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## Genetic differentiation in ponderosa pine along a steep elevational transect

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### Summary

Data are reported for 7 enzyme polymorphisms in ponderosa pine, *Pinus ponderosa* LAWS. var. *scopulorum* ENGELM., in 3 ecologically different localities along a steep elevational transect in the Front Range of Colorado. The highest and lowest sites of the transect differ by almost 1000 m. in elevation, but they are only 3 km apart. Heterozygote excesses associated with slope aspect are found at a peroxidase locus, and clinal differentiation is evident at a phosphoglucosyltransferase locus. Linkage disequilibrium, a measure of non-random association of genotypes at separate loci, is found at each of the localities.

**Key words:** ponderosa pine, genetic differentiation, linkage disequilibrium allozymes, elevational transect.

### Zusammenfassung

Es wird über 7 Enzym polymorphismen bei *Pinus ponderosa* LAWS var. *scopulorum* ENGELM. an drei ökologisch verschiedenen Orten berichtet. Diese liegen entlang eines Höhengradienten im Front Range von Colorado. Der maximale Höhenunterschied zwischen zwei Standorten beträgt 1000 m, wobei diese nur 3 km voneinander entfernt sind. Bei einem Peroxidase-Locus wurde ein Zusammenhang zwischen Heterozygotie und Hanglage festgestellt, eine klinale Variation wurde bei einem Phosphoglucosyltransferase-Locus deutlich. Kopplungsungleichgewichte, ein Maß für die nicht zufällige Zusammensetzung von Genotypen an einzelnen Loci wurden für jeden der Versuchsorte festgestellt.

### Introduction

Traditional views of the relative influence of gene flow and environmentally specific selection upon genetic differentiation and speciation led many biologists to assume that there would be little differentiation within continuous populations (MAYR, 1963). In spite of a growing body of evidence demonstrating that genetic differentiation does occur over short distances in many plants (JAIN and BRADSHAW, 1966; EHRLICH and RAVEN, 1969; GRANT and MITTON, 1977; MITTON *et al.*, 1977; WHITE, 1978) there still remains considerable skepticism that differentiation can occur with-

in continuous stands of long-lived woody perennials (ENDER, 1977). On the contrary, we have documented considerable genetic differentiation both between closely adjacent stands associated with ecological parameters such as slope aspect (MITTON *et al.*, 1977) as well as within stands associated with family structure (LINHART *et al.* 1980). Although the role of natural selection in molding population structure is strongly suggested by most of these studies, more empirical evidence is needed before the relative contributions of natural selection, migration, family structure, and stochastic forces can be assessed.

Presented here are results from a study of 7 protein polymorphisms in sample localities along an elevational gradient near Boulder, Colorado. A steep transect has been studied to maximize both the possibility of gene flow and the ecological and environmental distinction between the sample localities. Analyses are presented of both single gene polymorphisms and the joint distributions of pairs of polymorphic loci.

### Materials and methods

#### Description of sites

Ponderosa pine, *Pinus ponderosa* LAWS. vars *scopulorum*, is a long-lived, wind-pollinated temperate forest tree. It forms extensive, continuous stands in the Montane and Upper Montane region between 1829–2743 meters in the Colorado Rocky Mountains. On south-facing slopes it occurs in pure, open stands with a lush understory of grasses and herbs. On steep north-facing slopes, it is co-dominant with Douglas-fir, *Pseudotsuga menziesii*, forming dense stands through which little sunlight penetrates to the forest floor (MARR, 1967).

The transect studied here runs from east to west over a horizontal distance of 3 kilometers and 811 meters of elevational gain. The lowermost site (elev. 1767 m) is a savannah-like pure stand of relatively large diameter, short pines that are actively colonizing the grasslands of the Great Plains (ROBBINS and DODDS, 1908; ROACH, 1948). The slope is almost flat; trees are widely spaced and have an open-grown appearance. East of these trees, conifers are absent for hundreds of miles except for a few isolated stands. The highest elevation site (elev. 2579 m.), by contrast, consists of a

dense stand of small diameter, medium height pines growing at the upper limits of their range. Douglas-fir is the most abundant tree at this elevation and we were able to locate ponderosa pines only on a steep, exposed, north-facing saddle at the top of Bear Mountain, the highest mountain in the foothills near Boulder, Co. Here, ponderosa pine is associated primarily with limber pine, *Pinus flexilis* JAMES, which, in the Front Range, grows characteristically on ridges subjected to strong winds (MARR, 1967). The middle elevation site (elev. 2012 m.) is located at the rather abrupt interface between the steep slopes of Bear Mountain and the gentle slopes leading to the plains below. As far as is known, trees were present at this location when this area was first populated in the 1800's and they are believed to have been the source of pollen and seeds which produced those trees now colonizing the plains at lower elevations. The trees are mostly large diameter, tall ponderosa pine growing in a park-like stand in association with a few Douglas-fir. The slope is gentle and the site receives much shade for most of the afternoon.

#### Enzyme polymorphisms

Enzyme polymorphisms detected in mature needle tissue studied here are peroxidase (PER-2, 3 alleles), phosphohexose isomerase (PHI, 3 alleles), phosphoglucomutase (PGM-1, 3 alleles; PGM-2, 3 alleles), glutamate dehydrogenase (GDH, 2 alleles), colorimetric esterase (CE, 3 alleles), and fluorescent esterase (FE, 3 alleles). Mendelian inheritance for each of these loci has been demonstrated (MITTON *et al.*, 1979; O'MALLEY *et al.*, 1979) but linkage relationships among the loci are unknown.

Methods for the resolution and staining of these enzymes are unchanged from MITTON *et al.* (1977, 1979) except for PGM-1. The three alleles of this polymorphism resolve very close to one another, so that the assignment of genotypes can be difficult. We have circumvented this problem by scoring genotypes of this locus on two different buffer systems, a discontinuous lithium hydroxide buffer (SELANDER *et al.*, 1969, .035 M LiOH rather than .03 M) and a continuous pH 6.1 citric acid buffer (CLAYTON and TRETIAK, 1972). The lithium hydroxide buffer system clearly resolves the fastest migrating allele (# 1) from the others, but alleles 2 and 3 are not clearly separated. The citric acid buffer clearly resolves allele 3 from the other two alleles, but alleles 1 and 2 can not be clearly distinguished in this system. Comparison of allelic contents for each individual from both buffer systems allows an unambiguous assignment of genotype.

#### Statistical Analyses

Heterogeneity of allelic frequencies among sample localities is tested with the Chi-square test of WORKMAN and NISWANDER (1970), and linkage disequilibrium is tested from genotypic data by the method of HILL (1974). The fit of observed genotypic frequencies to those expected under the assumptions of the HARDY-WEINBERG Law is tested with a Chi-square test. Values for linkage disequilibrium are reported as  $D' = D/D_{max}$ , the linkage disequilibrium parameter standardized by the maximal degree of disequilibrium possible at those allelic frequencies. Linkage disequilibrium,  $D$ , for two loci, each with 2 alleles (A and a at one locus and B and b at a second locus), is defined as  $D = g_{AB}g_{ab} - g_{Ab}g_{aB}$ , where  $g_{AB}$  is the frequency of the gamete carrying alleles A and B. When genotypes at different loci are distributed independently,  $D = 0$ .

#### Results

Genotypic and allelic frequencies for 7 polymorphic loci from 3 localities are presented in Table 1. Previously, geographic and microgeographic variation for both allelic frequencies and genotypic proportions were reported for the PER-2 locus (MITTON *et al.* 1977), yet there is no significant

Table 1. — Genotypic and allelic frequencies of 7 protein polymorphisms at 3 localities in a elevational transect for ponderosa pine.

Locus	Site	Genotype						N	f(1)	f(2)	f(3)	$\chi^2_a$	$\chi^2_b$
		11	12	22	13	23	33						
PER-2	high	0	0	36	0	27	1	64	.00	.77	.23		
	mid	0	1	37	0	23	3	64	.01	.76	.23		
	low	0	3	36	0	24	1	64	.02	.78	.20		
PHI	high	0	1	63	0	0	0	64	.01	.99	.00		
	mid	0	3	59	0	2	0	64	.02	.96	.02	*	
	low	0	0	64	0	0	0	64	.00	1.00	.00		
PGM-1	high	0	0	61	0	3	0	64	.00	.98	.02		
	mid	0	4	50	0	8	2	64	.03	.88	.09		***
	low	1	3	32	3	13	12	64	.06	.62	.32	***	***
PGM-2	high	0	3	61	0	0	0	64	.02	.98	.00		
	mid	0	1	62	0	1	0	64	.01	.98	.01		
	low	1	3	57	0	3	0	64	.04	.94	.02		
GDH	high	28	29	7	0	0	0	64	.66	.34	.00		
	mid	33	29	2	0	0	0	64	.74	.26	.00		
	low	21	36	7	0	0	0	64	.61	.39	.00		
CE	high	36	9	0	19	0	0	64	.78	.07	.15		
	mid	26	10	1	26	0	1	64	.70	.09	.21		
	low	31	9	0	24	0	0	64	.74	.07	.19		
FE	high	3	25	23	3	9	1	64	.27	.62	.11		
	mid	4	24	28	3	5	0	64	.27	.67	.06		
	low	6	22	27	2	6	1	64	.28	.64	.08		

Note:  $\chi^2_a$  tests the fit of genotypic frequencies within elevations to Hardy-Weinberg expectations, and  $\chi^2_b$  tests heterogeneity of allelic frequencies among the three sample localities. \* =  $P < .05$ , \*\* =  $P < .01$ , \*\*\* =  $P < .001$ .

heterogeneity among these sample localities for this protein. The fit of genotypic proportions to those expected according to the HARDY-WEINBERG law is satisfactory at the middle locality, but at both of the other localities, there is an excess of heterozygotes which approaches statistical significance ( $P = .10$ ). If the highest and lowest samples are pooled, there is a significant excess of heterozygotes ( $p < .05$ ). This pattern of deviation from expectations is in agreement with previous observations (MITTON *et al.* 1977) which suggest that excess heterozygosity is associated with xeric habitats. Both the highest and the lowest sites receive sunshine all day while the middle site becomes shaded early in the afternoon. In addition, ponderosa pines at the high elevation site are growing on a ridge where they are exposed to strong westerly winds. The association of ponderosa pine with limber pine at this site strongly suggests a xeric habitat where evapotranspiration may be high.

Allelic frequencies at the PGM-1 locus change dramatically among the three localities. There is very little variation at the highest sample locality where the frequency of the 2 allele is .98, but variation increases with decreasing elevation ( $p < .001$ ). At the lowest site, where allele 3 increases dramatically in frequency from .02 to .32, there is a significant deficiency of heterozygotes. These data suggest that at the lower elevational limit of ponderosa pine in this area there is directional selection for allele 3 or for alleles at a locus in strong linkage disequilibrium with PGM-1.

The PHI locus is polymorphic, but not at all localities. Although these data are statistically heterogeneous, the degree of difference from site to site is small, and is probably not biologically significant. Genotypic observations fit those expected according to the HARDY-WEINBERG Law. No deviations from HARDY-WEINBERG proportions and no heterogeneity among sites were detected for PGM-2, GDH, CE or FE.

Loci were studied jointly to determine whether genotypes at one locus were distributed independently from other

loci. PHI and PGM-2 were not considered in these analyses due to their low variability. Alleles at each of the remaining loci were pooled to 2 alleles in such a way as to maximize the level of heterozygosity. The magnitudes of linkage disequilibrium are presented in Table 2. All possible pair-wise tests for linkage disequilibrium were performed, and five of these were statistically significant, which is more than the one or two expected by chance. Almost all of the significant values and most of the large values are between either PER or PGM-1 and other loci. These are the two loci for which, when looked at independently, our data suggest a role for natural selection in determining genotypic proportions. Thus, it is possible that significant values for associations between these loci and other loci are due, at least in part, to the action of natural selection.

### Discussion

Geographic variation for both morphological and protein characters in forest trees is a more common observation than is uniformity of characters (HAMRICK and LIBBY, 1972; TIGERSTEDT, 1973; FERET, 1974; MUHS, 1974; BERGMAN, 1975; HAMRICK, 1976; GRANT and MITTON, 1977; LUNDKVIST and RUDIN, 1977; MADSEN and BLAKE, 1977; MITTON *et al.*, 1977; FALKENHAGEN, 1978; KESSEL, 1979). This is certainly not unexpected, for many forms of natural selection and stochastic forces are expected to generate geographical variation under a broad range of ecological conditions and population structures (KIMURA and OHTA, 1971; ENDLER, 1977). Our interest centers partly on the demonstration of geographic variation for genetic loci in this wind-pollinated forest tree and also on the determination of the forces creating that differentiation. We chose this steep elevational transect in order to maximize both environmental differences and the potential for gene flow among the sites. The highest (2,579 m) and lowest (1,768 m) sites on this transect are separated in the horizontal axis by less than 3 km. The traditional view predicts that gene flow, particularly by pollen movement between these closely adjacent sites would prevent genetic differentiation. On the other hand, rapidly changing environmental conditions could produce asynchronous flow-

ering, restricting gene flow between the sites. Our data suggest that either gene flow is trivial between the sites or it is overridden by natural selection. In either case, geographical variation for the PGM-1 locus and heterozygote excesses for PER-2 are evident.

Although we are inclined to consider natural selection as the major force producing the differentiation found here, we cannot decisively rule out stochastic forces. The lowest site on the transect was established within the last 100 years, (BECKMAN, 1977), and it is not known what part founder effect played in this colonization. Similarly, Douglas-fir, lodgepole pine and limber pine were considerably more abundant than ponderosa pine at the highest site, so that stochastic forces may also have played a role in the establishment of this population.

Linkage disequilibrium, a measure of non-random association of genotypes (THOMSON, 1977), can be a sensitive measure of the action of natural selection. This measure is rarely different from zero in obligately out-crossed organisms (CLEGG, 1978). Substantial linkage disequilibrium among pairs of loci is found along this transect, and because the disequilibrium is generally found at the loci for which we have some evidence of the role of natural selection, it is probable that this disequilibrium is predominantly the product of natural selection. Linkage disequilibrium is expected, however, in finite populations (HILL and ROBERTSON, 1968; SVED, 1971) and as a consequence of inbreeding (ALLARD *et al.*, 1978; CLEGG *et al.*, 1972; HILL and ROBERTSON, 1966) so that processes other than natural selection may also be operating.

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Table 2. — Linkage disequilibrium for pairs of loci at three localities along an elevational transect for ponderosa pine.

LOCALITY					
high 2,579 meters					
	PGM-1	-1.000			
	GDH	-.027	-1.000*		
	CE	1.000	1.000	-.109	
	FE	-.059	1.000†	.266	.496
		PER	PGM-1	GDH	CE
mid 2,012 meters					
	PGM-1	-1.000			
	GDH	-.068	1.000†		
	CE	-.115	-.420*	-.409	
	FE	-.018	.268	.042	-.494*
		PER	PGM-1	GDH	CE
low 1,768 meters					
	PGM-1	-.101			
	GDH	-.465*	.106		
	CE	-.269	.657	.036	
	FE	.196	.218*	.197	.130
		PER	PGM-1	GDH	CE

Note: The linkage disequilibrium parameter presented here is  $D'$ , the degree of association relative to the maximum for those particular allelic frequencies;  $D' = D/D_{max}$ .  $D'$  retains the sign of  $D$ . + =  $P < .10$ , \* =  $P < .05$ .

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