic diversity showed no significant differences between male and female components within or between stands. The average proportion of heterozygous loci per embryo (H_e) was not significantly different between stands. For all stands combined, H_e was 0.012. Genotype proportions did not deviate from expected Hardy-Weinberg proportions.

Nearly 99 % of the genetic diversity resided within individual stands, with a significant 1.5 % due to differences between stands. Genetic distance between stands is not correlated with geographic distance. Considering the natural fire history of the stands, it is suggested that there is a great deal of gene flow into a stand during its early stage of development.

Key words: isozyme analysis, *Pinus ponderosa*, gene flow, population differentiation, heterozygosity, forest genetics.

Zusammenfassung

An Samen von *Pinus ponderosa* var. *scopulorum* ENGELM. von 50 Bäumen aus je sechs kleinen isolierten Beständen innerhalb eines Radius von 9 Kilometern um Colstrip, Montana, wurden 23 Isoenzym-Loci untersucht. Megagametophyten und Embryogewebe von jedem Samen wurden getrennt untersucht. Eine Bestimmung der genetischen Verschiedenheit hat keine wichtigen Unterschiede zwischen männlichen und weiblichen Bestandteilen innerhalb von Beständen oder zwischen Beständen gezeigt. Der durchschnittliche Anteil an heterozygoten Loci je Embryo (He) war zwischen Beständen nicht signifikant verschieden. Für alle Bestände kombiniert, war der durchschnittliche He-Wert 0.012. Die Verteilung der Genotypen wich nicht von der erwarteten Hardy-Weinberg-Verteilung ab.

Fast 99 % der genetischen Verschiedenheit für die 23 Loci sind innerhalb jedes einzelnen Bestandes zu finden, mit einer signifikanten, 1,5 %igen Differenz zwischen den Beständen. Die genetische Entfernung zwischen Beständen ist nicht mit der geographischen Entfernung korreliert. Wenn man die natürliche Waldbrand-Geschichte der Bestände betrachtet, ist anzunehmen, daß es in der Frühentwicklung eines Bestandes einen starken Genfluß gibt.

Introduction

The use of electrophoretic techniques to study isozyme variation in coniferous species has become widespread in recent years. Wind-pollinated conifers generally show a great deal of genetic variability (HAMRICK *et al.*, 1981), and less population differentiation than trees that are pollinated by other means (BROWN and MORAN, 1981). Many studies have investigated the distribution of isozyme variability in conifers over a broad geographic range (LUND-

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KVIST and RUDIN, 1977; O'MALLEY *et al.*, 1979; GURIES and LEDIG, 1981; YEH, 1981). Results indicate that most of the genetic variability exists within individual populations, with a small proportion due to differences between populations. Fewer studies have investigated genetic differentiation among populations over small areas; however differentiation between stands separated by distances of less than 1 kilometer has been found (SAKAI and PARK, 1971; MITTON *et al.*, 1977; LINHART *et al.*, 1981).

Differential selection of genotypes and random genetic drift are the primary forces responsible for genetic differentiation among populations. Conifers often occur in areas that have a great deal of topographic relief. Individual stands may be isolated by features that restrict seed and pollen movement (gene flow), and site conditions are highly variable. Determining the relative contributions of selection or genetic drift to population differentiation is difficult. Conifers occupying flat, consistent terrain, with little variation in site conditions, are usually found in nearly continuous stands. In this situation, selective forces are likely similar for various populations, however the difficulty of identifying populations and predicting the influence of gene flow is increased.

The naturally occurring stands of ponderosa pine *(Pinus ponderosa* var. *scopulorum* ENGELM.) found in southeastern Montana, near Colstrip, offer an exceptional opportunity to investigate population differentiation. This area is characterized by flat terrain, spotted with rocky hills. Ponderosa pine stands are usually small (0.5 to 15 hectares) and restricted to the rocky hills, with flat grasslands occupying the area between stands. Site conditions on the rocky hills are similar. It is possible to sample isolated stands, separated from each other by varying distances, to investigate genetic population structure.

This study examines isozyme variation in 23 loci coding for 15 enzymes in ponderosa pine from the Colstrip area in eastern Montana. Six small isolated stands, all located within a seven kilometer radius, were investigated to estimate the amount of genetic variation within stands and the amount of genetic differentiation between stands.

Materials and Methods

Open pollinated seeds were collected from 50 trees in each of six ponderosa pine stands located near Colstrip, Montana *(Figure 1).* All sample stands are isolated from other ponderosa pine populations by grasslands, with distances ranging from 100 meters to 850 meters. Distances between sample stands range from 850 meters to 13.6 kilometers. Sample stands consisted of ponderosa pine, with a few isolated juniper *(Juniperus scopulorum* SARG.). Stand area and density varies *(Table 1),* and all age classes are represented (RICHARDSON, 1981).

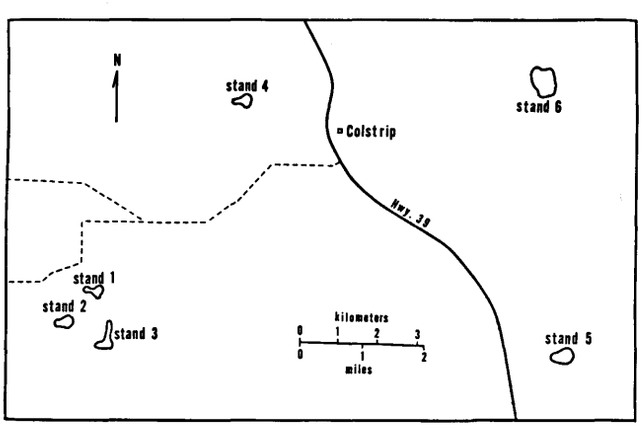

Figure 1. — Location of sample stands.

Table 1. — Size, area, and density of sample stands.

Stand number	Number of trees*	Stand area (hectares)	trees per hectare
1	260	4.4	59
2	230	3.1	74
3	920	8.4	110
4	400	3.4	118
5	2550	12.4	181
6	225	5.2	43

* Number of trees of reproductive age as estimated from air photos.

To estimate allele frequencies for the present stands, as well as genotypic frequencies in the seed crops, one seed from each tree was screened for allozyme variants in megagametophyte and embryo tissue. This procedure provides a sample of 50 genes per locus from trees comprising a stand, as well as 50 genes from the pollen fertilizing the stand. This sample size is sufficient to detect allele frequency differences between stands, or between a stand and the pollen fertilizing the stand. Genotype frequencies can be determined for the current seed crop, allowing interpretation of pollination patterns.

Fresh ponderosa pine seeds from the Colstrip area do not require stratification (WOODS and BLAKE, 1981); therefore, seeds were soaked in water for 19 hours, and germinated at room temperature on moist filter paper. Megagametophytes and embryos were dissected from the seeds when the radicle had extended about one centimeter, and were crushed in 0.2 to 0.5 ml. of distilled water in proportion to the amount of tissue present. Homogenates from the megagametophyte and embryo of each seed were absorbed into filter paper wicks, and placed separately in a starch gel for electrophoresis.

Two buffer systems were used with 12.5 percent starch gels:

I. Described by RIDGWAY *et al.* (1970).
   Gel buffer: 99 % 0.03 M tris, 0.005 M citric acid pH 8.5; 1 % tray buffer.
   Tray buffer: 0.06 M lithium hydroxide, 0.30 M boric acid, pH 8.1.
II. Described by CLAYTON and TRETIAK (1972).
   Gel buffer: 0.002 M citric acid, pH 6.1.
   Tray buffer: 0.04 M citric acid, pH 6.1.
   Both buffers are pH adjusted with N-3 (-aminopropyl)-morpholine.

Both systems were run at 50 milliamps and 150 to 200 volts for about four hours. The enzymes used are listed in *Table 2.* Enzyme stains, buffers and methods are described by ALLENDORF *et al.* (1977).

The nomenclature of isozymes is similar to ALLENDORF and UTTER (1979) and loci identification follows O'MALLEY *et al.* (1979). An abbreviation is given in capital letters to designate each enzyme; when not in full capitals the same abbreviations represent the loci coding for thes proteins.

Results and Discussion

Twenty-three loci coding for 15 enzymes were resolved with sufficient clarity to consistently determine allelic

Table 2. — Migration distances for alleles found at individual loci. Enzymes and buffer systems used.

Enzyme	Enzyme commission number	Buffer system	Locus	Alleles						
				A1	A2	A3	A4	A5	A6	A7
Adenylate kinase	2.7.4.3	II	Ak	100						
Alcohol dehydrogenase	1.1.1.1	II	Adh-1	100	120	71	84			
Aldolase	4.1.2.13	II	Ald	100						
Aspartate aminotransferase	2.6.1.1	I	Aat-1	100	187	67	96	267	147	
			Aat-2	100	120					
			Aat-3	100	96	76	107			
Glucosephosphate isomerase	5.3.1.9	I	Gpi-1	100	120					
			Gpi-2	100						
Glucose-6-phosphate dehydrogenase	1.1.1.49	I	G6p-2	100	90	108				
Glutamate dehydrogenase	1.4.1.2	I	Gdh	100	80					
Isocitrate dehydrogenase	1.1.1.42	II	Idh	100	106	83				
Malate dehydrogenase	1.1.1.37	II	Mdh-1	100	100a	80				
			Mdh-2	100	110	50				
			Mdh-3	100	97	111				
			Mdh-4	100	137	71				
Malic enzyme	1.1.1.40	II	Me	100	88	80	75	106	54	117
Mannosephosphate isomerase	5.3.1.8	I	Mpi	100						
Peptidase	3.4.-.-	I	Pep-1	100						
			Pep-3	100						
Phosphogluconate dehydrogenase	1.1.1.44	II	Pgd-1	100	125					
			Pgd-2	100	112					
Phosphoglucomutase	2.7.5.1	I	Pgm-2	100	96	106				
Sorbitol dehydrogenase	1.1.1.14	I	Sdh	100	81					

variants. Enzyme activity as indicated by staining intensity varied between enzyme systems, and between the loci coding for some enzymes. Most loci showed less activity in embryo tissue than in megagametophyte tissue. Enzyme activity showed little change with the level of radicle development. However, a radicle of about one centimeter provided a convenient amount of tissue to work with.

Table 3. — Genetic variation in open pollinated ponderosa pine (var. *scopulorum)* seeds from 6 stands located near Colstrip, Montana.

Stand	% loci polymorphic[a]			Alleles per locus - avg.			
	Mega.	Pollen	Embryos	Mega.	Pollen	Embryos	He[b]
1	52.17	52.17	60.87	2.00 ±0.30	1.87 ±0.23	2.17 ±0.30	12.83 ±3.87
2	65.22	60.87	69.57	2.09 ±0.26	1.87 ±0.19	2.22 ±0.27	13.17 ±3.48
3	52.17	65.22	69.57	1.96 ±0.28	1.96 ±0.21	2.17 ±0.26	12.69 ±3.48
4	56.52	52.17	60.87	1.96 ±0.23	1.83 ±0.24	2.04 ±0.25	11.53 ±3.63
5	56.52	43.48	56.52	2.00 ±0.27	1.65 ±0.21	2.00 ±0.27	10.44 ±3.45
6	56.52	47.83	56.52	1.96 ±0.29	1.74 ±0.24	2.00 ±0.32	13.90 ±4.11
Average	56.52 ±1.95	53.62 ±3.30	62.32 ±2.43	1.99 ±0.27c	1.82 ±0.22c	2.10 ±0.28c	12.43 ±3.67c
Combined	65.22	60.87	65.22	2.48 ±0.33	2.52 ±0.32	2.61 ±0.33	12.61 ±3.65

a. Frequency of the common allele is <0.99.
b. Mean expected heterozygosity over all loci.
c. Mean standard error for the 6 stands.

Genetic Variation Within Stands

The proportion of loci polymorphic (P) is defined as the proportion of loci in which the frequency of the common allele does not exceed 0.99. *Table 3* summarizes the values of P for megagametophytes and pollen, and for embryos (megagametophyte and pollen combined). The average percent of loci polymorphic over all stands is 56.52 for megagametophytes and 53.62 for pollen; the difference is not significant. In embryos, the average single stand value of P is 62.32 percent. When all six stands are considered as one population, P equals 65.22 percent.

These values of P are similar to those found for other coniferous species. In seven population of ponderosa pine var. *scopulorum* from eastern Colorado, Hamrick *et al.* (1981) found 68.4 percent of 22 loci to be polymorphic. A summary of genetic variability in 20 coniferous species by Hamrick *et al.* (1981) shows an average P of 67.7 percent, with a range from 0 for red pine (*Pinus resinosa* Ait.) to 100 percent for loblolly pine (*Pinus taeda* L.).

The average number of alleles per locus (A) is 1.99 for megagametophytes, and 1.82 for pollen *(Table 3).* Combining the megagametophyte and pollen for each seed (embryo) gives an average of 2.10 alleles per locus. No significant differences were found between values of A for pollen and megagametophyte tissue within a stand, or for values between stands. Combined data from all stands gave a value for A of 2.61.

Estimates of expected (Hardy-Weinberg) heterozygosity per locus (h_e), and average expected heterozygosity over all loci (H_e) (Nei, 1975) were calculated for individual stands, and for all stands combined. Levels of h_e varied a

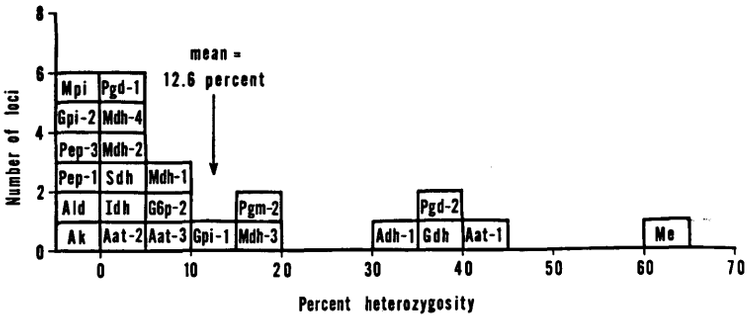

Figure 2. — Distribution of single locus heterozygosity, over all stands combined, for alleles determining electrophoretic variants in ponderosa pine from Colstrip, Montana.

great deal between loci within a stand, but very little between stands for a given locus. *Figure 2* shows the distribution of single locus heterozygosities for all stands combined.

Single stand values of H_e varied from 10.44 to 13.90 percent *(Table 3).* When all stands are combined as one population, H_e equals 12.61 percent. No significant differences in H_e were found between stands. The average heterozygosity in ponderosa pine (var. *scopulorum)* from Colstrip (12.6 %) is less than that found by ALLENDORF *et al.* (1982) in var. *ponderosa* from the northern Rocky Mountains (18.6 %).

Maintenance of Genetic Variation

A controversial problem in genetics is determining whether the protein variation in natural populations is maintained by balancing selection, or if it represents the drifting polymorphisms of neutral mutations. The level of genetic variation maintained in an equilibrium population under the neutral mutation hypothesis is dependant upon the effective population size (Ne) and the mutation rate (u). Using the infinite neutral alleles model, the theoretical average heterozygosity per locus is given by

$$H = M/(1 - M)$$

where $M = 4uNe$ (KIMURA and CROW, 1964). STEWART (1976) has shown that the theoretical variance of single locus heterozygosity is

$$V(H) = 2M/((1 + M)^2 (2 + M)(3 + M))$$

The value of M can then be estimated from observed values of H, and the theoretical V(H) determined. Comparisons between the observed V(H) (NEI and ROYCHOUDHURY, 1974) and theoretical V(H) can be used to test the neutral mutation hypothesis (NEI *et al.*, 1976).

For the combined sample of ponderosa pine from Colstrip, the observed V(H) is 0.031, and the theoretical V(H) is 0.033. The similarity of these values indicates the observed variance in heterozygosity can be reasonably explained by the neutral mutation hypothesis. Although certain kinds of selection and varying mutation rates per locus could produce the same effect (LI, 1978), it appears the observed patterns of isozyme variation are not primarily in response to varying selective forces between stands. However, the sampling strategy used is not sensitive to selective forces acting within stands. YEH (1981) also found close agreement between expected values of V(H) under the neutral-mutation theory, and observed values of V(H) for Douglas-fir (*Pseudotsuga menziesii* (MIRB.) FRANCO), lodgepole pine (*Pinus contorta* (ENGELM.) Critchfield) and sitka spruce (*Picea sitchensis* (BONG.) CARR.).

Genotypic Proportions

Allele frequency data from each stand were used to calculate the expected Hardy-Weinberg genotype proportions. LEVENE's (1949) correction was used to adjust for small sample size. Observed and expected genotype numbers were compared using chi-square analyses over all loci for each stand, and over all loci for all the stands combined. No significant differences were found between observed and expected genotype proportions at any locus for any single stand. When the data are combined for all stands, a significant excess of heterozygotes was detected at the Gdh locus. However, using the modified level of significance proposed by COOPER (1968) for simultaneous chi-square tests, the deviation from expected at this locus is not significant.

Observed genotypic proportions generally do not deviate from expected Hardy-Weinberg proportions for conifers (CONKLE, 1981; FERET, 1974, LUNDKVIST, 1979; and O'MALLEY *et al.*, 1979). WOODS *et al.* (1982), however report a significant excess of homozygotes in fully stocked, naturally established stands of ponderosa pine var. *ponderosa.* It is hypothesized that neighboring trees have a higher than normal probability of being related due to limited seed dispersion, and pollen from a given tree is more likely to fall on its near neighbors. This could result in an excess of homozygotes due to inbreeding.

The Colstrip stands normally have wide spacing between trees, and are subject to frequent wind. These factors would contribute to mixing the pollen, consequently gametes should come together in a nearly random fashion. The similarity of observed and expected genotype proportions in individual stands, as well as when all stands are considered as one population, suggests random mating is taking place.

Hierarchical Analysis of Gene Diversity

Electrophoretic data on gene diversity can be partitioned into different hierarchical levels of population structure (NEI, 1973). The total genetic diversity (H_t) is estimated by the expected Hardy-Weinberg proportions of heterozygotes, using mean allele frequencies at each locus for all stands, averaged over all loci. The mean genetic diversity within each stand (H_s) is equal to the average proportion of heterozygotes per locus found within individual stands. The total genetic diversity can be partitioned into genetic diversities within and between stands:

$$H_t = H_s + D_{st}$$

where D_{st} is the average gene diversity between stands. If all populations are members of a large panmictic unit and no genetic differentiation exists among them, then H_t

will equal H_s. The relative magnitude of genetic differentiation between stands (G_{st}) is given by

$$G_{st} = D_{st}/H_t$$

The sampling variance of G_{st} (V(Gst)) can be used to study the significance of population subdivision (CHAKRABORTY, 1974).

An analysis of electrophoretically detectable genetic diversity in seeds from the six ponderosa pine stands *(Table 4)* shows a great deal of variation among loci in the proportion of intra-stand genetic variation (G_{st}). The mean value of G_{st} over all loci is 0.0145, indicating approximately 1.5 percent of the total genetic variation is due to differences among stands. The standard error (square-root of $V(G_{st})$) is 0.005, showing a significant effect of subdivision between the sampled stands.

In ponderosa pine from the northern Rocky Mountains (var. *ponderosa)* about 12 percent of the allozyme variation was attributed to genetic differences among stands (O'MALLEY *et al.*, 1979). Using seedlings from the same ponderosa pine populations, MADSEN and BLAKE (1977) attributed 28.5 percent of the variability in two year height growth to among stand variation. The large amount of among stand variation in var. *ponderosa* compared to var. *scopulorum* from Colstrip is likely due to greater geographic distance between sample stands for var. *ponderosa,* the variety of elevations and site conditions, and the topographic barriers to pollen movement. Sample stands in the Colstrip area are relatively close together, and are not separated by topographic features which would restrict pollen movement.

Allelic Frequency Heterogeneity

When gene flow is restricted between populations, genetic differentiation may occur due to the forces of genetic drift or selection. The result of these forces is to alter gene frequencies within populations, resulting in genetic divergence between populations. If gene flow is great enough, genetic divergence will be reduced or eliminated. WRIGHT (1969) has shown that divergence among isolated populations is a function of the absolute number of migrants, and not of the proportion of migrants.

Table 4. — Genetic heterozygosity and the proportion of genetic differentiation among populations for 23 loci in ponderosa pine from Colstrip, Montana.

Locus	Total diversity (Ht)	Diversity within populations (Hs)	Differentiation between populations (Gst)
Ak	0.0000	0.0000	——
Adh-1	0.3255	0.3218	0.0116
Ald	0.0000	0.0000	——
Aat-1	0.3871	0.3808	0.0162
Aat-2	0.0198	0.0194	0.0202
Aat-3	0.0840	0.0826	0.0166
Gpi-1	0.1128	0.1117	0.0095
Gpi-2	0.0000	0.0000	——
G6p-2	0.0805	0.0796	0.0107
Gdh	0.3279	0.3229	0.0152
Idh	0.0264	0.0262	0.0057
Mdh-1	0.0649	0.0642	0.0104
Mdh-2	0.0231	0.0230	0.0055
Mdh-3	0.1723	0.1691	0.0184
Mdh-4	0.0133	0.0131	0.0151
Me	0.6585	0.6522	0.0096
Mpi	0.0000	0.0000	——
Pep-1	0.0000	0.0000	——
Pep-3	0.0000	0.0000	——
Pgd-1	0.0100	0.0098	0.0117
Pgd-2	0.3848	0.3742	0.0275
Pgm-2	0.1833	0.1815	0.0099
Sdh	0.0263	0.0261	0.0093
All loci combined	0.1261	0.1243	0.0145
Std. error	0.0365	0.0360	0.0050

Table 5. — Number of loci showing significant (Prob. $<$ 0.05) allele frequency differences, and the probability of allele frequency homogeneity over all loci, for pair-wise stand comparisons.

Stands	Number of significant loci			Probability of homogeneity[a]		
	Mega.	Pollen	Embryos	Mega.	Pollen	Embryos
1-2	0	2	2	0.054	0.032*	0.022*
1-3	0	0	1	0.100	0.150	0.045*
1-4	1	0	1	0.135	0.129	0.749
1-5	0	0	1	0.005*	0.010*	0.017*
1-6	2	0	1	0.088	0.126	0.133
2-3	0	0	1	0.147	0.234	0.233
2-4	2	0	2	0.008*	0.007*	0.080
2-5	0	0	2	0.075	0.141	0.030*
2-6	1	1	3	0.011*	0.002*	0.016*
3-4	0	0	0	0.550	0.672	0.566
3-5	0	0	0	0.631	0.670	0.666
3-6	1	0	3	0.022*	0.071	0.019*
4-5	1	0	2	0.002*	0.004*	0.010*
4-6	1	0	1	0.002*	0.009*	0.006*
5-6	0	0	4	0.011*	0.011*	0.001*

a. Chi-square contingency analysis of allele frequency homogeneity summed over all loci.
* Prob. $<$ 0.05.

Heterogeneity of allele frequencies has been detected among populations in several coniferous species. MITTON *et al.* (1977) observed allele frequency heterogeneity over relatively short distances in ponderosa pine var. *scopulorum* from Colorado. Significant allele frequency differences in var. *scopulorum* were also found between small populations all located within a two hectare area (LINHART *et al.*, 1981). FERET (1974) found significant allele frequency heterogeneity between three small populations of table mountain pine (*Pinus pungens* LAMB.) in Virginia. For 11 stands of pitch pine (*Pinus rigida* MILL.), distributed from Quebec to North Carolina, GURIES and LEDIG (1981) found variable population differentiation depending upon the locus tested. Most loci however, showed significant population heterogeneity.

Allele frequency differences at each locus between seeds from the six Colstrip stands were tested for significance using a contingency chi-square analysis. All pair-wise stand combinations were tested using data for megagametophytes, pollen and embryos (megagametophyte and pollen combined). *Table 5* shows the number of loci exhibiting significant allele frequency differences for pair wise comparisons between all stands. Loci which show significant allele frequency heterogeneity vary with the stands being compared. The large number of divergent loci found in the embryos, compared to the separate analyses of pollen and megagametophytes is likely due to larger sample size; 100 alleles per locus per stand, compared to 50.

Genetic differences between stands, over all loci combined, were tested for significance with chi-square contingency analyses *(Table 5).* Seven of the 15 possible pair-wise stand comparisons showed significant genetic differences over all loci for megagametophytes and for pollen. Nine of the 15 comparisons showed significant differences in the embryos. Again, the greater number of pair-wise comparisons showing significant allele frequency heterogeneity in the embryos is likely due to increased sample size.

Table 6. — NEI'S (1972) genetic distance (above diagonal) and geographic distance in kilometers (below diagonal) for pair-wise comparisons between six stands of ponderosa pine from Colstrip, Montana.

	Stand					
Stand	1	2	3	4	5	6
1		0.0040*	0.0098*	0.0078	0.0082*	0.0051
2	1.07		0.0061	0.0081	0.0051*	0.0042*
3	1.21	0.85		0.0040	0.0042	0.0057*
4	6.22	7.25	7.10		0.0075*	0.0076*
5	12.04	12.65	11.83	10.55		0.0051*
6	12.68	13.61	13.08	7.68	7.19	

* significant (prob. $<$ 0.05) allele frequency heterogeneity from the sum of contingency chi-square tests over all loci.

Genetic and Geographic Distance

A commonly used index of genetic differentiation between populations is that of genetic distance (D) (NEI, 1972). The accumulated number of gene differences per locus between populations is expressed as

$$D = -\ln I$$

where I is the normalized identity of genes.

Estimates of D were calculated over 23 loci, and are shown in *Table 6* along with straight-line geographic distances for pair-wise comparisons of stands. Values of D range from 0.0040 to 0.0098, with a mean of 0.0062. These values are similar to those obtained by GURIES and LEDIG (1981) for pitch pine, YEH and O'MALLEY (1980) and YANG *et al.* (1977) for Douglas-fir, and LUNDKVIST and RUDIN (1977) for Norway spruce (*Picea abies* KARST.). A comparison over 19 loci between ponderosa pine var. *ponderosa* from the northern Rocky Mountain area (ALLENDORF *et al.*, 1982) and all six Colstrip populations of var. *scopulorum* gives a D value of 0.0590. This value is large relative to the values of D normally found between populations within a coniferous species, however a large genetic distance is expected between taxonomic varieties.

Correlations between geographic (spatial) distance and genetic distance can be explained on the basis of isolation by distance. GURIES and LEDIG (1981) found no correlation between geographic and genetic distance in 11 populations of pitch pine. In Douglas-fir from coastal British Columbia, YEH and O'MALLEY (1980) found a significant correlation between geographic and genetic distance, however much of this may be due to differences between mainland and island populations. SCHAAL (1974) found genetic distance to be strongly correlated with geographic distance in *Liatris cylindracea* MICHX. over distances of several meters.

No correlation was found between geographic and genetic distance in the six ponderosa pine stands from Colstrip. This indicates genetic differentiation between stands is not primarily in response to restricted gene flow due to geographic distance, over the distances examined. The genetic heterogeneity among the stands could be due to differential selection of genotypes, or genetic drift. However, the similarity in stand structure, elevation, topography and soils, as well as the agreement between the observed and expected variance in heterozygosity V(H) discussed earlier) indicates genetic differences between stands is likely due to genetic drift.

Gene Flow Between Stands

Fire plays a large role in the ecology of ponderosa pine from the Colstrip area (RICHARDSON, 1981). Heavy burns that reduce a stand to a few trees are known to occur periodically. Due to the limited dispersal distances of seeds (DAHMS and BARRET, 1975), regeneration of a stand following a heavy burn would be primarily from the few trees left. The severe reductions in stand size are expected to result in a great deal of genetic drift due to the small sample of parent trees left to regenerate a stand following a fire. If gene flow into a regenerating stand is limited, a large amount of genetic divergence between stands would be expected.

ALLENDORF and PHELPS (1981) have shown that even with a great deal of gene flow between populations, allelic divergence will often occur. Because the Colstrip stands show allelic divergence at few loci, a large amount of gene flow in the form of pollen likely takes place between populations. Pollen movement would be aided by the flat topography and frequent winds found in this area. Wide spacing between trees within stands is common, and would allow wind and pollen to move easily through the stands. The exchange of pollen between stands would reduce the effect of genetic drift caused by bottlenecks in population size.

The large amount of gene flow into the isolated ponderosa pine stands may appear contradictory to the theory that considers a tree is pollinated primarily by its near neighbors (SHAW and ALLARD, 1981; LIBBY *et al.*, 1969). However, in a situation where a few isolated trees are left following a fire, and windy conditions are common, a great deal of effective pollen flow from outside sources is probable. As the stand regenerates, its diversity will increase due to the gene flow from other stands. This would increase a stands genetic diversity, and serve to greatly reduce differentiation caused by genetic drift. When the stand matures, it produces large amounts of pollen and trees would be pollinated primarily by their neighbors. Therefore, effective gene flow into a stand from outside sources would be reduced as the stand increases in size and density. The small amount of among stand genetic heterogeneity found in Colstrip ponderosa pine is explained by the gene flow into a stand during its early stage of development.

Ponderosa pine and other coniferous species found in areas with variable topography and site conditions may also be subject to large amounts of gene flow during the early development of a stand. Isolated trees left following a catastrophe such as fire would be pollinated by diverse outside sources. The gene flow into a stand would serve to increase the genetic diversity within a stand, and reduce differences between stands. Topographic barriers to gene flow would affect the pattern of genetic differentiation, and differences in site conditions would increase differentiation due to selective forces acting upon the new regeneration. When there are no barriers to pollen movement, large genetic differences between stands may be better explained by selection than by genetic drift.

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Index Selection For Increased Dry Weight in a Young Loblolly Pine Population

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Summary

Several selection models incorporating different traits into combined family-plus-individual multiple trait indexes were compared for their expected efficiency in increasing dry weight of individual trees. In our population, combined selection for volume with increased dry weight as the desired goal resulted in 10% less expected gain than combined selection based directly on dry weight. The most efficient single trait combined family-plus-individual selection model for dry weight used height as the selection criterion. Expected gain for this model was 8% greater than for combined selection with dry weight as the criterion because of the high genetic correlation of height and dry weight yield and the higher heritability of height.

Combining family and individual values for height, d.b.h. and volume in an index whose goal was dry weight increased expected gain over combined selection for dry weight by 17%. Extending the index to include specific gravity and dry weight increased expected efficiency to 120%. This small improvement in expected gain over the