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

Received 9 September 2003; revision received 8 December 2003; accepted 8 December 2003

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*,...
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-3_ homozygotes having the highest viability (Cobb et al. 1994). Growth rates differed two-fold among genotypes on lava soils, with _Gly-3_ 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-3_ 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 patterns 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

Glyceraldehyde 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 glyceral 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.htm) 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 meteorological 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

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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 Quaternary 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 1

<table>
<thead>
<tr>
<th>Locality</th>
<th>Apr</th>
<th>May</th>
<th>Jun</th>
<th>Jul</th>
<th>Aug</th>
<th>Precip</th>
<th>Elev</th>
<th>Soil</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Mount. Shavano</td>
<td>1.87</td>
<td>2.32</td>
<td>2.10</td>
<td>4.10</td>
<td>4.59</td>
<td>14.99</td>
<td>2896</td>
<td>schist</td>
</tr>
<tr>
<td>2. Kaufman Ridge</td>
<td>1.87</td>
<td>2.32</td>
<td>2.10</td>
<td>4.10</td>
<td>4.59</td>
<td>14.99</td>
<td>3018</td>
<td>schist</td>
</tr>
<tr>
<td>3. Garfield Mine</td>
<td>1.87</td>
<td>2.32</td>
<td>2.10</td>
<td>4.10</td>
<td>4.59</td>
<td>14.99</td>
<td>3109</td>
<td>schist</td>
</tr>
<tr>
<td>4. Canon City</td>
<td>3.98</td>
<td>5.27</td>
<td>4.25</td>
<td>6.28</td>
<td>6.53</td>
<td>26.31</td>
<td>1768</td>
<td>shale/sandstone</td>
</tr>
<tr>
<td>5. Pueblo</td>
<td>3.98</td>
<td>5.27</td>
<td>4.25</td>
<td>6.28</td>
<td>6.53</td>
<td>26.31</td>
<td>1737</td>
<td>shale/sandstone</td>
</tr>
<tr>
<td>6. Yellowjacket Canyon</td>
<td>2.35</td>
<td>2.59</td>
<td>1.28</td>
<td>3.37</td>
<td>4.01</td>
<td>13.61</td>
<td>1646</td>
<td>shale/sandstone</td>
</tr>
<tr>
<td>7. Owl Canyon</td>
<td>5.08</td>
<td>7.11</td>
<td>4.65</td>
<td>4.06</td>
<td>3.56</td>
<td>24.46</td>
<td>1829</td>
<td>shale/sandstone</td>
</tr>
<tr>
<td>8. San Cristobal</td>
<td>2.25</td>
<td>3.05</td>
<td>2.35</td>
<td>4.37</td>
<td>5.04</td>
<td>17.06</td>
<td>2335</td>
<td>shale/sandstone</td>
</tr>
<tr>
<td>9. El Malpais</td>
<td>1.85</td>
<td>1.50</td>
<td>1.45</td>
<td>4.88</td>
<td>6.91</td>
<td>16.59</td>
<td>2201</td>
<td>Quaternary volcanic</td>
</tr>
<tr>
<td>10. Sunset Crater</td>
<td>2.03</td>
<td>1.78</td>
<td>1.19</td>
<td>6.20</td>
<td>7.32</td>
<td>18.52</td>
<td>2350</td>
<td>Quaternary volcanic</td>
</tr>
<tr>
<td>11. Winona</td>
<td>2.34</td>
<td>1.45</td>
<td>1.65</td>
<td>6.70</td>
<td>6.22</td>
<td>18.36</td>
<td>1925</td>
<td>shale/sandstone</td>
</tr>
</tbody>
</table>

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.
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 $-0.064 \pm 0.023$, 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.

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

<table>
<thead>
<tr>
<th>Effect</th>
<th>d.f.</th>
<th>$\chi^2$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil</td>
<td>2</td>
<td>2.2</td>
<td>0.33</td>
</tr>
<tr>
<td>Precipitation</td>
<td>1</td>
<td>8.0</td>
<td>0.005</td>
</tr>
<tr>
<td>Elevation</td>
<td>1</td>
<td>0.4</td>
<td>0.51</td>
</tr>
</tbody>
</table>

d.f = degrees of freedom, $\chi^2$ = chi-square, $P$ = probability.

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 $-0.064 \pm 0.023$, 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).

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 $r = -0.92$ ($P < 0.001$).

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 (14.9 cm) is only 57% of that (26.3 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 pinyon, 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 pinyon 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 pinyon.

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 pinyon. 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.

Acknowledgements

We acknowledge support of NSF grants DEB 0111375 (to JBM) and DEB 020628 (to KLD and JBM), DEB 0075563 and Howard Hughes Medical Institute Grant #5203088 to Tom Whitham and Neil Cobb. Yan Linhart, Michael Grant, and Tom Whitham contributed comments on the manuscript. Doug Robertson helped identify soil types at the collection localities. Michael Grant helped with the logistic regression.

References


Jeff Mitton is an evolutionary geneticist whose research on plants and animals focuses on the forces that influence population structure. His research includes analyses of allozyme, chloroplast DNA, and mitochondrial DNA. Kristy Duran is a PhD student studying the evolutionary interactions of dwarf mistletoe, *Arceuthobium divaricatum*, and its six host species of pinyon pines.