GENETIC VARIATION WITHIN AND AMONG SUB-POPULATIONS OF THE ENDANGERED PLANT *LESQUERELLA KINGII* SSP. *BERNARDINA* (BRASSICACEAE)

WILLIAM J. STRAITS[1], DAVID S. GILL, and ANDREW J. LOWE
Department of Biology
California State University Fullerton, MH 282
Fullerton, California 92834

[[1]—Current address: Science Education Center,
The University of Texas at Austin, SZB 340,
Austin, Texas 78712]

ABSTRACT: *Lesquerella kingii* ssp. *bernardina* is an endangered plant endemic to carbonate soils of the San Bernardino Mountains, California. This study examined patterns of isozyme variation of the thirteen known sub-populations of this plant. Seven enzyme systems, yielding thirteen loci, were analyzed through starch gel electrophoresis. Genetic variation within sub-populations was greater than expected, indicating high levels of heterozygosity. There was little genetic differentiation among sub-populations, possibly suggesting high levels of gene flow or relatively recent sub-population derivation. Additional studies are needed to fully understand the ecology and population genetics of *Lesquerella kingii* ssp. *bernardina* and to provide guidance for future management decisions.

KEY WORDS: bladderpod, Brassicaceae, carbonate soils, Cruciferae, electrophoresis, genetic variation, isozymes, *Lesquerella kingii* ssp. *bernardina*, San Bernardino Mountains.

Lesquerella kingii Wats. ssp. *bernardina* Munz (Brassicaceae) is a herbaceous plant endemic to the dolomite and limestone outcrops of the San Bernardino Mountains, California. Its common name is the San Bernardino Mountains bladderpod, and will hereafter be refered to as LEKIB. This subspecies is restricted to specific habitat conditions, with all known individuals found on well drained slopes of carbonate soils which range from 2100–2700 m in elevation, habitat conditions found only infrequently in the San Bernardino Mountains (Barrows and Myers 1988). Total population size is estimated at 20,000 individuals (California Natural Diversity Database, as cited in USFWS 1994). Sub-populations are threatened by housing development as well as the proposed expansion of a major ski resort within the expanding Big Bear Lake area. The taxon is federally listed as an endangered species by the United States Fish and Wildlife Service (USFWS 1994).

Population genetics theory predicts that endemic species will have low levels of variation. This prediction has been supported by a number of studies comparing closely related widespread and endemic species (Karron 1987; Primack 1980; Soltis and Soltis 1991). Low levels of variation are often due to small population size and/or narrow species distributions or ranges. Ramifications of this low level of variation include lowered fitness, lowered adaptability, inbreeding depression, and greater effect of deleterious recessive mutations (Ledig 1986). Given these potentially negative consequences of low levels of variation it is critical for conservationists to have a knowledge of the variation within and among populations of endemic and rare species.

All known sub-populations of LEKIB are confined to thirteen localized sites (see Table 1). The purpose of this study was to assess the genetic variation within, and differentiation among, these thirteen sub-populations. Variation was assessed through examination of enzymes. Patterns of variability detected in this study could be quite important for the management of this subspecies.

Table 1. Description of sub-population study sites. The location, elevation, and aspect of the thirteen sub-populations of LEK1B.

Site number	Site acronym	Site elevation	Site aspect	Site location
1	BM1	2700 m	North	South of Big Bear Lake
2	BM2	2650 m	North	South of Big Bear Lake
3	BM3	2600 m	North	South of Big Bear Lake
4	BM4	2600 m	North	South of Big Bear Lake
5	BRR	2400 m	South	North of Big Bear Lake
6	BRW	2400 m	North	South of Big Bear Lake
7	DIV	2100 m	South	South of Big Bear Lake
8	DRW	2200 m	South	South of Big Bear Lake
9	LUT	2200 m	South	South of Big Bear Lake
10	NEW	2150 m	South	South of Big Bear Lake
11	SRR	2100 m	South	South of Big Bear Lake
12	SRW	2200 m	South	South of Big Bear Lake
13	VDC	2200 m	North	South of Big Bear Lake

METHODS

Leaf tissue of twenty individuals from each sub-population was randomly collected for isozyme analysis in September of 1993. Isozymes were studied through starch gel electrophoresis. Starch gels were prepared as outlined by Acquaah (1992). Morpholine-citrate and tris-EDTA-borate electrode buffer systems (Soltis et al. 1983) were used. Proteins were extracted through homogenization of leaf tissue in an extraction buffer adapted from Sosa and Garcia-Reina (1992). Filter paper wicks saturated with homogenate were inserted into the gel. Gels were then subjected to approximately 145V at a constant current of 40mA for a period of five hours, which allowed for sufficient migration and separation of isozymes. Seven enzyme systems were analyzed: malate dehydrogenase (MDH), isocitrate dehydrogenase (IDH), nicotinamide adenine dinucleotide dehydrogenase (NAD), aspartate amino transferase (AAT), malic enzyme (ME), glucose phosphate isomerase (GPI), and esterase (EST). Recipes and protocols for stains of the seven enzymes studied were based on the work of Soltis et al. (1983). Resultant data were analyzed for genetic variation within sub-populations and differentiation among sub-populations using Biosys I (Swofford and Selander 1989).

RESULTS

Assessment of genetic variation within sub-populations was based on several common measures: mean number of alleles per polymorphic locus, percent polymorphic loci, and the ratio of observed to expected Hardy-Weinberg frequencies (Table 2). The mean number of alleles per polymorphic locus ranged from 2.00 (BM1, BM2, BM3, SRR) to 2.22 (DRW, VDC), with a mean value of 2.09. Percentage polymorphic loci was 69.23 for all sub-populations except BM1, BM2, and SSR which had values of 53.85, 61.54, and 76.92, respectively. For all sub-populations the observed to expected Hardy-Weinberg ratio was greater than one. An observed to expected ratio of one indicates random mating, a ratio greater than one suggests an excess of heterozygosity as in predominantly outcrossing populations, and a ratio less than one suggests excess homozygosity as in predominantly inbreeding populations. Values ranged from 1.253 (SRR) to 1.896 (VDC and DIV), with a mean value of 1.765. As a composite index of variability within sub-population, mean rank of variability was calculated. The mean rank values are based upon the ranking of each sub-

Table 2. Summary of genetic variation. Average number of alleles per polymorphic locus, percentage polymorphic loci, and observed to expected Hardy-Weinberg frequencies are given for each sub-population. Larger values suggest greater variation. Mean values across all sub-populations given along bottom margin. Within each measure of genetic variability sub-populations were ranked; all ties were averaged. Mean variability ranking is given for each sub-population. See Table 1 for acronyms and descriptions of sub-population study sites.

Sub-population	Mean Number of alleles per polymorphic locus	Percentage of polymorphic loci	Observed to Expected Hardy-Weinberg Ratio	Mean rank of variability within each sub-population
BM1	2.00	53.85	1.667	2.17
BM2	2.00	61.54	1.759	2.83
BM3	2.00	69.23	1.881	6.50
BM4	2.11	69.23	1.774	7.00
BRR	2.11	69.23	1.881	8.50
BRW	2.11	69.23	1.775	7.33
DIV	2.11	69.23	1.896	9.50
DRW	2.22	69.23	1.809	9.00
LUT	2.10	69.23	1.615	4.83
NEW	2.11	69.23	1.860	8.00
SRR	2.00	76.92	1.253	5.50
SRW	2.11	69.23	1.877	9.00
VDC	2.22	69.23	1.896	10.83
— Mean —	— 2.09 —	— 68.05 —	— 1.765 —	—

population (least variable to most variable, the latter having larger values) for each of the measures of genetic variation.

Genetic differentiation among sub-populations was based on cluster analysis and Wright's F statistic (Wright 1965). A large F(st) value suggests a high degree of differentiation, whereas a small value indicates low differentiation. For the eleven loci studied the mean F(st) was 0.063, suggesting little differentiation among sub-populations (Table 3). Cluster analysis revealed that all sub-populations form one group at the 0.9425 level (Figure 1).

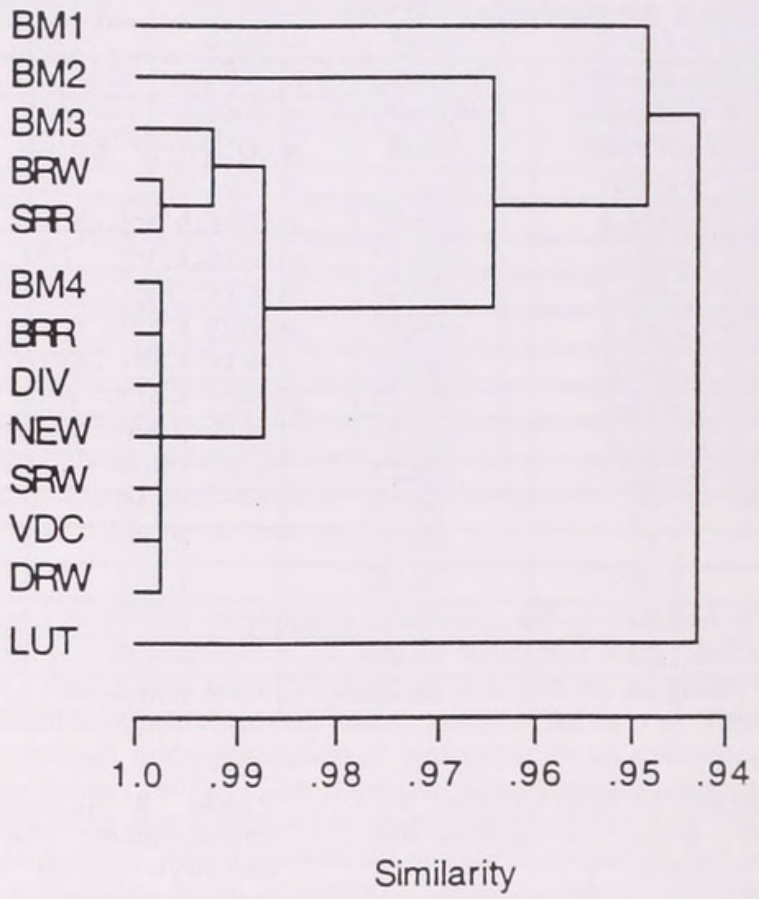
DISCUSSION

Endemic plant species on average have fewer alleles per polymorphic locus than widespread species (2.48 and 2.67, respectively) and fewer polymorphic loci (26%; widespread species 43%) (Hamrick et al. 1991). A large observed to expected Hardy-Weinberg ratio indicates a large number of heterozygous individuals within sub-populations. This ratio for widespread plant species is on average much

Table 3. Summary of genetic differentiation. The mean Wright's F statistics [F(st)] across all sub-populations sampled for each locus are given. Large F(st) values suggest greater partitioning of variation among sub-populations. The overall mean for all loci across all sub-populations is 0.063.

Locus	F (st)
IDH-1	0.071
IDH-2	0.043
NAD-1	0.006
MDH-1	0.007
MDH-2	0.015
AAT-1	0.085
ME-1	0.131
ME-2	0.001
GPI-1	0.021
GPI-2	0.135
EST-1	0.118
— Mean —	— 0.063 —

Figure 1. Cluster analysis using unweighted pair group method and Rogers genetic similarity coefficient. The diagram shows the relative distinctions among sub-populations based on multivariate analysis of all isozyme data. The more similar sub-populations unite farther to the left of the diagram and have correspondingly larger similarity values.



less than endemic species (0.63 and 1.05, respectively) (Hamrick et al. 1991). Percentage polymorphic loci and observed to expected Hardy-Weinberg ratio (Table 2) of LEKIB in this study suggest a relatively large amount of variation within each sub-population. The small number of alleles per polymorphic loci found in LEKIB relative to other plant species studied may be attributed to the relatively small numbers of individuals and enzyme systems analyzed. The consistency, however, of percentage of polymorphic loci and observed to expected Hardy-Weinberg ratio suggests that there is a great deal of heterozygosity within sub-populations.

Inspection of the cluster diagram based on isozyme data shows no clear distinctions among sub-populations (Figure 1). The F(st) values of the isozyme data also suggest little differentiation among sub-populations (Table 3). Geographically isolated sub-divisions of a population will often show a "spatial genetic structure" characterized by great allelic frequency differences [large F(st) values] (Heywood 1991). The small F(st) values for the loci studied contradict the prediction of genetic separation and, rather, suggest that a great deal of gene flow is occurring among sub-populations of LEKIB.

It is generally accepted that variation provides a species the potential to expand ecological and geographical range, as well as to adapt to changing environmental conditions (Allard et al. 1978; Heywood and Levin 1985; Rozema et al. 1978). Variation, the key to evolutionary change, may allow the better occupation of varying microhabitats (Silander 1985), survival of disturbance (Bremermann 1980; Futuyma 1983), as well as ecological suc-

cess of colonizing and established populations (Carson 1987). The greater the range of variation of a species which can be preserved, the greater the likelihood for long-term survival of the species.

The present existence of LEKIB is threatened by human activity. Pivotal to the preservation of this rare subspecies is the maintenance of variation. Ideally, the entire range of variation should be preserved. If due to political, social, economic, cultural, or other factors, the broad preservation of the sole population of LEKIB is not feasible, the following priority listing is given. The mean ranking based on the three measures of genetic variability supports the following ranking of sub-populations, from least variable to most variable: BM1, BM2, LUT, SRR, BM3, BM4, BRW, NEW, BRR, DRW, SRW, DIV, and VDC (Table 2).

Although this study attempts to rank the variation of sub-populations, it is important to note that all sub-populations are of consequence. For instance, while BM1 and BM2 have low overall variability, they may contain genotypes adapted to high elevation and/or north-facing slopes, and failure to conserve these lower variability sites may lead to the loss of genetic combinations important to survival at this extreme of distribution. Further, destruction of as few as one of the sub-populations may also disrupt gene flow and cause isolation of sub-populations. This fragmentation may result in a reduced effective population size, increased inbreeding, and subsequent inbreeding depression, as demonstrated in other taxa (Charlesworth and Charlesworth 1987). Much more needs to be understood if informed management decisions are to be made concerning the preservation of LEKIB.

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