

THE GENETIC CONSEQUENCES OF DISJUNCTION
IN CAMPANULA ROTUNDIFOLIA (ANGIOSPERMAE: CAMPANULACEAE)

A Thesis

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the Graduate Faculty of the Department of Biology
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of the Requirements for the Degree
Master of Science

by
Gwenlyn Kay Waller

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Department of Biology

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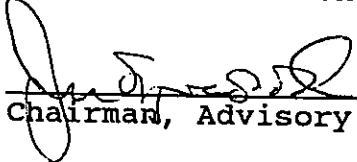
Master of Science

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ABSTRACT

It has been predicted that small, isolated populations will contain less genetic diversity and be genetically distinct from neighboring populations due to genetic drift. These predictions were tested for the flowering plant species Campanula rotundifolia by comparing three disjunct Missouri populations and eighteen non-disjunct, non-Missouri populations for patterns of isozyme variation in two enzyme systems (LAP and EST). The 21 populations are significantly differentiated from each other, with an estimated F_{st} of 0.173. Nei's genetic distance between populations is only weakly correlated with geographic distance ($r = 0.145$), and F_{st} estimates for local clusters of populations are as high as the total F_{st} , indicating that differentiation is mostly on a local scale. The median genetic distance between Missouri and non-Missouri populations was not significantly different from the median genetic distance between different non-Missouri populations, so there is no evidence that the disjunct Missouri populations have diverged to a greater extent than have non-disjunct populations. The median gene diversity (Nei) within the disjunct Missouri populations was not significantly less than that within non-Missouri populations, so there is likewise no evidence that the disjunct populations are genetically depauperate.

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Chairman, Advisory Committee

Southwest Missouri State University

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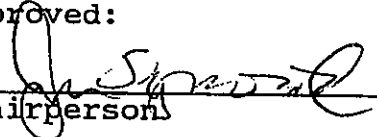
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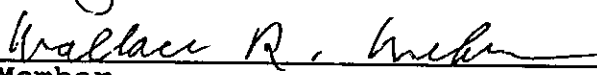
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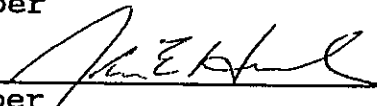
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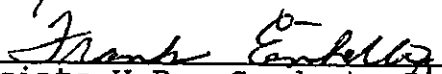

Associate V.P., Graduate Studies &
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INTRODUCTION

As land use pressures have increased, the effects of habitat fragmentation have become the focus of conservation biologists (Temple and Wilcox, 1986). Habitat fragmentation often results in smaller population sizes, disrupts migrating corridors, and disrupts mating and foraging patterns (Temple, 1986). This type of disturbance, both natural and man-induced, has generated heated controversy over refuge design (Diamond, 1976; Simberloff and Abele, 1982; Shaffer and Samson, 1985), and has given rise to the concept of minimum viable population sizes (Shaffer, 1981; Simberloff, 1988). The development of these ideas stems from the vulnerability of small populations to several stochastic factors (Shaffer, 1981). The study reported here addresses the effects of genetic stochasticity, or genetic drift, on small disjunct populations of the flowering plant species Campanula rotundifolia L. (harebell) located in the Missouri Ozarks.

The small sizes of these populations make them vulnerable to extinction. Usher (1987) identified six factors which concern conservationists when dealing with small populations: inbreeding, heterozygosity, bottleneck or founder effects, effective population size, genetic drift, and mutation rates. Many of these concerns are related. The greatest potential threats to viability of the

disjunct harebell populations are from a loss of genetic variation and an accumulation of inbreeding, both of which result from random genetic drift (Shaffer 1981; Charlesworth and Charlesworth, 1987; Usher, 1987; Simberloff, 1988; Lande and Barrowclough, 1989; Barrett and Kohn, 1991; Brussard, 1991; Ellstrand, 1992). Without genetic variation, evolutionary adaptation to a dynamic habitat cannot occur (Lande and Barrowclough, 1989). Inbreeding can be detrimental if it leads to inbreeding depression.

Crow (1986) defines genetic drift as a random change in allele frequencies that occurs over time in finite populations. These random changes become increasingly important as population size is reduced. Eventually, these changes will lead to the fixation of one allele and the loss of all others at a gene, which results in complete homozygosity (Franklin, 1980). Thus, small populations are particularly susceptible to the loss of genetic variation via genetic drift. For example, Bayer (1992), in his study of Antennaria arcuata, demonstrated that disjunct populations had less genetic variation when compared to more widespread species of Antennaria.

Genetic drift is more of a factor for isolated populations in which the effects of genetic drift are less likely to be offset by the introduction of new alleles via migration (Crow, 1986). Therefore, more isolated populations would be more likely to show genetic

differentiation between populations than less isolated populations of comparable size. For example, disjunct island populations of Campanula punctata are genetically distinct from mainland populations (Inoue and Kawahara, 1990).

Inbreeding occurs when two mating individuals share a common ancestor. In plants, inbreeding can occur through self-fertilization or through outcrossing among relatives, the latter being referred to as biparental inbreeding. Biparental inbreeding occurs whenever the inbreeding-effective size of the population, N_{ef} , is small. The effective size of a population may be small because of localized dispersal (isolation by distance; Wright, 1943; Heywood, 1993) or because the actual size of the population is small (Ellstrand and Elam, 1993). In small populations, kinship accumulates as alleles drift to fixation, and subsequent outcrossing has an equivalent effect as biparental inbreeding. Thus, sexual reproduction in small populations necessarily leads to the accumulation of inbreeding, regardless of the actual breeding system.

Inbreeding often results in a reduction of vigor and/or fertility because of the expression of deleterious recessive alleles that were otherwise concealed by a dominant allele in heterozygotes (Crow, 1986), a phenomenon referred to as inbreeding depression. Thus, inbreeding depression diminishes the viability of the population over all

conditions. Inbreeding depression has been shown to be significant in natural populations of many outcrossing plants (Charlesworth and Charlesworth, 1987; Gilpin, 1989; Holtsford and Ellstrand, 1990). Some studies have shown that long-distance outcrossing can also cause fitness depression if the parents occupy different specific habitats (Waser and Price, 1989; Ellstrand, 1992). However, outcrossing depression is not a factor when dealing with disjunct populations.

In addition to the effects of genetic drift and inbreeding, the genetic variation of small populations may be reduced due to natural selection. Because there is limited habitat, selection may act to produce a homozygous population by selecting traits that are favorable for a particular habitat (Karron, 1991).

Because of the small sizes of the disjunct populations of C. rotundifolia and the way evolutionary forces tend to act on small populations, I hypothesize that, on average, genetic variation will be reduced within the disjunct populations when compared to that found within populations in the normal range, and the disjunct populations will be less similar to populations from the normal range than populations from the normal range are to each other. The purpose of the study described here was to test these hypotheses using allozyme polymorphisms.

MATERIALS AND METHODS

Organism and Study Sites

The Ozark plateau is a unique, diverse mountain range which extends from eastern Oklahoma, through southern Missouri and northern Arkansas, to southern Illinois. Because of the geological and topographical complexity of the region, the occurrence of temporal climatic changes since the Pleistocene Epoch, and the lack of natural boundaries such as large rivers and mountains which allow species to migrate into the region, the Ozarks have become the home of relictual species which extended their range during the Pleistocene Epoch only to be isolated when the glaciers retreated as the result of a warming climate (Templeton et al., 1990). The harebell, Campanula rotundifolia, is just one example (Hensold et al., 1986; Autry, 1988; Nigh, 1988).

C. rotundifolia L. (Angiospermae: Campanulaceae) is a long-lived perennial, with ramets living for five or more years (Shetler, 1982). Observation of the plants along the Jack's Fork River indicate that plants can live at least three years at this location (G. Waller, pers. obs.). This species apparently originated within the Mediterranean Basin during or before the Miocene Epoch. Polyploidy is common throughout the range (Shetler, 1982; Great Plains Flora

Association, 1986). The breeding system of the harebell is almost exclusively outcrossing with little or no selfing occurring naturally (Shetler, 1982). The normal distribution of the harebell is almost completely within the boundaries of the glaciated regions. However, this species also occupies boreal regions south of the glacier front (Shetler, 1982) and disjunct protected sites are found along the Ozark National Scenic Riverway in southeastern Missouri (Steyermark, 1963) and on Fountain Bluff in southern Illinois (Mohlenbrock and Wilson, 1985) (fig. 1). Most of the disjunct populations are found on rocky bluffs along rivers (Shetler, 1982).

According to Shetler (1982), the harebell occupies several different habitats within its normal range. These habitats can be described as alpine or subalpine meadows or parks, open pine forests, gravel slides, shaded cliffs, or rocky shores. Within these habitats, the harebell can be a prominent species.

In Missouri, harebells can be found in three locations along the Ozark National Scenic Riverway: Jam-Up Cave Natural Area, Bear Hollow Bluff, and Middle Loader Hole Bluff (Nigh, 1988). The population at Jam-Up Cave Natural Area can be found growing on the rock bluff surrounding the mouth of Jam-Up Cave and on the bluff beyond the cave. It is a north-facing bluff with several moist ledges, and of the Missouri populations, is the largest. Bear Hollow Bluff

is also a north-facing bluff with moist ledges. The population size at this site is approximately 130 plants. Middle Loader Hole Bluff is a south-facing bluff where the harebells occur under an overhang which may provide the cooler microhabitat the plant seems to require. Only fourteen plants have been located at this site.

Chromosome numbers of the plants used in this study were not determined; however, reports of chromosome numbers of plants from the study sites or regions nearby indicate that $2N = 68$. These include the Pacific Northwest, Great Lakes, Northern Great Plains and Northern Atlantic East Coast regions. Chromosome numbers of $2N = 34$ and $2N = 102$ have been reported from New Hampshire and British Columbia, respectively (Shetler, 1982).

Leaf tissue of the harebell was collected from the following sites within the normal range of the plant: Isle Royale National Park, Apostle Island National Lakeshore, Pine Barrens National Forest, Wild River State Park, Effigy Mounds National Park, Acadia National Park, Allagash (Maine), Olympic National Park, Yellowstone National Park, and Glacier National Park (fig. 2). The three disjunct Missouri populations were also sampled. Habitat description and sample sizes are summarized in Table 1.

Sampling Procedures

Park employees collected from the non-Missouri sites by collecting stems with leaves from areas within their respective park. Forty stems were requested from various locations, taking care not to sample clones. The stems were wrapped in a moist paper towel and sealed in a plastic bag and shipped by an overnight express package, or brought to Missouri in a cooler with ice.

Missouri plants were sampled by selecting stems with leaves from plants that were found growing within reach on the bluff faces (within approximately 15 feet of the base of the bluff). At Middle Loader Hole, every plant found was sampled. Forty plants were sampled at Bear Hollow and thirty-seven were sampled at Jam-Up Cave. Only six plants were collected at Fountain Bluff in Illinois. Although they were collected, no electrophoretic analysis was performed on these plants due to the small sample size.

The plant tissue was freeze-dried upon receipt or upon collection to ensure preservation of enzymes until electrophoresis could be done.

Electrophoretic Analysis

Enzyme systems were screened for polymorphic loci by using electrophoretic methods described by Hebert and Beaton

(1989) for cellulose acetate plates. However, some modifications were made (Hampton, 1992; Appendix 1). Of the nineteen enzyme systems surveyed, only five were found to be polymorphic (Table 2). Of the five presumed polymorphic loci, only two, Leucine aminopeptidase (LAP) and esterase (EST), developed with scorable resolution. These two enzyme systems were run on a Trizma, EDTA and Boric Acid (TEB) buffer at 200 volts for 30 minutes. Wicks were moistened at 15 minutes to prevent the plates from drying. These loci displayed apparent polysomic inheritance, suggesting C. rotundifolia is a recent autopolyploid, and as a result it was not possible to distinguish individual genotypes. Therefore, the gels were scored according to the presence or absence of bands. Electrophoretic data were stored in a Minitab worksheet (Appendix 2).

Statistical Analysis

A series of standard descriptive statistics for genetic data were calculated, except all calculations were based on band frequencies instead of allele frequencies. Presence and absence of a band were treated as alternative "alleles", and separate bands were treated as separate "loci". Genetic variation within populations was estimated as Nei's (1973) gene diversity (H). Genetic differentiation among populations was estimated using Nei's (1973) genetic

distance (D) for pairwise comparisons and Wright's (1951) F_{st} for an overall value. Uncorrected F_{st} estimates were calculated as:

$$\hat{F}_{ST} = \frac{\sum_i \sum_j w_{ij} (p_{ij} - \bar{p}_i)^2}{\sum_i w_i \bar{p}_i (1 - \bar{p}_i)}$$

where

$$\bar{p}_i = \frac{\sum_j n_{ij} p_{ij}}{\sum_j n_{ij}}, \quad w_{ij} = \frac{n_{ij}}{\sum_i \sum_j n_{ij}}, \quad w_i = \frac{\sum_j n_{ij}}{\sum_i \sum_j n_{ij}},$$

and p_{ij} = the observed frequency of band i in population j , $\bar{p}_i$ = the mean observed frequency of band i across all populations, and n_{ij} = the sample size for band i in population j . This is essentially Nei's (1973) G_{st} , except that a weighting factor has been added that accounts for differences among populations in sample size. The null hypothesis that $F_{st} = 0$, or that banding phenotypes were randomly distributed among sampling locations, was tested by computer randomizations. On each randomization, banding phenotypes were randomly shuffled among samples and $\hat{F}_{ST}$ was recalculated. If the randomly generated $\hat{F}_{ST}$ values exceeded the observed $\hat{F}_{ST}$ value less than 5% of the time,

the null hypothesis would be rejected. The randomizations also provided an approximate estimate of the bias in $\hat{F}_{ST}$, which was subtracted from $\hat{F}_{ST}$ to yield an adjusted $\hat{F}_{ST}$.

Mann-Whitney tests (Sokal and Rohlf, 1987) were performed to determine if the median gene diversity (H) of the three Missouri populations is significantly different from that of all other populations, and to determine whether the median genetic distance between Missouri and non-Missouri populations is greater than the median genetic distance between different non-Missouri populations. In addition, a Spearman rank correlation analysis (Sokal and Rohlf, 1987) was performed to examine possible relationships between genetic distance and geographical distance.

RESULTS

The LAP enzyme system had seven distinguishable bands while the EST enzyme system had six distinguishable bands. Estimated band frequencies for the 21 populations sampled, based on a total of 388 individuals, are presented in Table 3 (LAP) and Table 4 (EST).

The adjusted $\hat{F}_{ST}$ value for the 21 populations is 0.173, which is significantly greater than zero ($p = 0.000$). Thus, there are significant differences among populations in their band frequencies. The adjusted $\hat{F}_{ST}$ value for the

Missouri populations was 0.271, which is also significantly greater than zero ($p = 0.000$), so differentiation exists among the disjunct populations themselves. For comparison, the adjusted $\hat{F}_{ST}$ value among local populations within Yellowstone, Glacier, and Olympic were 0.052, 0.171, and 0.247, respectively. All of these values are significantly different from zero ($p = 0.007, 0.000, 0.000$).

Pairwise genetic distances are presented in Table 6. The rank correlation between genetic distance and geographic distance is positive ($r = 0.145$, see fig. 3), and significant ($p = 0.035$). The median genetic distance between different non-Missouri populations was 0.0575 while the median genetic distance between non-Missouri and Missouri populations was 0.0740. There is no significant difference between these two numbers ($p = 0.4279$).

The gene diversities for the 21 populations are presented in Table 5. The median gene diversity of the three disjunct Missouri populations was 0.147 as compared to 0.196 for all other populations. There is no significant difference between these two values (Mann-Whitney U test, $p = 0.209$), indicating that the Missouri populations do not contain less gene diversity than the others.

DISCUSSION

The estimated F_{st} value of 0.173 indicates that there is some differentiation among the sampled populations of C. rotundifolia. This value is larger than the mean of F_{st} estimates for other outcrossing (mean $\hat{F}_{ST} = 0.118$), monoecious (mean $\hat{F}_{ST} = 0.092$), and long-lived species (mean $\hat{F}_{ST} = 0.077$), but smaller than the means reported for species with similar reproductive modes (mean $\hat{F}_{ST} = 0.300$), pollination mechanisms (mean $\hat{F}_{ST} = 0.262$), and seed dispersal mechanism (mean $\hat{F}_{ST} = 0.446$) (Loveless and Hamrick, 1984). Genetic distances do not indicate that the Missouri populations are genetically distinct from populations found within the normal, widespread range of this species. For example, the Missouri Bear Hollow population was more similar to the Yellowstone populations than to the neighboring Missouri Middle Loader Hole Bluff population (Table 6). The adjusted $\hat{F}_{ST}$ value for the Missouri populations (0.271) was greater than the overall $\hat{F}_{ST}$ value, indicating that Missouri populations are more differentiated from each other than from the other populations on average. However, there were similarly high levels of differentiation on a local scale within Glacier

and Olympic parks, indicating that, in general, most of the overall F_{st} is due to local differentiation. In addition, only a weak correlation was found between genetic distance and geographical distance, indicating that, in general, geographical distance is a poor indicator of genetic distance for this species. This is not consistent with results from other studies which indicate small, isolated populations are genetically distinct (eg., Inoue and Kawahara, 1990, Les et al., 1991).

The results also fail to support the prediction that the small, isolated Missouri populations are genetically depauperate, since gene diversities did not differ on average between Missouri and non-Missouri populations. However, it is interesting to note that the Middle Loader Hole Bluff population, which consisted of only fourteen individuals at the time samples were collected, had the lowest gene diversity of the 21 populations sampled.

The lack of evidence for enhanced levels of genetic drift in the disjunct Missouri populations of the harebell may be explained, in part, by the life history characteristics of this species. All else being equal, long-lived perennial, outcrossing, and widespread plants tend to maintain more allozyme genetic variation within populations, and show less differentiation among populations, than do plant species with other life history traits (Hamrick, Linhart, and Mitton, 1979). An outcrossing

mating system presumably reduces population differentiation because it encourages pollen flow among populations (Loveless and Hamrick, 1984).

Genetic drift would also be diminished by the polyploid nature of the harebell. For example, the effective size of a tetraploid population is twice that of a diploid population of the same actual size. The exact chromosome number found in Missouri plants has not been recorded. However, most of the counts indicate $2N = 68$ (recent tetraploid, ancient octaploid) throughout most of the North American range (Shetler, 1982). If the Missouri populations are tetraploid as well, this will double the effective size of each population. The ploidy history of the harebell is complex, and different theories of the process have been proposed (Shetler, 1982). However, it is believed that the tetraploid behaves as a diploid during meiosis (Shetler, 1982).

Because this study is based on only two presumed loci, it is also possible that the results are not representative of other loci. Slatkin (1985) points out that some gene loci can show extreme geographic differentiation while others do not. Also, because the observed isozyme banding patterns were too complex to allow unambiguous identification of genotypes, the possibility that isozyme polymorphism represents environmental variation in the activation of multiple loci cannot be ruled out. Thus, it

is possible that the observed geographical pattern reflects a combination of genetic and environmental differences.

Even if the isozyme variation is entirely genetic in origin, calculations based on banding patterns rather than allele frequencies are biased because alleles may be hidden in the phenotypic pattern. For a locus near Hardy-Weinberg proportions, band frequencies always overestimate allele frequencies because $p^2 + 2pq > p$. This will cause F_{st} to be slightly overestimated and gene diversities to be slightly underestimated. Thus, there may be slightly less differentiation among populations and slightly more diversity within populations than indicated by this study.

The results of this study indicate that the small disjunct Missouri populations have not diverged markedly as a result of genetic drift and do not appear to be genetically depauperate. However, this does not necessarily indicate that these populations are not at risk. First, because these are potentially very long-lived perennials, it is possible that too few generations have passed since the Pleistocene disjunction event for any significant genetic drift to have occurred. Second, the genetic data reveal what has happened to these populations in the past, and do not necessarily reflect current conditions. Recent changes in the environment may have severely impacted these populations, which would not yet show in the genetics. This is why current studies of population dynamics are so

important. In this light, demographic data are needed before any conclusions pertaining to the persistence of the Missouri populations can be made. We do not know if the populations are currently adding new individuals, nor do we know how the populations fluctuate from season to season. A large population increase was observed at the Missouri Bear Hollow Site (from approximately 15 plants to over 100 plants) from one growing season to the next. We do not know if these new individuals were indeed new or if they were merely too small and inconspicuous to be noticed the previous season. In addition, a flood occurred during the last season of the study and eliminated several plants from the Jam-up site. Other sites were not visited but were probably also affected. Certainly, a small population will be more susceptible to these random fluctuations than a large one (Shaffer, 1981).

It would be beneficial to determine the population dynamics of these small disjunct populations. Campanula rotundifolia appears to require a cool moist habitat. If this is true, the harebell would be sensitive to a warming climate and the relictual populations in Missouri would be at risk for local extinction.

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Table 1. Habitat description and sample sizes (N) of *C. rotundifolia* from collection sites.

<u>COLLECTION SITE</u>	<u>N</u>	<u>HABITAT DESCRIPTION</u>
Missouri		
Ozark National Scenic Riverway		
Jam-Up Cave Natural Area (OZ-JU)	34	North facing, moist bluff
Bear Hollow Bluff (OZ-BH)	38	North facing, moist bluff
Middle Loader Hole Bluff (OZ-ML)	14	South facing, dry to semi-dry
Michigan		
Isle Royale National Islands (ISLROY)	34	Meadows, rocky outcrops, forest openings, and lakeshores
Apostle Islands National Lakeshore (APOISL)	14	Rocky lakeshores
Pine Barrens U. S. Forest (PINBAR)	12	Open Pine Forest
Iowa		
Effigy Mounds National Monument (EFFMND)	31	Prairie
Montana		
Glacier National Park		
site 1 (GLAC#1)	9	Lodgepole pine forest
site 2 (GLAC#2)	11	Sub-alpine forest
site 3 (GLAC#3)	10	Prairie

Table 1. (continued)

Washington

Olympic National Park

Hurricane Hill (OLYM#1)	13	Exposed meadow hilltop
Obstruction Point (OLYM#2)	10	Rocky, south-exposed slope; sub-alpine
Hurricane Ridge Road (OLYM#3)	12	sub-alpine area
Switch Back, Obstruction Point Road (OLYM#4)	10	East-facing slope, meadow

Wyoming

Yellowstone National Park

Golden Gate (YELL#1)	12	West-facing slope, open lodgepole pine forest
Firehole Lake Drive (YELL#2)	12	Rocky barrens, silica base rock, very open area
Mt. Washburn (YELL#3)	13	Rich meadow
Biscuit Basin (YELL#4)	13	Open lodgepole pine forest

Maine

Allagash/St. John's River (ALLAGA)	34	Cobble beach river bank, barren open habitat
Acadia National Park (ACADIA)	25	Open granite ledges, dry; coastal grassy meadows

Minnesota

Wild River State Park (WLDRIV)	27	Open woods and prairie
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TABLE 2. Summary of enzymes surveyed

ENZYME	CODE	N	# OF BANDS	POYLMORPHIC ²
Colorimetric Esterase	EST	388	6	P*
Leucine Aminopeptidase	LAP	388	7	P*
Triose Phosphate Isomerase	TPI	71	6	P
6-Phosphogluconate Dehydrogenase	6PGD	72	2	P
Menadione Reductase	MNR	52	5	P
Phosphoglucomatase	PGM	41	2	M
Phosphoglucose Isomerase	PGI	30	3	M
Shikimate Dehydrogenase	SKDH	20	0 ¹	-
Malic Enzyme (decarboxylating) / Malate Dehydrogenase	ME/MDH	43	5	P
Acid Phosphotase	ACP	25	0	-
Aldolase	ALD	18	1	M
Alcohol Dehydrogenase	ADH	19	0	-
Glyceraldehyde-3-Phosphate Diaphorase	G3PD	16	0	-
Isocitrate Dehydrogenase	DIA	18	3	P
Aconitase	IDH	3	0	-
Xanthine Dehydrogenase	ACON	5	0	-
Formate Dehydrogenase	XDH	6	0	-
Aspartate Amino Transferase	FDH	6	0	-
	AAT	11	3	P

¹ 0 = weak or no activity.

² P = polymorphic bands observed; M = monomorphic; * identifies systems used in this study.

Table 3. Band frequencies for LAP enzyme system.
Sample sizes & abbreviations for collection
sites are as in Table 1.

Site	Phenotypic bands						
	<u>LAP-1</u>	<u>LAP-2</u>	<u>LAP-3</u>	<u>LAP-4</u>	<u>LAP-5</u>	<u>LAP-6</u>	<u>LAP-7</u>
OZ-JU	0.059	0.529	0.000	0.765	0.059	0.735	0.000
OZ-BH	0.079	0.158	0.053	0.816	0.316	0.684	0.000
OZ-ML	0.000	0.000	0.000	1.000	1.000	0.000	0.000
APOLSL	0.071	0.429	0.000	0.643	0.500	0.786	0.000
PINBAR	0.000	0.417	0.000	0.667	0.500	0.500	0.000
ISLROY	0.118	0.412	0.088	0.676	0.382	0.676	0.000
EFFMND	0.000	0.129	0.032	0.484	0.452	0.871	0.000
WLDRIV	0.185	0.481	0.111	0.333	0.556	0.704	0.037
YELL#1	0.083	0.333	0.000	1.000	0.167	0.417	0.083
YELL#2	0.167	0.250	0.000	0.667	0.500	0.333	0.000
YELL#3	0.000	0.692	0.000	0.923	0.385	0.462	0.231
YELL#4	0.000	0.308	0.000	0.692	0.538	0.231	0.000
GLAC#1	0.111	0.444	0.000	0.667	0.556	0.000	0.000
GLAC#2	0.273	0.818	0.000	1.000	0.091	0.000	0.000
GLAC#3	0.100	0.200	0.000	0.600	0.500	0.200	0.000
ALLAGA	0.176	0.382	0.000	0.735	0.588	0.294	0.000
ACADIA	0.320	0.400	0.040	0.560	0.600	0.080	0.000
OLYM#1	0.000	0.385	0.000	1.000	1.000	0.154	0.000
OLYM#2	0.000	0.000	0.000	1.000	1.000	0.000	0.000
OLYM#3	0.000	0.167	0.000	0.917	1.000	0.583	0.000
OLYM#4	0.800	0.000	0.000	1.000	1.000	0.700	0.000