Evolution, 45(4), 1991, pp. 989–999

GENETIC DIFFERENTIATION AND HETEROZYGOSITY IN PINYON PINE ASSOCIATED WITH RESISTANCE TO HERBIVORY AND ENVIRONMENTAL STRESS

Susan Mopper,[1] Jeffry B. Mitton,[2] Thomas G. Whitham, Neil S. Cobb, and Kerry M. Christensen
Department of Biological Sciences, Northern Arizona University, Flagstaff, Arizona 86011 USA

Abstract.—Arizona's Sunset Crater began erupting in 1064 AD and for the next 200 years buried over 2,000 km^2 in ash, cinders, and lava. Soil analyses indicate that pinyon pines (*Pinus edulis*) currently colonizing the cinder fields are faced with a highly stressful environment. Many of these pinyons suffer chronic, intense insect herbivory that reduces plant growth and eliminates female cone production. In contrast, herbivory among pinyons growing in neighboring sandy-loam soils is minimal. Furthermore, numerous trees within the heavily infested cinder field population suffer relatively low herbivory and maintain normal growth and reproduction. We used four polymorphic enzymes to examine the relationship between herbivore attack, environmental stress and genotypes of the adjacent cinder field, and sandy-loam soil pinyon populations. Our results demonstrate that 1) resistant trees display significant genetic differences and are more heterozygous for two enzymes associated with herbivory than susceptible trees; and 2) the cinder-soil pinyons exhibit significant genetic differences and are more heterozygous for an enzyme associated with environmental stress than the neighboring sandy-loam soil pinyons. We conclude that heterozygosity of specific or closely linked loci may facilitate pinyon resistance to herbivory and environmental stress, and that strong selection across narrow geographic boundaries resulted in rapid genetic differentiation of pinyon populations.

Key words.—Environmental stress, genetic differentiation, herbivory, heterozygosity, *Pinus edulis,* resistance.

Received July 5, 1990. Accepted October 20, 1990.

In 1064 AD, Sunset Crater began a 200 year eruption that eventually buried over 2,000 km^2 and killed most of the native vegetation (Krutch, 1974). The Sunset Crater pinyon population is therefore quite young, having invaded the cinder fields from neighboring populations only within the last 800 years. Sunset Crater pinyons suffer unusually high levels of chronic and severe herbivory by many insect herbivores (Whitham and Mopper, 1985; Mopper and Whitham, 1986; Mopper et al., 1990). Stem moth (*Dioryctria albovitella*) attack is so intense that it not only alters the architecture of infested trees, but it reduces reproduction and growth as well (Whitham and Mopper, 1985; Mopper et al., 1991). Nonetheless, numerous trees interspersed throughout the population are comparatively resistant to attack. In contrast to the widespread herbivory at Sunset Crater, pinyons growing in sandy-loam soils at the boundary of the cinder fields are rarely attacked and exhibit normal growth and reproduction.

Population genetics theory predicts that, under many selective regimes, fitness will increase with the number of heterozygous loci (Karlin and Lieberman, 1979*a,* 1979*b*; Ginzburg, 1979; Turelli and Ginzburg, 1983). In accordance with this prediction, many studies of protein variation have reported components of fitness to increase with heterozygosity (Mitton and Grant, 1984; Zouros and Foltz, 1987; Mitton, 1989, but see Endler, 1986). Empirical studies have reported individual heterozygosity to be positively correlated with viability (Mitton and Koehn, 1975; Koehn et al., 1976; Zouros et al., 1983), growth rate (Zouros et al., 1980; Pierce and Mitton, 1982; Koehn and Gaffney, 1984), fecundity (Rodhouse et al., 1986; Gajardo and Beardmore, 1989), mating success (Watt et al., 1985; Carter and Watt, 1988), and to be negatively correlated with routine metabolic costs or resting metabolic rate (Koehn and Shumway, 1982;

[1] All correspondence should be sent to S. Mopper. Present address: Department of Biological Science, Florida State University, Tallahassee, Florida 32306 USA.

[2] Present address: Department of Environmental, Population and Organismic Biology, University of Colorado, Boulder, Colorado 80309 USA.

Koehn et al., 1988). Particularly interesting are the studies suggesting an increase in physiological efficiency with heterozygosity (Koehn and Shumway, 1982; Mitton and Koehn, 1985; Hawkins et al., 1986, 1989), for they suggest a hypothesis relating genetic variation and plant resistance to herbivory.

The relationship between heterozygosity and resistance may be more apparent in stressful conditions (Parsons, 1971, 1973, 1987; Rainey et al., 1987). During droughts, or in stressful habitats, plant water budgets and subsequent production of defensive compounds, e.g., oleoresin, can be compromised (Lorio and Hodges, 1968). Under those circumstances, a heterozygous individual with greater metabolic efficiency may have the ability to resist herbivore attack compared with a susceptible homozygous neighbor.

Because they are wind-pollinated and predominantly outcrossing, conifers are generally thought to exhibit high genetic diversity within populations, and low diversity between populations (Hamrick 1979, 1983). This is particularly true for species exhibiting extensive, nearly continuous geographic distributions (Hiebert and Hamrick, 1983). Nevertheless, recent work has challenged this assumption and presented evidence of greater between-population differentiation among conifers than expected (Yeh et al., 1985, 1986). Despite high potential gene flow, other plant species have displayed significant genetic variation across narrow boundaries when faced with strong environmental selection (Jowett, 1964; Jain and Bradshaw, 1966; McNeilly and Bradshaw, 1968; Endler, 1977).

In view of the potential strong selection pressures of insect herbivory and environmental stress at Sunset Crater, we developed several predictions regarding the genetic differences of resistant and susceptible cinder field pinyons and the adjacent sandy-loam soil populations. 1) Resistant and susceptible trees differ genetically. 2) Resistance is positively associated with heterozygosity. 3) The cinder field and sandy-loam soil pinyon populations differ genetically. 4) Trees growing under stressful conditions are more heterozygous than trees growing in more benign habitats.

Methods

Characteristics of Cinder and Sandy-Loam Soils

Several studies have demonstrated how soil characteristics influence plant stress and herbivore attack (White 1969, 1976; Mattson 1980; Waring and Cobb, 1992). We therefore estimated the relative stress conditions of the cinder and sandy-loam soils by measuring soil moisture and nutrients. We collected 10 g soil samples in airtight containers from the midcanopy line beneath 20 resistant and 20 susceptible cinder-soil trees and from 20 sandy-loam soil trees. We used half of each sample for soil moisture analysis and half for nutrient analysis.

Because herbivore-infested trees develop a distinctly dense and shrubby canopy in contrast to open-canopied pinyons with low herbivory (Whitham and Mopper, 1985), we selected resistant and susceptible cinder-field trees on the basis of architectural phenotype. From 1982 to 1986, shrubby trees averaged 25.87 ± 5.22% and open canopied trees averaged 9.78 ± 3.17% annual stem destruction by *D. albovitella.* Insect abundance declines sharply on pinyons growing in adjacent sandy-loam soils and averages about 2%. Therefore, all of these pinyons appear resistant to herbivory and exhibit the typical open canopy architecture of unattacked trees. All study trees were between 100 and 200 years old.

To determine soil moisture we weighed the samples, oven dried, then reweighed them. The Bilby Research Center in Flagstaff, Arizona determined the amounts of soil NO_3 and NH_4 using a modified micro-Kjeldahl digestion method and Technicon Autoanalyzer II (Parkinson and Allen, 1975). We compared cinder and sandy-loam soil moisture and nutrients with a one-way analysis of variance followed by a Tukey test for pairwise comparisons (Zar, 1984).

Characteristics of Pinyons in Cinder and Sandy-Loam Soils

Pinyon Xylem Pressure. — As an additional measure of plant stress we estimated the xylem pressure of pinyons growing in the cinder and sandy-loam soils (Scholander et al., 1965; Waring and Cleary, 1967). Because of diurnal fluctuation in pressure, we recorded all measurements at the two sites concurrently from 1 to 3 P.M. when plants generally exhibit the greatest moisture stress. Each measurement is an average of the xylem pressure recorded for three needle fascicles from each of nine resistant and eight susceptible cinder-soil pinyons and 19 sandy-loam soil pinyons. We compared the xylem pressures using a one-way analysis of variance followed by a Tukey test for pairwise comparisons.

Pinyon Resin Production.—The amount of resin produced by a tree can be an indication of its resistance to herbivore attack (Mason, 1969; Raffa and Berryman, 1982). To determine the amount of wound response resin produced by resistant and susceptible trees growing in the cinder soils and in the adjacent sandy-loam soils, we clipped off the ends of five terminal stems per tree and collected and weighed the resin appearing at the wound after 10 minutes. We collected resin from 13 resistant and 12 susceptible cinder-soil trees and 5 trees from the sandy-loam soils. Because resin production can vary temporally, we collected resin samples concurrently from 10 A.M. to 12 P.M. at both sites. We calculated the average resin produced by each tree and conducted a one-way analysis of variance, followed by a Tukey test for pairwise comparisons.

Genetic Comparison of Cinder and Sandy-Loam Soil Pinyons

To examine the genetic diversity of cinder and sandy-loam soil trees we homogenated pinyon needles using the methods of Mitton et al. (1979) and conducted horizontal starch gel electrophoresis to reveal genetic variation for four polymorphic loci. We resolved isocitrate dehydrogenase (IDH), peroxidase (PER), and glucose phosphate isomerase (PGI) with a continuous tris-citrate pH 6.3 buffer system (Selander et al., 1971), and glycerate dehydrogenase (GLY) with a discontinuous tris-citrate pH 7.5 buffer system (Mitton et al., 1977).

We scored PGI, GLY, IDH, and PER for needles collected from 57 resistant and 65 susceptible cinder-soil trees, and scored GLY and PER for 56 trees from the sandy-loam soil pinyon populations. Not all individuals resolved clearly enough to score for each locus so sample sizes vary slightly. All data pertaining to soil moisture and nutrients, xylem pressure, and resin production analyses were collected from a subset of these trees.

TABLE 1. Analysis of variance tests for soil and pinyon characteristics on cinder and sandy-loam soils.

Source	*df*	MS	*F*	*P*
Soil moisture				
Site	2	87.15	71.76	0.000
Residual	27	1.21		
Soil NO_3				
Site	2	0.59	3.97	0.032
Residual	24	0.15		
Soil NH_4				
Site	2	40.18	14.35	0.000
Residual	26	2.80		
Xylem water pressure				
Site	2	96.60	8.05	0.001
Residual	33	12.00		
Resin production				
Site	2	61.75	9.65	0.001
Residual	26	6.40		

We tested the heterogeneity of allele frequencies by the method of Workman and Niswander (1970) and employed chi-square tests to compare the fit of observed to expected gene frequencies under the assumptions of Hardy-Weinberg equilibrium (Speiss, 1977). We calculated *F*, the fixation index (Wright, 1965) as:

$$F = 1 - \frac{\text{observed heterozygosity}}{\text{expected heterozygosity}}$$

and tested its significance with chi-square analysis (Nei, 1987). To determine if resistant, susceptible, and sandy-loam soil trees differed in heterozygosity for PER, IDH, GLY, and PGI, we used chi-square tests.

RESULTS

Characteristics of Cinder and Sandy-Loam Soils

The cinder soils of Sunset Crater are water and nutrient poor relative to neighboring sandy-loam soils and probably represent a significant environmental stress for the res-

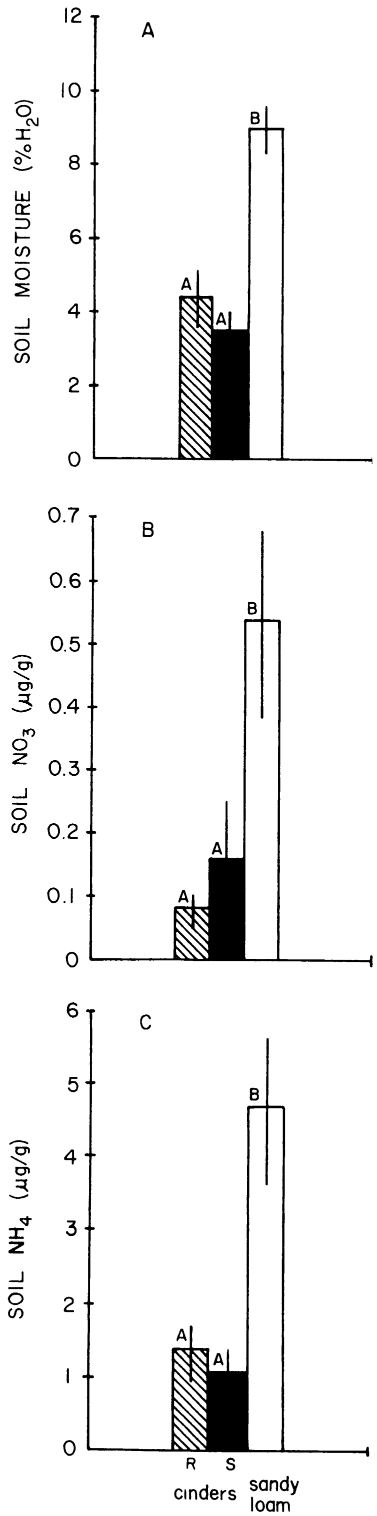

FIG. 1. Amount of moisture (A), NO_3 (B), and NH_4 (C) in the cinder soil beneath resistant (R, hatched bar) and susceptible (S, closed bar) pinyons and in the neighboring sandy-loam soil (open bar). Bars represent means plus or minus one SE, letters above designate statistically significant differences between groups.

ident pinyon population (Table 1, Fig. 1). The sandy-loam soils contained at least twice the amount of moisture, ammonia, and nitrates as did the cinder soils. In contrast, we found no significant differences in either nutrients or moisture between the cinder soils beneath resistant and susceptible trees.

Characteristics of Pinyons in Cinder and Sandy-Loam Soils

Pinyon Xylem Pressure and Resin Production.—Consistent with our finding of low soil moisture, pinyons growing in cinder soils exhibited significantly greater water stress compared with pinyons growing in sandy-loam soils (Table 1, Fig. 2A). However, the xylem pressures of resistant and susceptible cinder-field trees did not differ significantly, a result that reflects the similar moisture contents of soils beneath the two tree types (Fig. 1).

As predicted by plant defense theory, resistant trees produced twice as much wound-response resin as susceptible trees, a significant difference (Fig. 2B). Furthermore, susceptible trees produced twice the amount of resin as the sandy-loam soil trees, but the variation among samples was high and the difference was not significant (Table 1).

Genetic Comparison of Resistant and Susceptible Cinder Field Trees

The genetic differences between resistant and susceptible trees at Sunset Crater were consistent with our predictions of a relationship between herbivory, environmental stress, and genetic differentiation. Although resistant and susceptible pinyons grow side-by-side in the same soil, there were significant differences in their genotypes and allelic frequencies for the PER and IDH allozymes (Table 2).

The genotypic frequencies of resistant trees were significantly more heterozygous for PER and IDH than were susceptible trees (Table 3, Fig. 3). Furthermore, as indicated by the fixation index (*F*), susceptible trees deviated significantly from Hardy-Wein-

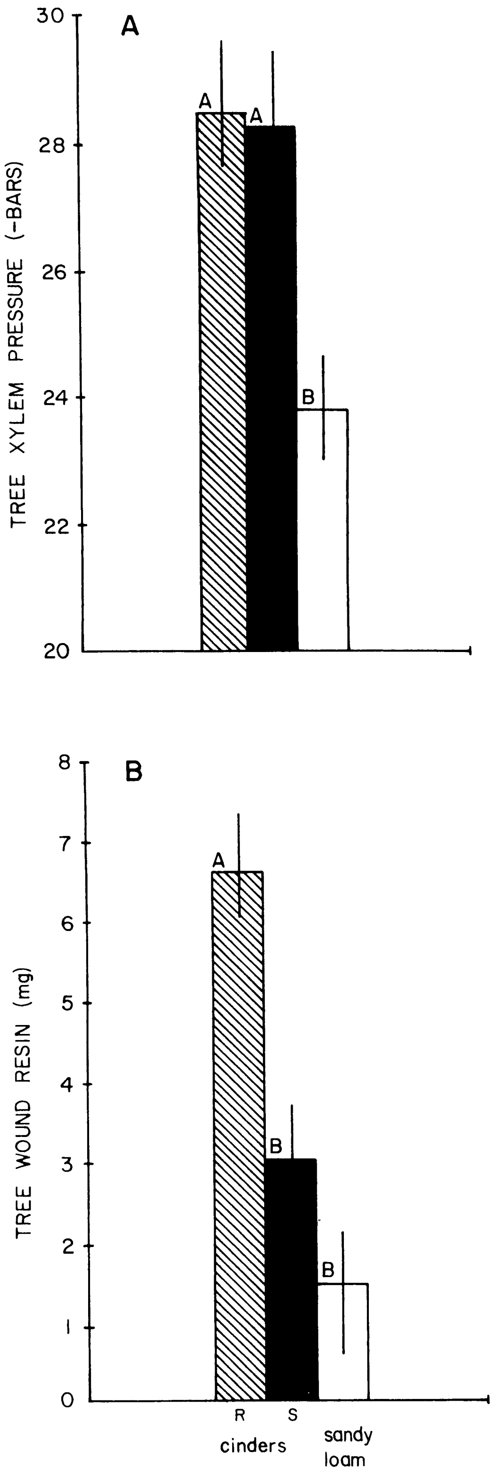

FIG. 2. Water stress (A) as estimated by xylem pressure (A) and the amount of wound resin (B) of resistant (R, hatched bar) and susceptible (S, closed bar) cinder-soil pinyons, and pinyons in the neighboring sandy-loam soil habitat (open bar). Bars represent means plus or minus one SE, letters above designate statistically significant differences between groups.

berg equilibrium for PER (Table 4). The large positive *F*-statistic of 0.365 indicated a significant excess in the frequency of homozygotes among susceptible individuals compared with the predictions of the Hardy-Weinberg model, and suggests the possibility of inbreeding depression among the susceptible trees.

GLY genotypes were virtually identical for resistant and susceptible trees (Table 2, Fig. 3), therefore the enzyme does not appear to be related to herbivory. Because of their similarity, we pooled the GLY data from resistant and susceptible cinder-soil trees for statistical comparison with the sandy-loam soil pinyons.

Genetic Comparison of Cinder and Sandy-Loam Soil Pinyons

Although GLY does not appear to be related to herbivory based on the data from cinder-soil trees, there were significant differences in genotypes and allelic frequencies of cinder-soil and sandy-loam soil trees (Table 5, Fig. 4). Sunset Crater trees were significantly more heterozygous than sandy-loam trees (Table 3). This pattern suggests a relationship between GLY, or an associated gene, and adaptation to different environments or soil types.

Neither genotypes and allelic PER frequencies nor the heterozygosity of sandy-loam trees differed significantly from cinder-soil resistant trees (Table 5, Fig. 4). However, the genotypes and allelic frequencies of sandy-loam and susceptible cinder-soil trees did differ significantly. Sandy-loam trees exhibited much greater heterozygosity than susceptible trees and were in fact out of Hardy-Weinberg equilibrium as indicated by the highly negative *F*-statistic (Table 4).

DISCUSSION

Genetic Differences and Herbivory

Cinder Soil Pinyons.—Our results demonstrate significant genetic differences between individuals resistant and susceptible

TABLE 2. Comparisons of genotypic and allelic frequencies of PER, IDH, GLY, and PGI between resistant and susceptible pinyon pines at Sunset Crater.

Enzyme	Group genotypic frequencies: 11	12	22	23	24	N	χ^2	P	Allelic frequencies: f(1)	f(2)	f(3)	f(4)	χ^2	P
PER														
Res	0.019	0.423	0.558	—	—	52	8.9	<0.025	0.231	0.769			1.7	NS
Sus	0.083	0.183	0.733	—	—	60			0.175	0.825				
IDH														
Res	0.357	0.518	0.125	—	—	56	6.3	<0.05	0.616	0.384			5.3	<0.025
Sus	0.587	0.333	0.079	—	—	63			0.754	0.246				
GLY														
Res	0.175	0.579	0.246	—	—	57	0.5	NS	0.465	0.535			0.4	NS
Sus	0.226	0.565	0.210	—	—	62			0.508	0.492				
PGI														
Res	0.018	0.088	0.772	0.070	0.053	57	2.4	NS	0.061	0.877	0.035	0.026	2.1	NS
Sus	0.000	0.062	0.862	0.046	0.031	65			0.031	0.931	0.023	0.015		

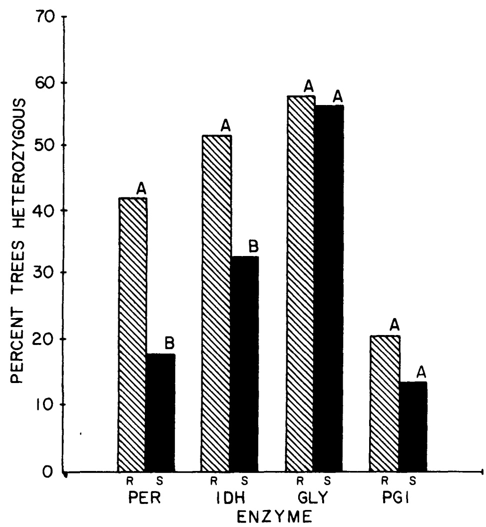

FIG. 3. The percent of resistant (R, hatched bar) and susceptible (S, closed bar) cinder-soil trees heterozygous for PER, IDH, GLY and PGI. Letters above bars designate statistically significant differences between groups.

to insect attack and support the prediction that heterozygous pinyon pines possess an advantage over homozygotes when herbivory is severe. In the Sunset Crater population, herbivore-resistant trees exhibited different genotypic and allelic frequencies (Table 2) and were significantly more heterozygous for PER and IDH than susceptible trees (Fig. 3). When both IDH and PER were successfully scored for an individual, 2 of 58 (3.5%) susceptible trees and 9 of 50 (18%) resistant trees were double heterozygotes. This pattern supports the prediction of increased individual fitness as the number of heterozygous loci increases and the hypothesis of physiological and energetic advantages associated with heterozygosity in forest trees (Mitton and Jeffers, 1989).

Resistant trees produced over twice the amount of resin in response to wounding than susceptible trees (Fig. 2B). The ability of resistant trees to produce more resin is consistent with the heterosis hypothesis because genetic heterozygosity can reduce protein turnover and energetic costs in other organisms (Mitton et al., 1986; Hawkins et al., 1986, 1989). Lower routine metabolic costs could help conserve energy that can

TABLE 3. Statistical comparisons of the number of heterozygote and homozygote individuals represented among the resistant and susceptible cinder and sandy-loam soil.

Protein	Group	No. heterozygotes	No. homozygotes	χ^2	*P*
PER	Resistant	22	30	7.7	0.006
	Susceptible	11	49		
IDH	Resistant	29	27	4.1	0.044
	Susceptible	21	42		
GLY	Resistant	33	24	0.0	NS
	Susceptible	35	27		
PGI	Resistant	12	45	1.1	NS
	Susceptible	9	56		
PER	Resistant	22	30	3.4	NS
	Sandy-loam	32	21		
PER	Susceptible	11	49	21.1	0.0001
	Sandy-loam	32	21		
GLY	Cinders	68	51	4.9	0.028
	Sandy-loam	22	34		

then be allocated for plant defense (Mitton, 1989).

Sandy-Loam Soil Trees

Sandy-loam soil trees suffer the least herbivory and exhibit the highest levels of PER heterozygosity. This is consistent with the negative relationship between PER heterozygosity and herbivory displayed by the resistant and susceptible cinder-soil trees. However, while insects may act as a selective force against homozygotes within the cinder-soil pinyon population, levels of herbivory are so low on the sandy-loam soils that it is unlikely that insects currently play an important role in selection. Why the sandy-loam trees are so highly heterozygous for PER is unknown. Perhaps heterozygosity for PER (or a related gene) has other advantages. Further studies are warranted to understand this pattern.

Sandy-loam soil trees produce the least amount of wound response resin, yet suffer the lowest levels of herbivory (Fig. 2A). This is inconsistent with the positive relationship between resin production and herbivore resistance that we observed in cinder-soil trees. Sandy-loam soil trees also have smaller resin canals than cinder-soil trees (Mopper, unpubl. data). If resin production is an evolutionary response to the pressures of herbivory, it has little advantage when herbivory is very low. The energy saved could be channelled into other functions.

Among coniferous species, evidence is accumulating that PER may be directly influenced by natural selection rather than being in disequilibrium with other genes under selection (Beckman and Mitton, 1984), but much more empirical work is required to determine the role, if any, played by PER in plant resistance. In addition, not all resistant trees were heterozygous for PER and IDH. When both IDH and PER were successfully scored for an individual, 31 of 58 (53.5%) susceptible trees and 15 of 50 (30%) resistant trees were double homozygotes.

Overdominance alone may not impart re-

TABLE 4. Values for the fixation index, *F*, and the χ^2 test of significance of deviation from zero (Nei, 1977). Asterisks indicate significance deviation from Hardy-Weinberg expectations at the $P = 0.005$ level.

	PER		IDH		GLY		PGI	
	F	χ^2	*F*	χ^2	*F*	χ^2	*F*	χ^2
Cinder soil								
Resistant	0.192	1.92	−0.095	0.51	−0.164	1.53	0.064	0.23
Susceptible	0.365	7.99**	0.102	0.66	−0.129	1.03	−0.045	0.13
Sandy-loam soil								
	−0.386	7.90**			0.162	1.47		

TABLE 5. Comparisons of genotypic and allelic frequencies of GLY and PER between resistant, and susceptible cinder and sandy-loam soil pinyons.

Protein group	Genotypic frequencies 11	12	22	*N*	χ^2	*P*	Allelic frequencies f(1)	f(2)	χ^2	*P*
PER										
Res	0.019	0.423	0.558	52	3.5	NS	0.231	0.769	2.2	NS
Sandy-loam	0.019	0.604	0.377	53	21.6	0.0001	0.321	0.679	6.5	0.021
Sus	0.083	0.183	0.733	60			0.175	0.825		
GLY										
Sandy-loam	0.429	0.393	0.179	56	9.9	0.007	0.321	0.679	5.8	0.018
Cinders	0.202	0.571	0.227	119			0.487	0.513		

sistance, nor the lack of it result in susceptibility. Instead, resistance may arise when a number of traits occur together, such as a combination of certain genotypes as well as heterozygosity of some genes. Because resistance and susceptibility are the end points of a range of infestation levels, trees with only a portion of these genetic traits may display a partially resistant phenotype. Directional selection of an allele can also result in high proportions of heterozygous genotypes in a population. The genetic information presented here represents only a small fraction of the pinyon genome. Clearly, further studies on seeds or seedling pinyons before selection and on older trees after selection are required to accurately estimate genotypic fitness.

Genetic Differences and Environmental Stress

In addition to the intense herbivory suffered by many Sunset Crater pinyons, the volcanic cinder soil also represents a severe environmental stress. Compared with the sandy-loam soils, the Sunset Crater soil contained much less water and nutrients (Fig. 1). These stressful conditions are reflected in the significantly more negative xylem pressures exhibited by cinder-soil pinyons when compared with sandy-loam soil pinyons (Fig. 2A).

Associated with the stressful cinder field environment was a much greater degree of GLY heterozygosity in both resistant and susceptible trees compared with trees growing in sandy-loam soils (Fig. 4). The influence of soil type on the genetic divergence of neighboring plant populations has been documented for other plant species. Local adaptation was detected in northern Arizona in a subspecies of rabbit brush (*Chrysothamnus nauseosus* spp. *hololeucus*), which is associated predominantly with cinder soils (G. W. Fernandes, unpubl. data), as well as for *Agrostis* (Jowett, 1964; Jain and Bradshaw, 1966; McNeilly and Bradshaw, 1968).

Rapid Evolution of a Long-Lived Conifer

Our study reveals that rapid differentiation can occur in a long-lived plant species despite potentially high gene flow. The difference in genetic composition of the cinder and sandy-loam soil populations may have arisen from immediate environmental or

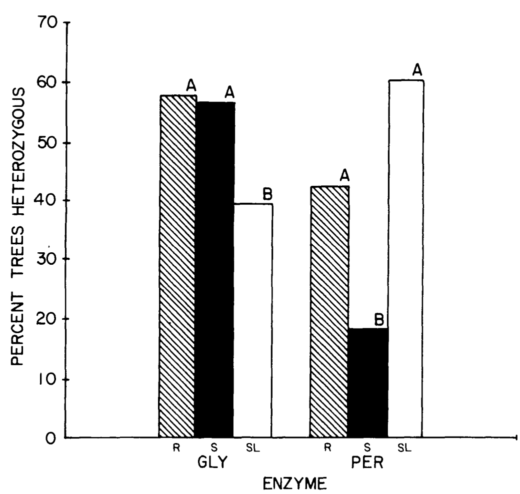

FIG. 4. The percent of resistant (R, hatched bar) and susceptible (S, closed bar) pinyons cinder-soil and sandy-loam soil trees (SL, open bar) heterozygous for GLY. Letters above bars designate statistically significant differences between groups.

herbivore selection of certain genotypes during the invasion of the cinder fields by the sandy-loam pinyons 800 years ago. Or, the colonizers may have been identical to the original population and the variation observed today the result of a slightly slower process (several generations) of selection.

Taxonomic studies document local adaptation since the eruption of other cinder-soil plant species. For example, *Penstemon clutei* and *Camissonia gouldi* are endemics found only on the volcanic Sunset Crater soils (Raven, 1969; McDougall, 1973). Conifers usually exhibit high genetic diversity within populations and low diversity between relatively continuous populations (Hamrick, 1979, 1983; Guries, 1984), patterns that contrast with the genetic differentiation displayed by nearby pinyon populations in northern Arizona.

Acknowledgments

This research was supported by U.S.D.A. grants 84-CRCR-1-1443 and GAM-8700709, and NSF grants BSR-8604983 and BSR-8705347 to T.G.W. and S.M., and by the University of Colorado faculty fellowship to J.B.M. We thank G. Waring and two anonymous reviewers whose comments improved this manuscript. We are grateful to the U.S. Forest Service and the Sunset Crater National Monument personnel for their cooperation.

Literature Cited

BECKMAN, J. S., AND J. B. MITTON. 1984. Peroxidase allozyme differentiation among successional stands of ponderosa pine. Am. Midl. Nat. 112:43–49.

CARTER, P. A., AND W. B. WATT. 1988. Adaptation at specific loci. V. Metabolically adjacent enzyme loci may have very distinct experiences of selective pressures. Genetics 119:913–924.

ENDLER, J. A. 1977. Geographic Variation, Speciation, and Clines. Princeton Univ. Press, Princeton, NJ.

———. 1986. Natural Selection in the Wild. Princeton Univ. Press, Princeton, NJ.

GAJARDO, G. M., AND J. A. BEARDMORE. 1989. Ability to switch reproduction mode in *Artemia* is related to maternal heterozygosity. Mar. Ecol. Prog. Ser. 55:191–195.

GINZBURG, L. R. 1979. Why are heterozygotes often superior in fitness? Theor. Popul. Biol. 15:264–267.

GURIES, R. P. 1984. Genetic variation and population differentiation in forest trees. Proc. North Am. For. Biol. Workshop, 8th, pp. 119–131.

HAMRICK, J. L. 1979. Genetic variation and longevity, pp. 84–113. *In* O. Solbrig, S. Jain, G. Johnson, and P. Raven (eds.), Topics in Plant Population Biology. Columbia Univ. Press, N.Y.

———. 1983. The distribution of genetic variation within and among natural plant populations, pp. 335–348. *In* C. M. Schonewald-Cox, S. M. Chambers, B. MacBryd, and W. L. Thomas (eds.), Genetics and Conservation, A Reference for Managing Wild Animal and Plant Populations. W. A. Benjamin Inc., Menlo Park, CA.

HAWKINS, A. J. S., B. L. BAYNE, AND A. J. DAY. 1986. Protein turnover, physiological energetics and heterozygosity in the blue mussel, *Mytilus edulis*: The basis of variable age-specific growth. Proc. R. Soc. London B 229:161–176.

HAWKINS, A. J. S., B. L. BAYNE, A. J. DAY, J. RUSIN, AND C. M. WORRALL. 1989. Genotype-dependent interrelations between energy metabolism, protein metabolism and fitness, pp. 283–292. *In* J. S. Ryland and P. A. Tyler (eds.), Reproduction, Genetics and Distributions of Marine Organisms. Olsen and Olsen, Fredensborg, Denmark.

HIEBERT, R. D., AND J. L. HAMRICK. 1983. Patterns and levels of genetic variation in great basin bristlecone pine, *Pinus longaeva*. Evolution 37:302–310.

JAIN, S. K., AND A. D. BRADSHAW. 1966. Evolutionary divergence among adjacent populations: I. The evidence and its theoretical analysis. Heredity 21: 407–441.

JOWETT, O. 1964. Population studies on lead tolerance of *Agrostis tenuis*. Evolution 15:70–87.

KARLIN, S., AND U. LIEBERMAN. 1979*a*. Central equilibria in multilocus systems I. Generalized nonepistatic selection regimes. Genetics 91:777–798.

———. 1979*b*. Central equilibria in multilocus systems II. Bisexual generalized nonepistatic selection models. Genetics 91:799–816.

KOEHN, R. K., W. J. DIEHL, AND T. M. SCOTT. 1988. The differential contribution by individual enzymes of glycolysis and protein catabolism to the relationship between heterozygosity and growth rate in the coot clam, *Mulinia latoralis*. Genetics 118: 121–130.

KOEHN, R. K., AND P. M. GAFFNEY. 1984. Genetic heterozygosity and growth rate in *Mytilus edulis*. Mar. Biol. 82:1–7.

KOEHN, R. K., R. MILKMAN, AND J. B. MITTON. 1976. Population genetics of marine pelecypods. IV. Selection, migration and genetic differentiation in the blue mussel *Mytilus edulis*. Evolution 30:2–32.

KOEHN, R. K., AND S. A. SHUMWAY. 1982. A genetic/physiological explanation for differential growth rate among individuals of the American oyster, *Crassostrea virginica*, (Gmelin). Mar. Biol. Letters 3:35–42.

KRUTCH, J. W. 1974. The Paradox of a Lava Flow. Southwest Parks and Monuments Association, Phoenix, AZ.

LORIO. P. L., JR., AND J. D. HODGES. 1968. Microsite effects of oleoresin exudation pressure of large loblolly pines. Ecology 49:1207–1210.

MASON, R. R. 1969. A simple technique for measuring oleoresin exudation flow in pines. For. Sci. 15:56–57.

MATTSON, W. J., JR. 1980. Herbivory in relation to plant nitrogen content. Annu. Rev. Ecol. System. 11:119–161.

McDougall, W. B. 1973. Seed Plants of Northern Arizona. Museum of Northern Arizona, Flagstaff, AZ.

McNeilly, T., and A. D. Bradshaw. 1968. Evolutionary processes in populations of copper tolerant *Agrostis tenuis* Sibth. Evolution 22:108–118.

Mitton, J. B. 1989. Physiological and demographic variation associated with allozyme variation. *In* D. Soltis and P. Soltis (eds.), Isozymes in Plants, Discoides Press.

Mitton, J. B., C. Carey, and T. D. Kocher. 1986. The relation of enzyme heterozygosity to standard and active oxygen consumption and body size of tiger salamanders, *Ambystoma tigrinum*. Physiol. Zool. 59:574–582.

Mitton, J. B., and M. C. Grant. 1984. Relationships among protein heterozygosity, growth rate, and developmental stability. Ann. Rev. Ecol. Syst. 15:479–499.

Mitton, J. B., and R. M. Jeffers. 1989. The genetic consequences of mass selection for growth rate in Engelmann spruce. Silvae Genetica 38:6–12.

Mitton, J. B., and R. K. Koehn. 1975. Genetic organization and adaptive response of allozymes to ecological variables in *Fundulus heteroclitus*. Genetics 79:97–111.

———. 1985. Shell shape variation in the blue mussel, *Mytilus edulis,* and its association with enzyme heterozygosity. J. Exp. Mar. Biol. Ecol. 90:73–80.

Mitton, J. B., Y. B. Linhart, J. L. Hamrick, and J. S. Beckman. 1977. Observations on the genetic structure and mating system of ponderosa pine in the Colorado Front Range. Theoret. Appl. Genet. 57:5–13.

Mitton, J. B., Y. B. Linhart, K. B. Sturgeon, and J. L. Hamrick. 1979. Allozyme polymorphisms detected in mature needle tissue of ponderosa pine. J. Hered. 70:86–89.

Mopper, S., J. Maschinski, N. Cobb, and T. G. Whitham. 1991. A new look at habitat structure: Consequences of herbivore-modified plant architecture, pp. 260–280. *In* S. Bell, E. McCoy, and H. Mushinsky (eds.), Habitat Complexity: The Physical Arrangement of Objects in Space. Chapman and Hall, N.Y.

Mopper, S., and T. G. Whitham. 1986. Natural bonsai of Sunset Crater. Natural History 95:42–47.

Mopper, S., T. G. Whitham, and P. W. Price. 1990. Plant phenotype and interspecific competition between insects determine sawfly performance and density. Ecology 71:2135–2144.

Nei, M. 1987. Molecular Evolutionary Genetics. Columbia Univ. Press, N.Y.

Parkinson, J. A., and S. E. Allen. 1975. A wet oxidation procedure suitable for the determination of nitrogen and mineral nutrients in biological material. Communications in Soil Science and Plant Analysis 6:1–11.

Parsons, P. A. 1971. Extreme environment heterosis and genetic loads. Heredity 26:479–483.

———. 1973. Genetics of resistance to environmental stresses in *Drosophila* populations. Annu. Rev. Genet. 7:239–265.

———. 1987. Evolutionary rates under environmental stress. Evolutionary Biology 21:311–347.

Pierce, B. A., and J. B. Mitton. 1982. Allozyme heterozygosity and growth in the tiger salamander, *Ambystoma tigrinum*. J. Hered. 73:250–253.

Raffa, K. F., and A. A. Berryman. 1982. Physiological difference between lodgepole pines resistant and susceptible to the mountain pine beetle and associated organisms. Envir. Entomol. 11:486–492.

Rainey, D. Y., J. B. Mitton, and R. K. Monson. 1987. Associations between enzyme genotype and dark respiration in perennial ryegrass, *Lolium perenne*. Oecologia 74:335–338.

Raven, P. H. 1969. A revision of the genus *Camissonia* (Onagraceae). Contributions from the U.S. National Herbarium 37:361–396.

Rodhouse, P. G., J. H. McDonald, R. I. E. Newell, and R. K. Koehn. 1986. Gamete production, somatic growth and multiple locus heterozygosity in *Mytilus edulis* L. Mar. Biol. 90: 209–214.

Scholander, P. F., H. T. Hammel, E. D. Bradstreet, and E. A. Hemmingsen. 1965. Sap pressure in vascular plants. Science 148:339–346.

Selander, R. K., M. H. Smith, S. Y. Yang, W. E. Johnson, and J. B. Gentry. 1971. Biochemical polymorphism and systematics in the genus *Peromyscus* I. Variations in the old-field mouse (*Peromyscus polionotus*). Studies in Genetics VI. Univ. Texas Publ. 7103, pp. 49–90.

Speiss, E. B. 1977. Genes in Populations. John Wiley and Sons, N.Y.

Turelli, M., and L. Ginzburg. 1983. Should individual fitness increase with heterozygosity? Genetics 104:191–209.

Waring, G. L., and N. S. Cobb. 1992. The impact of plant quality on herbivore population dynamics: The case of plant stress. *In* E. A. Bernays (ed.), Focus on Insect-plant Interactions, Vol. III, CRC Press, Boca Raton, FL. *In press.*

Waring, R. H., and B. D. Cleary. 1967. Plant moisture stress: Evaluation by pressure bomb. Science 155:1248–1254.

Watt, W. B., P. A. Carter, and S. M. Blower. 1985. Adaptation at specific loci. IV. Differential mating success among glycolytic allozyme genotypes of colias butterflies. Genetics 109:157–175.

White, T. C. R. 1969. An index to measure weather-induced stress of trees associated with outbreaks of psyllids in Australia. Ecology 50:905–909.

———. 1976. Weather, food and plagues of locusts. Oecologia (Berlin) 22:119–134.

Whitham, T. G., and S. Mopper. 1985. Chronic herbivory: Impacts on architecture and sex expression of pinyon pine. Science 228:1089–1091.

Workman, P. L., and J. D. Niswander. 1970. Population studies on southwestern Indian tribes. II. Local differentiation in the Papago. Am. J. Hum. Genet. 22:24–49.

Wright, S. 1965. The interpretation of population structure by *F*-statistics with special regard to systems of mating. Evolution 19:395–420.

Yeh, F. C., W. M. Cheliak, B. P. Dancik, K. Illingworth, D. C. Trist, and B. A. Pryhitka. 1985. Population differentiation in lodgepole pine, *Pinus contorta* spp. *latifolia*: A discriminant analysis of allozyme variation. Can. J. Genet. Cytol. 27: 210–218.

YEH, F. C., M. A. K. KAHLIL, Y. A. EL-KASSABY, AND D. C. TRUST. 1986. Allozyme variation in *Pices mariana* from Newfoundland: Genetic diversity, population structure, and analysis of differentiation. Can. J. For. Res. 16:713–720.

ZAR, J. H. 1984. Biostatistical Analysis. Prentice Hall, Inc., Englewood Cliffs, NJ.

ZOUROS, E., AND D. W. FOLTZ. 1987. The use of allelic isozyme variation for the study of heterosis, pp. 1–59. *In* M. C. Rattazzi, J. G. Scandalios, and G. S. Whitt (eds.), Isozymes: Current Topics in Biological and Medical Research. Volume 13. Alanr. Liss, Inc., N.Y.

ZOUROS, E., S. M. SINGH, D. W. FOLTZ, AND A. L. MALLET. 1983. Post-settlement viability in the American oyster (*Crassostrea virginica*): An overdominant phenotype. Genet. Res. Camb. 41:259–270.

ZOUROS, E., S. M. SINGH, AND H. E. MILES. 1980. Growth rate in oysters: An overdominant phenotype and possible explanations. Evolution 34:856–867.

Corresponding Editor: P. W. Hedrick