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Genetic variation in piñon pine, *Pinus edulis*, associated with summer precipitation

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Abstract

Three previous reports of microgeographical variation of glycerate dehydrogenase (*Gly*) frequencies in piñon, *Pinus edulis*, established the hypothesis that *Gly* frequencies contribute to adaptation to heterogeneous environments, specifically to variation in soil moisture. In each of these studies, the frequency of the *Gly-3* allele or of *Gly-33* homozygotes was higher on dry sites than on nearby moist sites. Here we attempt to extend these observations by testing the hypothesis that *Gly* frequencies respond to soil moisture variation on a range-wide scale. *Gly* frequencies were surveyed in 11 natural populations, and the frequency of the *Gly-3* allele varied from 0.27 to 0.65 among the sample sites. Elevation varied from 1650 to 3100 m, and summer precipitation, defined as precipitation from April to August, varied from 13.7 to 26.4 cm. The soil types at the collection sites were schist, quaternary volcanic or a mixture of shale and sandstone. Logistic regression revealed that *Gly* frequencies did not respond to either elevation or soil type, but were related to summer precipitation ($P < 0.01$). The correlation between summer precipitation and the frequency of the *Gly-3* allele was $r = -0.92$ ($P < 0.001$). Thus, the patterns of differentiation on microgeographical scales are consistent with greater differentiation on a range-wide scale.

Keywords: allozymes, geographical variation, piñon pine, selection

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Introduction

Repeated patterns of genetic variation reveal evolutionary genetic responses to physical components of the environment (Linhart & Grant 1996). For example, clinal variation associated with latitude on several continents revealed selection on alcohol dehydrogenase variation in *Drosophila melanogaster* (Oakeshott *et al*. 1982; Kreitman *et al*. 1992). Peroxidase variation in ponderosa pine, *Pinus ponderosa*, responds to temperature variation that varies with elevation and slope aspect in six canyons along the Front Range in Colorado (Mitton *et al*. 1977; Beckman & Mitton 1984). In Engelmann spruce, *Picea engelmannii*, allelic frequencies at *PGM* vary consistently between adjacent wet and dry sites, indicating that selection of *PGM* genotypes varies with the normal pattern of environmental patchiness for water availability (Stutz & Mitton 1988; Mitton *et al*. 1989). Similarly, repeated patterns of microgeographical differentiation of glycerate dehydrogenase (*Gly*) frequencies are associated with moisture availability in piñon pine, *P. edulis* (Mopper *et al*. 1991; Cobb *et al*. 1994; Mitton *et al*. 1998), indicating that the piñon *Gly* polymorphism plays a role in adaptation to heterogeneous environments.

At Sunset Crater, near Flagstaff, AZ, a mosaic of normal sandy soils and lava soils provides a natural laboratory. Because lava soils retain little moisture and have low levels of nutrients, they provide stressful conditions for plant communities. Studies comparing population samples between lava and normal soils in nearby sites reported that *Gly* frequencies differed between environments (Mopper *et al*. 1991; Cobb *et al*. 1994), with the frequency of *Gly-3* allele or the *Gly-33* homozygote consistently higher on lava soils. This same pattern of geographical variation of allelic frequencies was reported among sampling sites at an isolated population on the northern periphery of the distribution in Owl Canyon, northwest of Fort Collins, CO. Within this population, relatively wet and dry sites were identified by the size and density of the trees, and the associated plant community. Relatively dry sites had smaller trees and lower tree densities, and three species were diagnostic for dry sites: mountain mahogany, *Cercocarpus montanus*,

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yucca, *Yucca glauca* and prickly pear cactus, *Opuntia compressa*. Although distances between wet and dry sites were on the order of a few hundred metres, the dry sites, like the lava soils in Arizona, had higher frequencies of the *Gly-3* allele (Mitton *et al*. 1998).

In each of the three studies of *Gly* allelic frequencies (Mopper *et al*. 1991; Cobb *et al*. 1994; Mitton *et al*. 1998), the relatively wet and dry sites were separated by 100 m to 21 km, which is sufficiently close to be connected by gene flow via wind-borne pollen. Consequently, the differentiation cannot be attributed to genetic drift or to unique historical events that produced microgeographical variation. Furthermore, the same pattern of differentiation was reported in each study, suggesting that a common pattern of natural selection was involved in the study sites.

On dry sites, growth rates, viabilities and stomatal sizes and shapes differ among piñon *Gly* genotypes. Comparisons of young and mature trees on lava soils revealed heterogeneous viabilities among *Gly* genotypes, with *Gly-33* homozygotes having the highest viability (Cobb *et al*. 1994). Growth rates differed two-fold among genotypes on lava soils, with *Gly-33* homozygotes having the highest growth rates (Cobb *et al*. 1994). Measurements from scanning electron micrographs revealed that stomatal length and width varied among genotypes (Mitton *et al*. 1998), and that the stomates of *Gly-33* homozygotes, which had the highest growth and viability on dry sites, were distinctively long and narrow. The long, narrow stomates may release less water during respiration, or they may be able to close more quickly, or more completely, during bouts of water stress.

Summer precipitation limits the geographical range of piñon (Pearson 1920; Quinn 1974). Piñon seeds germinate best in bare mineral soil, so sites with relatively dry spring and early summer months are optimal, for these conditions limit cover by grasses and shrubs. Piñon is common in sites with 12–25 cm of summer rain, but if precipitation exceeds this range, piñon may be crowded out by ponderosa pine (Pearson 1920; Quinn 1974). Given the importance of summer precipitation for limiting the distribution of piñon, we hypothesized that summer precipitation would also influence population structure.

The purpose of this study was to determine whether the patterns of microgeographical variation in Arizona and Colorado predict similar patters of genetic variation on a greater geographical and environmental scale. This study examines piñon *Gly* variation among populations occupying a variety of environmental conditions. Samples were selected at both the upper and lower limits of the elevational distribution, and from relatively wet and dry sites. Because *Gly* variation has been reported to vary with soil moisture as a function of soil type (Mopper *et al*. 1991; Cobb *et al*. 1994), the soils at the collection sites were identified. We used logistic regression to measure the response of *Gly* frequencies to summer precipitation, elevation, and soil type, and to test the hypothesis that *Gly-3* frequencies decrease with increasing summer precipitation.

Materials and methods

Glycerate dehydrogenase is a dimer; homozygotes and heterozygotes are single- and triple-banded, respectively. Three alleles segregate at this locus (*Gly-1*, *Gly-2* and *Gly-3*), with *Gly-1* having the fastest migration rate, and *Gly-3* the slowest. Because *Gly-1* was occasionally rare (frequency < 0.05) but usually absent in our samples, *Gly-1* was pooled with *Gly-2* to avoid statistical problems. *Gly-2* and *Gly-3* correspond to alleles *F* and *S* in Cobb *et al*. (1994).

Electrophoretic surveys of *Gly* variation were based on needle samples collected from natural populations and refrigerated for up to 3 weeks before homogenation (Mitton *et al*. 1979). Starch gel electrophoresis was carried out with a discontinuous buffer system (Poulik 1957) for ~2.5 h at 50 mA. *Gly* activity was detected in starch gels after by applying a staining solution in an agar overlay. Ten millilitres of staining solution (30 mg glyceric acid, 10 mg βNAD, 10 mg MTT, 2 mg PMS in a Tris–HCl buffer, pH 8.0 or 9.0) were mixed with 10 mL of 1% agar heated to 60 °C, then poured over a freshly sliced gel.

Precipitation data (Table 1) were taken from a web site maintained by the Western Regional Climate Center (http://www.wrcc.dri.edu/summary/climsmco.html) at the Desert Research Institute, Reno, NV. For some of the piñon sample localities (Fig. 1), a meteorological station was sufficiently close to provide a reasonable estimate of precipitation. However, some of the sample localities were not close to a meteorological station, and for these sample localities we used an average of the two, three or four closest localities for the estimates of precipitation.

For the sample sites at Mount Shavano, Kaufman Ridge and Garfield Mine, we used the means from the meteorological stations at Buena Vista, Antero Reservoir, Saguache and Twin Lakes. For sample sites at Cañon City and Pueblo, we used the means of the values from Colorado Springs, Cañon City, Rye and Pueblo. The values for San Cristobal are the means of the values from Cerro and Taos. The data for Owl Canyon are from the meterological station in Fort Collins, and the data for El Malpais are from El Morro. The data for Sunset Crater are from a meteorological station at Sunset Crater, and data for the Winona site are from Burrus Ranch.

We defined summer precipitation as precipitation from April to August. The website provided monthly mean precipitation for each of these months, based on records extending back 20–60 years.

Some of the genetic data (Table 2) examined in this study are taken from previous publications. The population samples for Sunset Crater and Winona are taken from Cobb *et al*. (1994), and the data for Owl Canyon were taken from

Table 1 Monthly precipitation values (cm), elevations, and soil types for 11 piñon sample localities in Colorado, New Mexico and Arizona

Locality	Apr	May	Jun	Jul	Aug	Precip	Elev	Soil
1. Mount. Shavano	1.87	2.32	2.10	4.10	4.59	14.99	2896	schist
2. Kaufman Ridge	1.87	2.32	2.10	4.10	4.59	14.99	3018	schist
3. Garfield Mine	1.87	2.32	2.10	4.10	4.59	14.99	3109	schist
4. Canon City	3.98	5.27	4.25	6.28	6.53	26.31	1768	shale/sandstone
5. Pueblo	3.98	5.27	4.25	6.28	6.53	26.31	1737	shale/sandstone
6. Yellowjacket Canyon	2.35	2.59	1.28	3.37	4.01	13.61	1646	shale/sandstone
7. Owl Canyon	5.08	7.11	4.65	4.06	3.56	24.46	1829	shale/sandstone
8. San Cristobal	2.25	3.05	2.35	4.37	5.04	17.06	2335	shale/sandstone
9. El Malpais	1.85	1.50	1.45	4.88	6.91	16.59	2201	Quaternary volcanic
10. Sunset Crater	2.03	1.78	1.19	6.20	7.32	18.52	1850	Quaternary volcanic
11. Winona	2.34	1.45	1.65	6.70	6.22	18.36	1925	shale/sandstone

Precip = summer precipitation, defined as the sum of precipitation from April to August. Elev = elevation is in metres; precipitation is in cm. Monthly precipitation data are from a web site maintained by the Western Regional Climate Center (http://www.wrcc.dri.edu/summary/climsmco.html). Locality numbers correspond to those in Fig. 1 and Table 2. See text for further explanation.

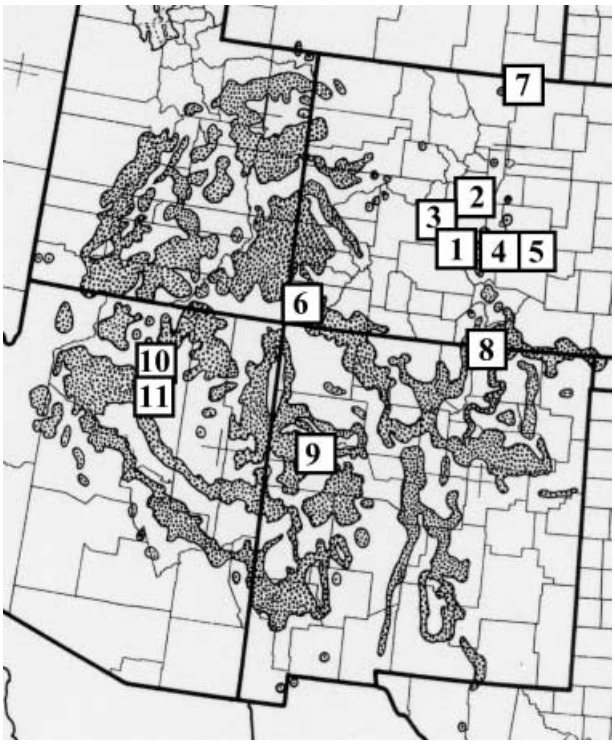

Fig. 1 The distribution of piñon, Pinus edulis, in southwestern North America and the sample sites for the survey of glycerate dehydrogenase variation: 1, Mount Shavano; 2, Kaufman Ridge; 3, Garfield Mine; 4, Cañon City; 5, Pueblo; 6, Yellowjacket Canyon; 7, Owl Canyon; 8, San Cristobal; 9, El Malpais; 10, Sunset Crater; 11, Winona.

Mitton *et al*. (1998). The data for the eight other population samples are new data, and have not been published elsewhere.

Soil type (Table 1) for each of the collection sites was taken from a map of the soils of the southern Rocky Mountain region (Bennison 1990). The data from Cobb *et al*. (1994) were broken into the Sunset Crater site, on lava soils, and Winona, just 21 km to the south, on soils derived from shale and sandstone.

Homogeneity of allelic frequencies among sites was tested with a row-by-column test of independence (Spiess 1977). A logistic regression (Hosmer & Lemeshow 1989) was used to determine whether the frequency of the *Gly-3* allele responded to summer precipitation, elevation, and soil type.

Results

The sample localities are widespread geographically (Fig. 1) and are diverse in an ecological sense. Annual precipitation varies among the 11 sites from 24.5 to 40.5 cm (data not shown), while summer rainfall, defined as precipitation from April to August, varies from 13.6 to 26.3 cm (Table 1). Elevation ranges from 1646 m at Yellowjacket Canyon, at the lower limit of piñon distribution on the western slope of the Rocky Mountains, to 3110 m at the upper limit of piñon on the eastern slope. Three soil types were found at the sample sites. Three sites at high elevation on the eastern slope of the Continental Divide in Colorado were on schist. Two sites, Sunset Crater in Arizona and El Malpais in New Mexico, were on Quarternary volcanic soils. All of the other sites were on soils derived from shale and sandstone.

Gly frequencies were heterogeneous among the sample localities ($\chi^2 = 77.2$, $P < 0.001$, Table 2). The frequency of *Gly-3* ranged from a low of 0.27 at Pueblo to a high of 0.65 at Yellowjacket Canyon. All of the population samples have genotypic frequencies that fit Hardy–Weinberg expectations; this observation is consistent with the belief that piñon is wind pollinated and predominantly outcrossed.

Table 2 Allelic and genotypic frequencies for glycerate dehydrogenase in piñon, *Pinus edulis*, in 11 sample localities

	Genotypes					
	22	23	33	f(2)	f(3)	χ^2
1. Mount Shavano, CO	10	26	18	0.43	0.57	
2. Kaufman Ridge, CO	9	19	21	0.38	0.62	
3. Garfield Mine, CO	5	6	7	0.44	0.56	
4. Cañon City, CO	10	11	5	0.60	0.40	
5. Pueblo, CO	16	9	3	0.73	0.27	
6. Yellowjacket Canyon, CO	3	19	14	0.35	0.65	
7. Owl Canyon, CO	96	107	22	0.66	0.34	
8. San Cristobal, NM	24	49	17	0.54	0.46	
9. El Malpais, NM	48	115	61	0.47	0.53	
10. Sunset Crater AZ	81	135	78	0.51	0.50	
11. Winona, AZ	99	138	63	0.56	0.44	77.2

f(2) and f(3) are the frequencies of alleles *Gly-2* and *Gly-3*. χ^2 measure the heterogeneity of allelic frequencies among the sample localities ($P < 0.001$). Locality numbers correspond to those in Table 1 and Fig. 1.

Table 3 Logistic regression analysis of *Gly-2* frequencies in 11 populations of piñon

Effect	d.f.	χ^2	*P*
Soil	2	2.2	0.33
Precipitation	1	8.0	0.005
Elevation	1	0.4	0.51

d.f = degrees of freedom, χ^2 = chi-square, *P* = probability.

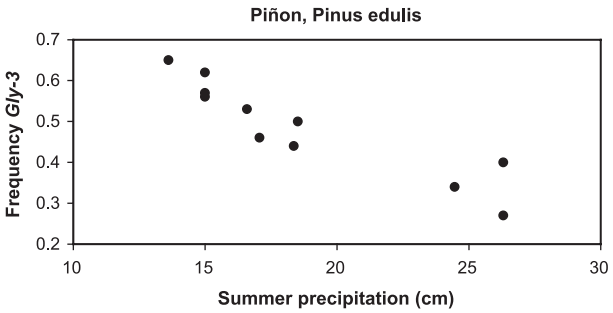

Fig. 2 Piñon *Gly-3* frequencies plotted as a function of summer precipitation for 11 sample localities in CO, AZ and NM. The correlation between *Gly-3* frequency and summer rainfall is $r = -0.92$ ($P < 0.001$).

The logistic regression (Table 3) explained a significant proportion of the variation in *Gly* frequencies with the three variables, summer precipitation, elevation and soil type ($P < 0.001$). The regression analysis indicated that *Gly* frequencies were not related to either elevation or soil type, but the relationship with summer precipitation was significant ($P < 0.01$). The coefficient for summer precipitation was -0.064 ± 0.023, indicating that when the effects of soil type and elevation were held constant, an increase of 1 cm of summer precipitation decreased the probability of finding the *Gly-3* allele by 0.064.

Discussion

The purpose of this study was to determine whether the range-wide pattern of variation of allelic frequencies was consistent with microgeographical patterns, and to specifically test the hypothesis that the frequency of *Gly-3* decreases with increasing summer precipitation. The pattern of genetic variation reported here across a substantial portion of the range of the species is consistent with previous reports (Mopper *et al*. 1991; Cobb *et al*. 1994; Mitton *et al*. 1998). Once again, the frequency of *Gly-3* was higher on relatively dry sites (Fig. 2).

Frequencies of the *Gly* alleles varied by 0.38 among localities, which is much more than the variation reported from any of the studies of microgeographical variation. This greater range of genetic variation might be attributed to either a greater range of environmental variation, or to the greater geographical distances in this study, or both. The distances examined in this study would limit gene flow among populations, providing a greater opportunity for differentiation. Within the range of environmental variation sampled in this study, the response of *Gly* frequencies to summer precipitation was approximately linear (Fig. 2).

Significant differentiation was detected between the upper and lower limits of piñon distribution in the Colorado Front Range. *Gly* frequencies are homogeneous between Cañon City and Pueblo, which are ~50 km apart. Frequencies are also homogeneous among Mount Shavano, Kaufman Ridge and Garfield Mine, which are 10–50 km apart. But *Gly* frequencies are significantly differentiated in the 110 km between the cluster of populations at the upper limit and the pair of populations at the lower limit;

average *Gly-3* frequencies at the upper and lower limit are 0.59 and 0.34 ($\chi^2 = 19.8$, $P < 0.001$). This differentiation is not related to elevation per se (Table 3). Summer precipitation at the upper limit (14.99 cm) is only 57% of that (26.31 cm) at the lower limit. Although precipitation usually increases with elevation in the Rocky Mountains, this anomalous pattern is attributable to a rain shadow east of the Continental Divide, and perhaps also the inability of upslope storms from the Gulf of Mexico to reach the high elevations on the eastern slope.

In contrast to this genetic differentiation in piñon, a study of limber pine, *Pinus flexilis*, revealed no allozyme differentiation over a similar gradient in the Colorado Front Range (Schuster *et al*. 1989; Latta & Mitton 1997; Schuster & Mitton 2000). Chloroplast DNA and allozyme loci exhibit no differentiation between the isolated escarpment population at Pawnee Buttes and upper tree line on Sheep Mountain, a distance of > 160 km along an elevational transect of ~1900 m. Those studies concluded that gene flow via windborne pollen was extensive in limber pine. Gene flow in limber pine and piñon are probably similar in magnitude. Because the pattern of geographical variation reported here is consistent with three previous reports of microgeographical variation, we conclude that this pattern was not created by genetic drift among isolated populations, but is attributable to natural selection associated with moisture availability.

In a recent review of geographical variation in plants, Linhart & Grant (1996) summarized concordant studies of geographical variation and microgeographical variation in both slender wild oats, *Avena barbata* (Clegg & Allard 1972; Hamrick & Allard 1972) and ponderosa pine (Mitton *et al*. 1977; Beckman & Mitton 1984). They concluded

> … in both *A. barbata* and *P. ponderosa*, large-scale patterns and small-scale patterns are consistent with each other. This provides strong inferential evidence that selection produced the differentiation observed. Hypotheses of isolation by distance, genetic drift, and gene flow are all falsified in these particular examples because none predict these patterns of similarity across the different spatial scales....

This conclusion also applies to the *Gly* polymorphism in piñon.

The *Gly* polymorphism fits the definition of a bioindicator polymorphism; Müller-Starck & Schubert (2000) defined bioindication as the response of biological systems to environmental heterogeneity or anthropogenic stresses. Similar bioindicator loci in forest trees include the isocitrate dehydrogenase polymorphism in silver fir, *Abies alba*, which has clinal variation with latitude (Bergmann & Gregorius 1993), and the leucine aminopeptidase, peroxidase-B, phosphoglucomutase-A and malate dehydrogenase-C polymorphisms in European beech, *Fagus sylvatica*, which respond to a variety of environmental stresses (Müller-Starck & Schubert 2000).

We do not have a mechanistic hypothesis for how the *Gly* polymorphism influences the development of stomatal size and shape in piñon. However, we hypothesize that the relationship between *Gly* frequencies and summer precipitation is mediated by the differences among *Gly* genotypes in the size and shapes of their stomates (Mitton *et al*. 1998), and we predict that dry sites will have longer, narrower stomates than relatively moist sites.

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References

Beckman JS, Mitton JB (1984) Peroxidase allozyme differentiation among successional stands of ponderosa pine. *American Midland Naturalist*, **112**, 43–49.

Bennison AP (1990) *Geological Highway Map of the Southern Rocky Mountain Region: Utah, Colorado, Arizona, New Mexico*. American Association of Petroleum Geologists, Tulsa, OK.

Bergmann F, Gregorius H-R (1993) Ecogeographical distribution and thermostability of isocitrate dehydrogenase (IDH) alloenzymes in European silver fir. *Biochemical Systematics and Ecology*, **21**, 597–605.

Clegg MT, Allard RW (1972) Patterns of genetic differentiation in the slender wild oat species, *Avena barbata*. *Proceedings of the National Academy of Sciences of the USA*, **69**, 1820–1824.

Cobb NS, Mitton JB, Whitham TG (1994) Genetic variation associated with chronic water and nutrient stress in pinyon pine. *American Journal of Botany*, **81**, 936–940.

Hamrick JL, Allard RW (1972) Microgeographical variation in allozyme frequencies in *Avena barbata*. *Proceeding of the National Academy of Sciences of the USA*, **69**, 2000–2004.

Hosmer DW, Lemeshow S (1989) *Applied Logistic Regression*. Wiley, New York.

Kreitman M, Shorrocks B, Dytham C (1992) Genes and ecology: two alternative perspectives using *Drosophila*. In: *Genes in Ecology* (eds Berry RJ, Crawford TJ, Hewitt GM), pp. 281–312. Blackwell Scientific, Oxford.

Latta RG, Mitton JB (1997) A comparison of population differentiation across four classes of gene marker in limber pine (*Pinus flexilis* James). *Genetics*, **146**, 1153–1163.

Linhart YB, Grant MC (1996) Evolutionary significance of local differentiation in plants. *Annual Review of Ecology and Systematics*, **27**, 237–277.

Mitton JB, Grant MC, Yoshino AM (1998) Variation in allozymes and stomatal size in pinyon (*Pinus edulis*, Pinaceae), associated with soil moisture. *American Journal of Botany*, **85**, 1262–1265.

Mitton JB, Linhart YB, Hamrick JL, Beckman JS (1977) Observations on the genetic structure and mating system of ponderosa pine in the Colorado Front Range. *Theoretical and Applied Genetics*, **7**, 5–13.

Mitton JB, Linhart YB, Sturgeon KB, Hamrick JL (1979) Allozyme polymorphisms detected in mature needle tissue of ponderosa pine. *Journal of Heredity*, **70**, 86–89.

Mitton JB, Stutz HP, Schuster WSF, Shea KL (1989) Genotypic differentiation at PGM in Engelmann spruce from wet and dry sites. *Silvae Genetica*, **38**, 217–221.

Mopper S, Mitton JB, Whitham TG, Cobb NS, Christensen KM (1991) Genetic differentiation and heterozygosity in pinyon pine associated with resistance to herbivory and environmental stress. *Evolution*, **45**, 989–999.

Müller-Starck G, Schubert R (2000) Genetic markers as a tool for bioindication in forest ecosystems, pp. 227–237. In: *Forest Conservation Genetics: Principles and Practice* (eds Young A, Boshier D, Boyle T). CSIRO Publishing, Collingwood, Australia.

Oakeshott JG, Gibson JB, Anderson PR, Knibb WR, Anderson DG, Chambers GK (1982) Alcohol dehydrogenase and glycerol-3 phosphate dehydrogenase clines in *Drosophila melanogaster* on different continents. *Evolution*, **36**, 86–96.

Pearson GA (1920) Factors controlling the distribution of forest types. *Ecology*, **1**, 139–159.

Poulik MD (1957) Starch gel electrophoresis in a discontinuous system of buffers. *Nature*, **180**.

Quinn JA (1974) Factors contributing to the discontinuous distribution of piñon pine in the Front Range of Colorado. Masters thesis, University of Colorado at Boulder.

Schuster WSF, Alles DL, Mitton JB (1989) Gene flow in limber pine: evidence from pollination phenology and genetic differentiation along an elevational transect. *American Journal of Botany*, **76**, 1395–1403.

Schuster WSF, Mitton JB (2000) Paternity and gene dispersal in limber pine (*Pinus flexilis* James). *Heredity*, **84**, 348–361.

Spiess EB (1977) *Genes in Populations*. Wiley, New York.

Stutz HP, Mitton JB (1988) Genetic variation in Engelmann spruce associated with variation in soil moisture. *Arctic and Alpine Research*, **20**, 461–465.

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