An Analysis of Genetic Architecture in Populations of Ponderosa Pine[1]

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Abstract: Patterns of genetic variation were studied in three populations of ponderosa pine in Colorado by using electrophoretically variable protein loci. Significant genetic differences were found between separate clusters of trees and between age classes within populations. In addition, data indicate that differential cone production and differential animal damage have genetic components. These results suggest that many diverse phenomena can affect allele frequencies within populations and may contribute to the high levels of genetic variability detected within populations of this species.

Assessments of genetic variation in forest trees have been aided greatly by the use of electrophoretic analyses of protein polymorphisms. Many such studies have concentrated on the distribution of this variation over a broad geographic range (for example, Guries and Ledig 1981, Lundqvist and Rudin 1977, and Yeh 1981). Results document that substantial variation exists in trees and that this variation is distributed in patterns determined primarily by the heterogeneity of the physical environments. These findings agree with data on patterns of geographic variation collected by earlier forest geneticists (Langlet 1971, Libby and others 1969). Small-scale patterns—over a distance of 1 km or less—are less well documented but increasing evidence suggests that differentiation can occur in forest trees over distances as small as a few hundred meters (Barber 1965, Benson and others 1967, Herman and Lavender 1967, Mitton and others 1977, Grant and Mitton 1977). Again, this differentiation is associated with significant environmental heterogeneity produced by elevation, slope, or exposure.

The results mentioned above describe the organization of genetic variation between populations but say little about variation within populations. Within-population variation is particularly worthy of interest at this time, since several studies have suggested that in some forest trees, including ponderosa pine, such variation is significantly greater than variation between populations (Lines and Mitchell 1966, Madsen and Blake 1977, O'Malley and others 1979).

Many variables can affect the patterning of genetic variation within populations, including differential predation by animals and restricted pollen and seed dispersal. Also, trees are long-lived and environmental conditions change with time. As a result, different genotypes may reproduce in different years and natural selection operating on seedlings may involve different forces in different years. Consequently, generations of different genetic constitutions may exist within multiaged stands.

This paper reports a study dealing specifically with patterns of intrapopulation variation. In 1976, we began studying populations that occupy relatively small areas, from 2 to 5 hectares, within which the heterogeneity of the physical environment is limited. We investigated three populations of ponderosa pine (*Pinus ponderosa* var. *scopulorum* Engelm.) in Colorado for (a) spatial distribution of genotypes within a given population and (b) genetic differences between age groups within a given population. We also investigated whether (c) genetic differences exist between trees that reproduce heavily and those that do not and, finally, (d) whether animal damage in the form of deer browsing or woolly aphid infestation is related to genetic constitution of the trees.

MATERIALS AND METHODS

All populations in our study grow in the Front Range of the Rocky Mountains, near Boulder, Colorado. None of them have been heavily disturbed by human activities, such as heavy logging or tree planting nearby. One population (Boulder Canyon) has been analyzed in detail; comparative data for some of the phenomena being studied are available from the Glacier Lake and Shanahan Mesa populations.

Genetic Methods

All genetic analyses are made on the basis of enzyme polymorphisms detectable in extracts of mature needle tissues. Because we are studying patterns of variation, only polymorphic loci are investigated.

The enzyme polymorphisms being studied include one peroxidase (PER-2, 3 alleles), one phosphohexose isomerase (PHI, 3 alleles), two phosphoglucomutase loci (PGM-1, 2 alleles and PGM-2, 3 alleles), one glutamate dehydrogenase (GDH, 2 alleles), one colorimetric esterase (C.E., 3 alleles), and one fluorescent esterase (F.E., 3

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alleles). Patterns of Mendelian inheritance are reported elsewhere (Mitton and others 1977, 1979).

Methods of preparation of needle extracts, resolution and staining are reported in the following publications: Mitton and others (1977) for PER-2; Mitton and others (1979) for all other loci.

Heterogeneities of allele frequencies were tested with the chi-square test of Workman and Niswander (1970).

Boulder Canyon Population

This population, within a pure ponderosa pine stand on the south-facing slope of Boulder Canyon at 1738 m, consists of all individuals—with the exceptions of a few seedlings—within an area of about 2 hectares (*fig. 1*). The population is located in the ponderosa pine belt typical of the lower elevations of the Colorado Front Range.

We numbered and mapped all pines in the population. We recorded age, diameter, pollen and cone output, presence or absence of infestations by woolly aphids (*Pineus coloradensis* Hopk.), browsing by deer (*Odocoileus demionus* Raf.) and the genetic constitution at all seven polymorphic protein loci.[4]

We compared the genetic constitutions of the six clusters of trees in the population. In 1977, we compared trees producing at least some pollen with those that did not, and we also counted all mature female cones. We compared prolific cone producers with poor cone producers, correcting for the effects of age and size on cone production (Linhart and others 1979a).

We analyzed browsing by mule deer, and we compared the genetic constitutions of heavily browsed trees to those of lightly or unbrowsed trees. We also compared trees that had woolly aphids on their foliage in 1979 with those that did not.

Glacier Lake Population

This population is also on a south-facing slope, at 2590 m. Here the forest is composed primarily of ponderosa pine, with Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco), limber pine (*Pinus flexilis* James), and juniper (*Juniperus scopulorum* Sarg.) intermixed. We tagged and mapped approximately one-third of the ponderosa pines within a 4-ha area. In contrast to the Boulder Canyon population, some disturbance occurred here in about 1900 when scattered logging was done in the area. Furthermore, during our study, an outbreak of bark beetles (*Dendroctonus ponderosae* Hopk.) killed more than one-third of the trees. As a result, the spatial structure of this population is not as stable as it is in the Boulder Canyon population. In this population we investigated primarily the spatial distribution of genetic variation at three loci: PER, C.E. and R.E.

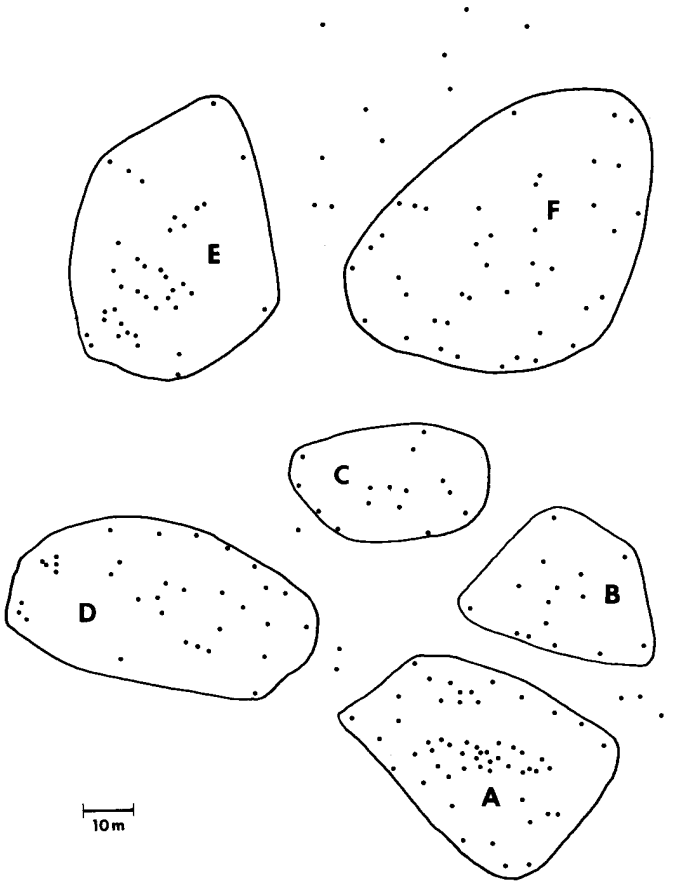

Figure 1—Approximate distribution of trees in a stand of ponderosa pine in Boulder Canyon, Colorado. Six clusters have been defined and are labeled A through E. In the text, these are called the Boulder Canyon Population. Age structures of all clusters are also shown.

Shanahan Mesa Population

This population, at an elevation of 1700 to 2000 m, is on an east-facing mesa at the base of the Front Range. The mesa was grass-covered until about 120 years ago when ponderosa pine started to colonize the area from seed dispersed from stands growing on the steep slopes above the mesa. In one area, a second generation of saplings has become established under the closed canopy of the mature trees (Beckman 1977).

We compared variation at the PER locus in the original colonists, which grew up in the open grassland, with variation of their offspring, which grew up under their canopy.

RESULTS AND DISCUSSION

Spatial Clustering and Genetic Differentiation

Both the Boulder Canyon and the Glacier Lake populations have clusters of trees that are easily identified.

[4]Linhart, Y.B., J.B. Mitton, and K.B. Sturgeon. Some genetic features of clustering in a ponderosa pine population. (Manuscript in preparation.)

Open spaces are found between clusters. In each population, some trees, located either between clusters or at the edge of the mapped area, are not clearly assignable to a given cluster and, therefore, are omitted from the comparisons. In both populations we found significant differences in allele frequencies among the various clusters (*table 1*). Genetic heterogeneity is particularly striking in the Boulder Canyon population where nearby clusters can vary by as much as 20 percent at GDH, 21 percent at C.E., 22 percent at PER-2, and 29 percent at F. E. We found little intrapopulation variation at the other three loci tested—PGM-1, PGM-2, and PHI have their most common alleles at frequencies of 88 to 100 percent in all clusters. Similar results are found in the Glacier Lake population (*table 1*).

Similar genetic differentiation between carefully mapped, nearby clusters of plants has also been documented for *Linanthus parryae* (Gray) Greene (Epling and Dobzhansky 1942), *Liatris* (Schaal 1975), *Pinus sylvestris* L. (Rudin and others 1977), and *Picea abies* (L.) Karst. (Tigerstedt 1973). Tigerstedt believed that evidence for such heterogeneity was lacking within the populations he studied; however, an analysis of his figure 4 shows that, if one divides that population into two segments by an E-W line running through the center of the mapped area, the fast allele at the LAP locus is at a frequency of 0.67 in one area composed of 24 trees, and at a frequency of 0.39 in the other area composed of 23 trees. This difference is significant ($X^2 = 16.49$, 1 d.f., $P < 0.005$). Some evidence also exists for clustering and genetic heterogeneity in local populations of other trees. These include *Pseudotsuga menziesii* (Mirb.) Franco (Rehfeldt 1978, Shaw and Allard *1981), Cupressus macrocarpa* Hartw. (Kafton 1977), *Shorea leprosula* Miq. (Dipterocarpaceae), and *Xerospermum intermedium* Radlk. (Sapindaceae) (Gan and others 1977) and *Fagus* (Stern and Roche 1974). No evidence was found, however, for such clustering in populations of *Pinus rigida* Mill. (Guries and Ledig 1977) or *Pinus taeda* L.[5]

The genetic differentiation observed between nearby clusters probably results in part from the genetic relatedness of trees within a cluster. Such relatedness may occur when most seeds from a given parent tree are dispersed within a small area. Even if the pollen that fertilized the parent tree is a completely random sample of the pollen available in the stand, the group of trees arising from the seed rain of a single parent is a group of half-sibs. Also, those wind-dispersed seeds that are least likely to be transported any distance are those produced in the lower portion of a crown; those seeds also happen to be the ones that are most likely to have been produced by self-pollination (Fowler 1965). This fact may contribute to the genetic relatedness among individuals within a group.

Inbreeding, in the absence of selection, would be expected to produce deficiencies of heterozygotes relative to Hardy-Weinberg expectations. The distribution of genotype frequencies within given clusters compared with frequencies expected under Hardy-Weinberg equilibrium showed few significant departures from expectations. In the Glacier Lake population, no departures were observed. In Boulder Canyon, at the PER-2 locus, group B had 13 heterozygotes in a total of 15 trees ($X^2 = 7.13$, 2 d.f., $P < 0.05$), and group D had 24 heterozygotes in a total of 33 trees ($X^2 = 13.75$, 2 d.f., $P < 0.005$). Both of these departures result from excesses of heterozygotes. There are only two significant deviations in the 24 tests (6 clusters x 4 highly variable loci) that we performed, and these observations may be fortuitous. They may also reflect

[5]Personal communications from F. T. Ledig, and from M. Thompson Conkle, geneticists, Forest Service, U.S. Dep. Agric., July 26, 1979.

Table 1—*Allele frequencies ± standard errors for the most common allele at several protein loci among clusters within two populations of ponderosa pine*

Locus (allele)	Cluster							
	A	B	C	D	E	F	X^2	P
	Boulder Canyon Population							
PER-2 (2)	0.787± 0.04	0.567± 0.08	0.750± 0.08	0.712 ± 0.06	0.737 ± 0.05	0.721± 0.05	11.39 (10 d.f.)	<0.20
GLU (1)	.723± .04	.633± .08	.532± .09	.697 ± .06	.711 ± .05	.733± .05	5.34 (5 d.f.)	< .40
F.E. (2)	.630± .05	.667± .08	.750± .08	.742 ± .05	.461 ± .06	.547± .05	21.72 (10 d.f.)	< .025
C.E. (1)	.722± .04	.767± .08	.563± .09	.773 ± .05	.697 ± .05	.767± .05	25.01 (10 d.f.)	< .01
Total trees	54	15	16	33	38	43		
	Glacier Lake Population							
PER-2 (2)	0.709±0.05	0.783±0.06	0.725±0.06	0.833 ±0.07	0.727 ±0.05	0.833±0.07	1.28 (4 d.f.)	<0.90
F.E. (2)	.535± .05	.522± .08	.500± .08	.714 ± .07	.583 ± .06	.667± .08	17.30 (8 d.f.)	< .05
C.E. (1)	.628± .05	.696± .06	.550± .08	.785 ± .07	.693 ± .06	.667± .08	12.42 (8 d.f.)	< .20
Total tree[1]	43	23	20	14 to 15	31 to 44	18		

[1] For clusters D and E, this number is variable because some trees died before their genotype at a given locus could be ascertained.

different selection pressures: other populations of ponderosa pine at comparable elevations also show excesses of heterozygotes at the PER-2 locus (Mitton and others 1977).

The extent of differentiation between clusters reported here is high: there may be greater genetic differences between some clusters *within* these pine populations than are found between populations separated by many kilometers in other species. One way to compare levels of differentiation is by estimating genetic distance, D (Nei 1972). Such comparisons must be done with caution because values of D are affected by the relative proportion of monomorphic loci used in the calculations. Because we used only polymorphic loci in our calculations of D, our results can be compared to those of Lundqvist and Rudin (1977) who used four polymorphic loci in studying *Picea abies* (L.) Karst. populations throughout Sweden, and those of Levin (1977) who used seven polymorphic loci in studies of *Phlox drummondii* Hook. subspecies throughout Texas. Lundqvist and Rudin reported D values of 0.002 to 0.039 between native populations. Levin reported a mean distance, $\overline{D}$ of 0.02 between all subspecies.

The Boulder Canyon clusters had a $\overline{D}$ of 0.0 15, with a range of 0.004 to 0.035; Glacier Lake had a $\overline{D}$ of 0.022, with a range of 0.006 to 0.055.

Genetic Differentiation Between Age Classes

The Shanahan Mesa population provides detailed evidence of how selection pressures within a population can change during a few years as a result of changes in the environment. Such changes in selection pressures produced significant genetic differentiation between age classes. The analysis was with the PER-2 locus, which has three alleles. Other studies have shown that allele 2 is found most frequently in the moister, cooler conditions prevalent at higher elevations (above 2400 m) and north-facing slopes. Alleles l and 3 are found most frequently in the warmer, more xeric conditions prevalent at lower elevations (below 1800 m) and on south-facing slopes (Mitton and others 1977). Also, 23 heterozygotes occur in frequencies significantly higher than those predicted by Hardy-Weinberg expectations in all populations growing in relatively warm, xeric conditions. Beckman (1977) studied the kinetics of the allozymes and found that the biochemical evidence was consistent with results from field observations: a 22 genotype produces an enzyme that functions best at cool temperatures; 13 and 33 genotypes produce enzymes that function best at warm temperatures; and a 23 genotype functions well over a broader range of temperatures than do the other genotypes.

At Shanahan Mesa, two well-defined size and age classes exist. In certain areas dominant trees that form the canopy are more than 40 years old, have colonized the open grasslands, and have had to survive in the relatively xeric conditions of these grasslands. Beneath them grow their offspring—trees that developed in the much moister, cooler conditions provided by a closed canopy. Under this canopy, snow accumulates and can remain for several weeks. In contrast, the snowfalls on the adjacent prairie are blown away by frequent winds or melted by the sun within days. Significant differences exist between these two groups at the PER-2 locus. The old colonizers have significantly higher frequencies of alleles 1 and 3 (0.200) than do the younger trees (0.060); this difference is significant ($X^2 = 6.40$, $P < 0.025$). In addition, there are significant excesses of heterozygotes in the old colonizers, whereas genotypic frequencies in the young trees conform to Hardy-Weinberg expectations (Beckman 1977). The associations between allele 2 and cool conditions, and alleles 2 and 3 and hot, xeric conditions are so consistent, that we believe the results at Shanahan Mesa truly reflect genetic differentiation in response to environmental conditions that changed during the span of 120 years. In the Boulder Canyon population, the genetic make-up of the major age classes was compared at seven loci. There were no striking genetic differences between them.

Genetic Aspects of Differential Reproduction

Natural selection results in part from differential reproduction of genotypes (Darwin 1859), but only a few published reports document differential reproduction of genotypes in natural populations (Eanes and others 1977, Clegg and others 1978). We wish to know if reproduction in individual trees is related to their allozyme genotypes. Because it is difficult to estimate accurately total pollen production for a population, we compared the genetic constitution of trees that produced pollen with those that did not. Three loci—PER-2, F.E. and C.E.—were analyzed in the Boulder Canyon and Glacier Lake .populations, and PER-2 only was analyzed in a third population near Glacier Lake. Results were the same for all comparisons: no genetic differences were detected between trees producing pollen and those that did not in 1977 (Linhart and others 1979b).

Female cone production can be measured accurately in the trees we study because the stands are open, and the trees are no taller than 15 m. Cone production is affected by both tree size and age in conifers (Schubert 1974, Dorman 1976). To describe cone production as a function of age and size, we performed a multiple regression analysis of cone production, using age and diameter of trees as independent variables. To get an accurate estimate of differential cone production in the Boulder Canyon population, we compared the trees whose cone production deviated most strikingly from that expected for their diameter and age. We compared the 50 trees that most exceeded their expected cone production to the 50 trees that were farthest below expectations. Results show that in 1977, a mast year, significant differences existed at several loci between high cone producers and low cone producers (*table 2*).

That differential cone production in trees has a significant genetic component is not unexpected. Much

agricultural research on selection for higher yields in plants and animals has underscored the importance of this genetic component (Allard 1960). Recent studies using allozyme markers have usually (Schall and Levin 1976, Eanes and others 1977, Clegg and others 1978), though not always (Christiansen and Frydenberg 1976), found genetic differences between reproducing and nonreproducing individuals. However, this genetic component needs more recognition in forestry research: a recent exhaustive review of research on reproduction in southern pines (Dorman 1976) shows that little of that research has focused on the genetic and evolutionary implications of differential reproduction.

Animal Damage and Its Genetic Implications

Patterns of deer browsing in 1977 and the presence of woolly aphids in 1979—the only years for which we have analyzed data so far—have distributions that are affected by the genetic constitutions of trees in the Boulder Canyon population. A comparison of the genetic constitution of heavily browsed trees with that of unbrowsed or lightly browsed trees at three loci showed that, although at PER-2 and F.E. we found no statistically significant differences, at C. E. the frequencies of alleles I and 2 combined were 0.791 in the heavily browsed and 0.672 in the lightly browsed trees ($X^2 = 4.635$, $P < 0.05$). In addition, a preference by deer for particular genotypes has been shown in *Pseudotsuga menziesii* (Mirb.) Franco (Radwan 1972).

Woolly aphids are common on ponderosa pine in the Central Rocky Mountains. We scored all trees in the Boulder Canyon population for presence or absence of woolly aphids by sampling 40 branches per tree. Trees with aphids (N =51) were compared with trees without aphids (N = 166) in the frequencies of the most common allele and of all other alleles pooled together. Significant differences were found at the F. E. locus. The frequencies of allele 2 was 0.500 in trees with aphids, and 0.651 in noninfested trees ($X^2 = 6.82$, $P < 0.01$). These data suggest that patterns of animal attacks on trees are not random. Similar results have been obtained in ponderosa pine by Edmunds and Alstadt (1978) in a study of the black pine leaf scale, *Nuculapsis californica*, and by Sturgeon (1979) in a study of the bark beetle, *Dendroctonus brevicomis.*

Table 2—*Comparisons between individuals with low and high relative cone productions in a population of ponderosa pine, at the Boulder Canyon population*

Locus	Allele	Cone production		Chi square	Significance
		Low F ±S.E.	High F ±S.E.		
PER	1	0.07±0.026	0.01±0.010	5.5	P<0.07
	2	.71 ± .045	.70 ± .046		
	3	.221 ± .041	.29 ± .045		
GLU	1	.66 ± .047	.74 ± .044	1.6	P < .50
	2	.34 ± .047	.26 ± .044		
F.E.	1	.20 ± .040	.31 ± .046	6.6	P < .05
	2	.74 ± .044	.57 ± .050		
	3	.06 ± .024	.12 ± .035		
PHI	1	.01 ± .010	.01 ± .010	1.4	P < .50
	2	.98 ± .014	.99± .010		
	3	.01 ± .010	.00		
C.E.	1	.67 ± .047	.82 ± .038	5.9	P < .05
	2	.05 ± .044	.03 ± .017		
	3	.28 ± .045	.15 ± .035		
PGM-1	1	.03 ± .017	.08 ± .027	2.5	P < .50
	2	.97 ± .017	.92 ± .027		
PGM-2	1	.09 ± .029	.06 ± .024	2.2	P < .50
	2	.90 ± .030	.94 ± .024		
	3	.01 ± .010	.00		

Synthesis

The genetic clustering reported in the species cited in this report presents a different picture from the one observed in some other conifers studied to date. For example, no subdivision has been detected in *Pinus rigida* Mill. (Guries and Ledig 1977) or *Pinus taeda* L.[5] Rudin and others (1977) studying *Pinus sylvestris* L. and Tigerstedt (1973) studying *Picea abies* (L.) Karst. show that in these species the subdivision is not very pronounced. These differences may result, in part, from study techniques: our study at Boulder Canyon involves mapping of all individuals established within a given area, with a sample size of more than 200 individuals; the studies cited earlier typically involved selected individuals and sample sizes of 60 to 70 trees. More importantly, the differences probably reflect real differences in biological features of the species concerned. Ponderosa pine in the Rocky Mountains often grows in all-aged stands which are composed of patches of relatively uniform size and age (Schubert 1974). In species where such mosaics do not occur, and where stands are primarily single-aged and single-sized, less opportunity for such differentiation exists. This may be particularly true in species such as *Pinus rigida* Mill. that depend on fire for their establishment, so that extensive stands get started under similar conditions.

Because heterogeneity of the physical environment is limited within a site, causes of the heterogeneity must be sought elsewhere. We believe that clusters are probably composed of genetically related individuals, given the short dispersal distances of both tree seeds (Levin and Kerster 1974) and viable pollen (Coles and Fowler 1976, Muller 1977). Differentiation among clusters may also occur if different clusters, which have different age structures (*fig. 1*), come from genetically-different parents, are exposed to different selective pressures in their early years, or both. Comparisons between major age classes in ponderosa pine (Beckman 1977) have shown major age-associated genetic differences within some populations.

Animal predation may also contribute to the genetic structure of the Boulder Canyon population. Both deer and woolly aphids show preferences for some phenotypes, and it is likely that other animals with more destructive habits could practice similar discrimination.

The extent of differentiation in allele frequencies observed between clusters within the Boulder Canyon ($\overline{D}$ =0.015) and Glacier Lake populations ($\overline{D}$ = 0.022) are similar in magnitude to the differentiation between the Boulder Canyon and Glacier Lake populations ($\overline{D}$ = 0.030). These populations are separated by more than 1000 m in elevation and several kilometers in their linear distance. This suggests that a substantial proportion of total genetic variation can be found within populations of ponderosa pine and supports the findings of Madsen and Blake (1977) and O'Malley and others (1979). Similar results for other conifers have been obtained by Yeh (1981), Lines and Mitchell (1966), and Fins (1979). However, this observation cannot be applicable to all classes of genetic variation, otherwise we would not have witnessed the repeated disasters produced by planting seeds of far-away sources in a given area; nevertheless, it probably applies to at least some of the variability that forest geneticists are intent upon capturing.

CONCLUSIONS

The populations of ponderosa pine we have studied show a great deal of intra-population differentiation which is not obviously associated with heterogenity of the physical environment. Similar results in other herbaceous and woody species suggest that such differentiation is a common feature of the genetic structure of plant populations.

The highly localized heterogeneity we have demonstrated, along with the genetic differences observed between heavy and light cone bearers have practical implications. Whenever seed collections or regeneration after logging depend on a few trees with large numbers of cones, the genetic base of the following generations may be severely restricted. Genetic analyses, provenance studies, and seed orchards cannot produce accurate pictures of total genetic variation if they depend only on progenies from trees producing many seeds in a given year.

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