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ALLOZYME VARIATION IN A RARE PLANT SPECIES, *PEDIOMELUM PIEDMONTANUM* (FABACEAE), FROM THE LOWER PIEDMONT PLATEAU OF GEORGIA

N. Fitzpatrick, A. Godfrey, J. Legler and T. D. Schwaner
Department of Biology
North Georgia College & State University
Dahlonega, GA 30597

Address correspondence To:
T. D. Schwaner
College of Sciences
The University of Findlay
Findlay, OH 45840-3695
schwaner@findlay.edu

ABSTRACT

A single population of *Pediomelum piedmontanum* (Fabaceae) occurs in Columbia County, Georgia. We examined allozyme variation in this population for 20 loci in eleven enzyme systems. Genetic diversity was low (P = 20.0%; A = 1.20; overall average HO = 0.038). Allelic frequencies in a large sample from one of two subpopulations predicted a significant deficiency of heterozygotes for all polymorphic loci ($P < 0.001$), inconsistent with Hardy-Weinberg expectations. Allelic frequencies for a smaller sample from the other subpopulation predicted a significant deficiency of heterozygotes for one polymorphic locus ($P < 0.025$) and non-significant excesses of heterozygotes for two others. Genetic differentiation among subpopulations was low ($F_{ST} = 0.0142$). Factors responsible for the extreme rarity of *P. piedmontanum* are unknown, but low genetic variation in the small, isolated Georgia population is most likely due to historical factors, genetic drift and perhaps inbreeding.

Key words: allozymes, genetic diversity, *Pediomelum piedmontanum*, Fabaceae

INTRODUCTION

Genetic variation is an important component to the adaptation and potential long-term survival of plant species (1). Breeding system, past and present levels of gene flow among populations, geographic distributions, isolation, historical events, and human disturbance of the habitat all affect genetic diversity within a species and its populations (2,3). Rare species, particularly those distributed in small, isolated populations, may experience genetic drift and/or inbreeding that lead to random fixation of alleles and reduced heterozygosity (4). A significant loss of genetic variation can contribute

to the extinction of small populations (5) and the loss may occur before the endangered status of the species is apparent (6).

Pediomelum piedmontanum Allison, Morris & Egan sp. nov. (7) is a newly described species of perennial herbaceous legume apparently endemic to rocky, open areas and adjacent open woodlands in the lower Piedmont Plateau of Georgia and South Carolina. The species is known from only three populations, two in Richland County and Lexington County, South Carolina, and one in Columbia County, Georgia (7). The Columbia County population covers only a few hectares, in acidic soil of low fertility, in a serpentinite outcrop. Casual estimates suggest only a few hundred individual plants at the Georgia location. The species appears to be insect pollinated and is most vigorous in relatively open, sunny patches (7).

We used allozyme electrophoresis to address the following questions: What are the levels of genetic variation in the Georgia population? Is any genetic sub-structuring detectable within the population at this site?

MATERIALS AND METHODS

One leaf was taken from each of 249 individual plants at two sampling sites (A-B, Table 1), approximately 1 km apart. Leaves of any plant less than 0.5 m from another were ignored to avoid sampling vegetative clones. Each leaf was placed in a plastic Zip-lock bag, labeled with a letter and number, immediately placed on ice, and kept refrigerated until homogenized. One or two leaflets (depending on relative size) were coarsely chopped with a clean razor blade, placed in a centrifuge tube with 50-100 µL cold "microbuffer" (8), and homogenized with a small sample laboratory homogenizer. Each homogenate was centrifuged at 5°C for 15 min at 5000 X G, and the supernatant transferred to a clean cryotube for storage at -75°C until use. Samples were run on Cellogel (Chemetron, Milan) using standard procedures (Richardson et al. (9). The only deviation from these protocols was to run the gels for one hr, rather than two, to avoid running fast-moving allozymes off the end of the gel.

Table I. Estimated allele frequencies at 4 polymorphic loci at two sample sites for the Columbia County, Georgia, population of *Pediomelum piedmontanum*. Site A is a more or less contiguous patch of plants; site B is about 1 km from site A. Number of individuals sampled at each site (n); H_O = number of observed heterozygotes and H_E = number of expected heterozygotes, per locus; Mean H_O = observed heterozygosity and Mean H_E = expected heterozygosity, per site, averaged over all 20 loci examined.

		Sample Sites	
Locus	Alleles	A (n = 234)	B (n = 15)
Aat-1	a	0.363	0.200
	b	0.637	0.800
	H_O	62	2
	H_E	108.4	5.00
Aat-2	a	0.325	0.367
	b	0.675	0.633
	H_O	62	7
	H_E	102.8	7.2
Gpi-3	a	0.066	0.067
	b	0.934	0.933
	H_O	17	2
	H_E	29.0	1.9
Pgm	a	0.855	1.000
	b	0.145	0.000
	H_O	34	0
	H_E	58.2	0.0
Mean H_O		0.038	0.037
Mean H_E		0.064	0.047

After differential staining, products of a multi-system enzyme were numbered sequentially according to their distance (in this case anodally) from the loading site (*Aat-1*, *Aat-2*, etc.). Patterns for polymorphic loci were either monomeric or dimeric (i.e., heterozygotes exhibited a two-banded or three-banded pattern, respectively). Fastest and slowest moving bands at a locus were labeled alphabetically (*Aat-1a*, *Aat-1b*, etc.)

Percentage of polymorphic loci (P; 0.95 criterion), average number of alleles per locus (A), and average heterozygosity over all loci and subpopulations

were calculated by hand using standard formulas (10). Expected numbers of heterozygotes (H_E) were computed using Levene's correction (11) and deviations from predictions of Hardy-Weinberg equilibrium (HWE) were tested using an exact test with a Markov chain algorithm (11). A fixation index (F_{IS}) was used to determine departures from HWE predictions for heterozygosity for each locus, and F_{ST} (unbiased estimate θ) was calculated to determine the degree of differentiation among subpopulations (11, 12).

RESULTS

Of the 20 loci examined, 15 (*Acon, Est-1, Est-2, Gpi-1, Gpi-2, Gpi-4, Hex, Mdh-1, Mdh-2, Mdh-3, Me-1, Me-2, Mpi, 6Pgd-1, 6Pgd-2*) were monomorphic. One locus (*Idh*) exhibited a frequency of 0.966 for the most common of three alleles and was not considered to be polymorphic. The four remaining (*Aat-1, Aat-2, Gpi-3* and *Pgm*) were polymorphic, with two alleles per locus (P = 4/20 = 20.0%; A = 48/40 = 1.20). Observed heterozygosities over all loci (see Table 1) were similar but low for subpopulations A (H_O = 0.038) and B (H_O = 0.037). Allele frequencies showed significant differences among the two sample sites for *Pgm* (Fisher's exact test, $P < 0.02$), probably because *Pgm* was fixed in site B. However, allele frequencies for *Aat-1*, *Aat-2* and *Gpi-3* for sample site A (alleles a and b = 0.363 and 0.637, 0.325 and 0.675, and 0.067 and 0.993) and sample site B (a and b alleles = 0.200 and 0.800, 0.367 and 0.633, and 0.067 and 0.933) were similar (P = 0.10; Table 1). The average observed and expected heterozygosity values for the total *P. piedmontanum* data set, obtained by averaging values for the two sampling sites, were 0.038 and 0.056, respectively. Average expected heterozygosity differed between sample sites (site A, mean H_E = 0.064; site B, mean H_E = 0.047) and both expected values were higher than for observed values (site A, mean H_O = 0.038; site B, mean H_O = 0.037). Expected numbers of heterozygotes predicted by allele frequencies for all loci in the larger subpopulation (A) were significantly greater than observed numbers (F_{IS} values between +0.398 and +0.429, all $P < 0.001$). A deficiency in heterozygotes was also predicted by allele frequencies for *Aat-1* in the smaller subpopulation (B) and a non-significant excess of heterozygotes predicted at Aat-2 and Gpi-3 (*FIS* = -0.013 and 0.021, respectively). Genetic differentiation, calculated over the four polymorphic loci, among the two subpopulations was very low (F_{ST} = 0.0142).

DISCUSSION

Genetic variation within the Columbia County, Georgia population was low. Only 20% of loci examined were polymorphic and the mean number of alleles per locus was 1.20, compared with means of 36% loci polymorphic (n = 24, range 3.4% - 90.5%) and 2.4 alleles per locus (n = 24, range 2 - 3.2) in a list of restricted plants (see 13, Table 6.1, pp 90-91). Low values as in *P. piedmontanum* have been found for even more isolated populations of plants (e.g. 13 and references cited therein). Moreover, all polymorphic loci

in the larger subpopulation (A) and one in the smaller subpopulation (B) were significantly deficient in expected numbers of heterozygotes.

Very little is known about the origins and relationships of *P. piedmontanum*. Morphological comparisons suggest that its sister species may be distributed farther west of the Mississippi River (see reference 7 and citations therein for a discussion of known relationships within the genus), in which case the Georgia and South Carolina populations may be relicts, occupying their current locations for a very long time. Whether or not these populations may initially have been larger and were later reduced in size is unknown, but their apparent confinement to rocky areas and serpentinite soils that may restrict the growth of other plants (7) suggests that they have always been small and relatively isolated.

Perhaps the most important factors underlying the genetic structure of plant populations are time of origin, size, degree of isolation, and mating habits (3). Small, isolated populations experience genetic drift and the loss of heterozygosity over time (14). Also, mating in small plant populations is likely to involve inbreeding due to the sessile habit, restricted gene dispersal through pollen and seeds, and high levels of self-fertilization and sib-mating in plants. *P. piedmontanum* has deep taproots that resist fire, suggesting they regenerate annually (7). However, "papilionoid flowers" and the presence of numerous winged insects, "including bees (Bombus spp.)" and several species of butterflies, suggested that the species exhibits "entomophyly" (7). Seed set was observed in October 2006 (A. Godfrey, personal observation). Consequently, the restricted population could be a mix of older perennials and newer seeded plants. Small populations become inbred more rapidly than large populations and inbreeding reduces heterozygosity. Although insect pollination is likely in *P. piedmontanum* (7), for very small and presumably old populations, like the one in Columbia County, the influence of size on inbreeding may overwhelm effects brought about by the system of mating alone (5). Very low genetic differentiation among subpopulations separated by 1 km at the Georgia locality suggests that allele and genotypic diversity can be maintained between close populations of *P. piedmontanum*.

Studies using other molecular markers (e.g., the random amplified polymorphic DNA or RAPID method) may detect more variation to help determine the role of small population size, insect pollination, and vegetative reproduction to mating habits and presumed inbreeding. Demographic analysis would better define population size and age structure. Also, a comparison involving other *P. piedmontanum* populations may reveal correlations between variance in morphological features and genetic variation. Obviously, there is much more to be learned about *P. piedmontanum* in Georgia, and this study is a limited view of one aspect of its biology.

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