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Genetic Differentiation of Rare and Common Varieties of *Eriogonum Shockleyi* (Polygonaceae) in Idaho Using ISSR Variability

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GENETIC DIFFERENTIATION OF RARE AND COMMON VARIETIES
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IN IDAHO USING ISSR VARIABILITY

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ABSTRACT.—Idaho populations of *Eriogonum shockleyi* are divided taxonomically into 2 varieties: *E. shockleyi* var. *packardae*, which is endemic to Idaho, and the typical variety, which is widespread in the western United States. Recent morphological investigations of *E. shockleyi* in Idaho have identified potentially reliable morphological characters for field identification of the subspecific taxa. This paper investigates the genetic basis for the separation of the 2 varieties of *E. shockleyi* using inter simple-sequence repeats (ISSR) markers. Although we found some morphological differences between the populations that correlated with the 2 varieties, we identified no molecular markers in this study to distinguish between them. Morphological measurements obtained in the field indicate that although a population may have an overall average morphology that defines the variety, some individuals in nearly all populations have putative diagnostic characters that define the other variety. The morphological characters used to distinguish the 2 varieties are most likely the result of environmental variability and could result from differences in precipitation and soil water retention. Alternatively, high levels of outcrossing through pollen flow could be obscuring selection for morphological characters at particular sites.

Key words: *Eriogonum shockleyi*, ISSR, variety, rare plants, Polygonaceae, populations.

Increasing pressures on natural resources have raised questions and concern regarding the status and management of rare populations and species of plants. Of critical concern regarding the status of rare plants is whether they are genetically distinct from more common and widespread congeners and conspecifics (Falk and Holsinger 1991). Genetic variation and the distribution of this variation in rare plant populations provide information for understanding the origin and evolution of rare plants as well as management considerations.

One plant species that merits further study is *Eriogonum shockleyi* S. Wats (Polygonaceae). *Eriogonum shockleyi* is a widespread species in the western United States, known from California to Colorado, south to Arizona and New Mexico, and north to Idaho. This species commonly occurs on barren rocky, clay, or sandy substrates in shrubland or pinyon-juniper communities (Reveal 1985). The species has been separated into 2 varieties, *E. shockleyi* var. *shockleyi* S. Wats and *E. shockleyi* var. *packardae* Reveal. *Eriogonum shockleyi* var. *packardae* is a rare Idaho endemic found from the Halverson Lakes region of Ada County along the

Snake River to the Bruneau River drainage of Owyhee County (Fig. 1, Table 1).

The 2 varieties have a history of being difficult to distinguish (DeBolt and Rosentreter 1988). Both are mound-forming perennials with densely compact rosettes. Recent morphometric analysis of these 2 varieties has identified 4 morphological characters that readily delimit them (Moseley and Reveal 1996). These 4 characters are peduncle length, leaf blade width, leaf blade length, and petiole length. In general, *E. shockleyi* var. *shockleyi* has longer peduncles and larger leaves than does *E. shockleyi* var. *packardae* (Moseley and Reveal 1996). These 4 characters readily separated all Idaho populations of *E. shockleyi* into the 2 varieties, with the exception of a single population from Upper Sugar Valley, Owyhee County (Fig. 1). This last population was identified as an intermediate between the 2 varieties during morphometric analyses of herbarium material, although morphologically the individuals were readily identified as one or the other in the field (Moseley and Reveal 1996).

The morphological distinctiveness and geographic disjunction of the 2 varieties of *E. shockleyi* raise the possibility that these represent a

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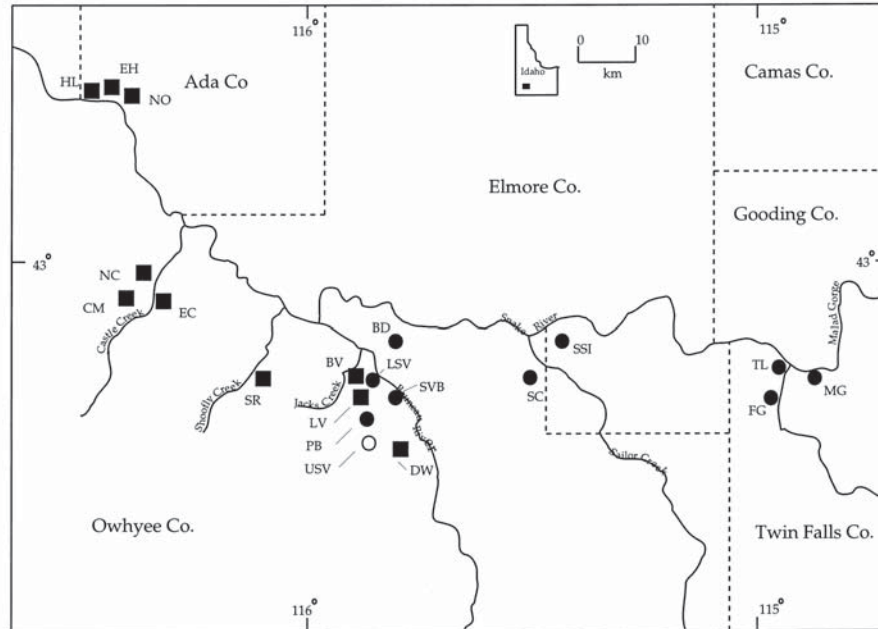


Fig. 1. Map of southern Idaho showing localities for all *Eriogonum shockleyi* var. *packardae* (shown with squares) and *E. shockleyi* var. *shockleyi* (shown with circles) populations sampled as part of this study. The population represented by a mixture of the 2 varieties is shown with an open circle (USV). Abbreviations for populations follow Table 1.

progenitor-derivative pair (Gottlieb 1973a, 1973b, 1974). In progenitor-derivative pairs, the rare taxon is generally thought to have evolved from an isolated population of its more common and widespread relative. Isolation may have occurred as the result of climate changes (Smith and Pham 1996), substrate differences (Gottlieb 1973b), polyploidy (Ownbey 1950, Soltis and Soltis 1991), chromosome rearrangements (Gottlieb 1974), or long-distance dispersal into new habitats (Kliman and Hey 1993). If this progenitor-derivative hypothesis is correct, the genetic variation found in populations of *E. shockleyi* var. *packardae* is expected to be a subset of that found in *E. shockleyi* var. *shockleyi* (Gottlieb 1973a, 1974).

The best means of assessing genetic variation of rare plants is to examine directly the level and structure of genetic diversity of several populations of both varieties. Molecular methods have provided many insights into the genetic diversity of rare plants (Waller et al. 1987, Lesica et al. 1988, Hickey et al. 1991, van Buren et al. 1994, Smith and Pham 1996, Hickerson and Wolf 1998, Archibald et al. 2001). In this paper we make use of inter simple-sequence repeats (ISSRs). These markers provide considerable amounts of variability within populations and have gained a reputa-

tion for their repeatability. The markers utilize regions of DNA that are segments of short repetitive sequences. For example, a segment of DNA may have a sequence of GGA repeated any number of times. This variability in the size of these segments (number of times the sequence is repeated) is often used as a population level marker (microsatellite; Sunnucks 2000). ISSR variability instead makes use of the spaces between these repeats. The primers for ISSR markers incorporate simple-sequence repeats and are anchored on one end with a random tag from outside the repeating region (Zietkiewicz et al. 1994). Amplified products are the areas between the primer binding sites, and the data used in the analysis are whether 2 repeat sites are in close enough proximity to produce a band of DNA during the amplification procedure. Sites that differ in the length of DNA between them will be scored as different loci.

ISSRs have not had the breadth of use that RAPDs have, but studies utilizing these data report a high degree of resolution among populations and individuals (Charters et al. 1996, de la Hoz et al. 1996, Tsumura et al. 1996, Yang et al. 1996, Nagaoka and Ogihara 1997, Wolfe et al. 1998). In their study of diploid hybrid speciation in *Penstemon*, Wolfe et al.

TABLE 1. Sampled populations of *Eriogonum shockleyi*. Taxonomic classification follows that of Moseley and Reveal (1996) with the exception of OR that was classified by the first author based on population averages of morphological measurements. Full descriptions and coordinates of localities are available in Moseley and Reveal (1996) with the exception of OR, which was found at 41°37.111'N, 114°49.631'W, 1713 m elevation.

Variety	Abbreviation	Idaho county	Site	
<i>E. shockleyi</i> var. <i>packardae</i>	EH	Ada	East of Halverson Lakes	
	HL	Ada	Halverson Lakes	
	NP	Ada	North of Priest Ranch	
	BV	Owyhee	Bruneau Valley Rim	
	CM	Owyhee	Castle Creek Mines	
	DW	Owyhee	Deer Water	
	EC	Owyhee	East of Castle Creek	
	LV	Owyhee	Little Valley	
	NC	Owyhee	North of Castle Creek	
	SR	Owyhee	Shoofly Road	
	USV	Owyhee	Upper Sugar Valley	
	OR	Elko, NV	O'Neill Basin Road	
	<i>E. shockleyi</i> var. <i>shockleyi</i>	SSI	Elmore	South of Schoff's Island
		MG	Gooding	Malad Gorge
BD		Owyhee	Bruneau Dunes	
LSV		Owyhee	Lower Sugar Valley	
PB		Owyhee	Prominent Buttes	
SC		Owyhee	Lower Sailor Creek	
SVB		Owyhee	Sugar Valley Badlands	
FG		Twin Falls	Fossil Gulch	
TL		Twin Falls	Lower Salmon Falls	
				Dam Transmission Line

(1998) scored 270 bands across 5 taxa, of which 4 were fixed across all populations of all taxa. Other bands provided sufficient variation such that individual DNA accessions could be genotyped with 1 to 3 ISSR primers, and markers for all species were found with these data. Likewise, 41 ISSR markers from 3 primers successfully distinguished among 34 lines of Chinese sorghum (Yang et al. 1996), and either of 2 primers successfully distinguished 16 of 20 cultivars of *Brassica napus* ssp. *oleifera* (Charters et al. 1996). Thus, the variability detected in ISSR markers has utility to recognize different lineages, cultivars, species, and groups of closely related species (Wolfe et al. 1998).

This paper specifically seeks to determine (1) the genetic variability in the rare variety *E. shockleyi* var. *packardae* using ISSR markers, (2) degree of genetic distinctiveness between *E. shockleyi* var. *packardae* and its more common conspecific, *E. shockleyi* var. *shockleyi*, and (3) degree of morphological distinctiveness between the 2 aforementioned varieties.

METHODS

Material Acquisition

All known populations that were located in a 1995 survey of *Eriogonum shockleyi* var.

packardae (Moseley and Reveal 1996) from Idaho were sampled (Fig. 1, Table 1), with the exception of 1 population near Perjue Canyon, Owyhee County. This population is located near the Shoofly Road; therefore, its omission from the analysis is not likely to seriously bias the conclusions. An additional population from Nevada that fit the description of *E. shockleyi* var. *packardae* (R. Moseley personal communication) also was sampled, although the variety was not known previously to occur outside of Idaho. Twelve populations were assigned to *E. shockleyi* var. *packardae* (Table 1).

We also sampled 9 of 11 known populations of *E. shockleyi* var. *shockleyi* in Idaho (Moseley and Reveal 1996, Fig. 1, Table 1). Two populations near Horse Hill, Owyhee County, were omitted (Moseley and Reveal 1996, Narad et al. 1997). Their omission is unlikely to affect the results, however, as other populations near the Horse Hill population were sampled. From each of the 21 populations, we randomly selected 28 individuals.

DNA Analysis

DNA was extracted from all individuals using Qiagen DNeasy plant miniprep kits following the manufacturer's instructions. All amplifications used 1 µL of template DNA in

25 μ L reactions. Eight primers were used in all reactions: (1) (CT)₈TG, (2) (CT)₈RG, (3) (CA)₆RY, (4) (GT)₆RG, (5) (GT)₆AY, (6) CAA(GA)₆, (7) (GT)₆ YR, and (8) (CA)₆RG. Reaction conditions were 1X magnesium-free buffer provided by manufacturer, 3 mM MgCl₂, 0.16 mM each of dCTP, dATP, dGTP, and dTTP, 0.1 mg mL⁻¹ BSA, 0.4 pmol primer, and 0.5 μ L Taq polymerase (Promega). Amplification profiles varied in annealing temperature, depending on the primer, and were optimized for each of the primers to produce clear and repeatable amplifications. They otherwise consisted of 1.5 minutes at 94°C, followed by 35 cycles of 40 seconds at 94°C, 45 seconds at 44°C, 1.5 minutes at 72°C followed by 45 seconds at 94°C, 45 seconds at 44°C, and 5 minutes at 72°C. Ultimately, we used 3 different annealing temperatures for the different primers: 44°C was used for primers 1, 3, 5, and 7; 42°C for primers 2, 6, and 8; and 48°C for primer 4.

Total amplified reaction mixes were run on 1.4% agarose gels for 5 hours at 35 volts. After electrophoresis, gels were stained with ethidium bromide and photographed. Standard markers were included in the first and last lane of each gel. Initial surveys of each ISSR primer, using a subset of the individuals in the analysis, indicated that bands were fully repeatable in duplicate gels.

Data Scoring

Gels were scored by first determining the size of the amplified fragment and then scoring each individual for presence or absence of each band. Primer 2 never successfully amplified population MG (Table 1) despite several attempts at altering reaction conditions and annealing temperatures. Likewise, primer 6 did not amplify populations SSI and LSV (Table 1). When we obtained no amplification products from individuals, these individuals were considered to be missing data and were not scored as homozygous recessives for the absence of the amplified bands.

Molecular Data Analysis

Each amplified fragment of a particular size was scored as a distinct locus. Because ISSR markers act as dominants, heterozygous individuals all will be counted as dominants with the marker present. We converted presence of each locus into a population percentage based

on the number of individuals with the marker in each population for all populations. These data were then analyzed in BIOSYS-1 (Swofford and Selander 1981) to determine gene diversity statistics (Nei 1973) and genetic relatedness. Since primers 2 and 6 had missing data for entire populations, these data were removed from the analysis. Percent loci polymorphic was calculated with and without the data from primers 2 and 6.

Gene diversity statistics (Nei 1973) were calculated for each ISSR marker scored using the WRIGHT78 program of BIOSYS-1 (Swofford and Selander 1981). Because of limitations in using amplified gene regions to assess genetic diversity (heterozygotes remain undetected due to inheritance of markers as dominants), we expected assessments of diversity using these markers to be lower than when compared to studies with codominant loci such as isozymes (Novak et al. 1996, Smith and Pham 1996). In addition, markers with high frequencies (1-3/N, 0.995 in this study) were omitted from the analysis, as recommended by Lynch and Milligan (1994), based on theoretical examination of the use of dominant markers in estimating genetic diversity.

Genetic relatedness for all populations was analyzed using the CLUSTER program of BIOSYS-1 (Swofford and Selander 1981).

Morphological Characters

Four morphological characters have been identified as readily separating *Eriogonum shockleyi* var. *shockleyi* from *E. shockleyi* var. *packardae* in Idaho (Moseley and Reveal 1996): peduncle length, leaf width, leaf length, and petiole length. These 4 morphological characters were measured for each individual on fresh plant material. We averaged these morphological data for each of the characters for each population and calculated a standard deviation for each mean. Significant differences between means were determined using a *t* test (Snedecor and Cochran 1980).

Analysis of variance (ANOVA) was used to determine if each of the morphological variables differed between varietal designation. Regression analysis was used to determine if morphological variables depended on longitude of the populations or precipitation received by the population. Since the goal was to determine a correspondence to longitude and precipitation for the entire species, all

TABLE 2. Summary of morphological data gathered for all populations of *Eriogonum shockleyi* sampled from Idaho. Populations are listed in order of increasing peduncle length. Classification to variety follows Moseley and Reveal (1996). Superscript letters denote groups of means that are not significantly different from each other at the $P = 0.05$ level using a t test.

Variety	Population	Peduncle length (cm) mean \pm s	Leaf length (mm) mean \pm s	Leaf blade width (mm) mean \pm s	Petiole length (mm) mean \pm s
<i>packardae</i>	NC	4.5 ^a \pm 2.0	6.2 ^b \pm 1.1	1.9 ^{bcd} \pm 0.5	1.7 ^{abc} \pm 0.6
<i>packardae</i>	SR	4.9 ^a \pm 3.1	6.5 ^{bcd} \pm 1.8	1.7 ^{ab} \pm 0.5	1.9 ^{bcd} \pm 1.0
<i>packardae</i>	NP	5.0 ^a \pm 2.3	6.8 ^{bcd} \pm 1.1	1.8 ^{abc} \pm 0.4	2.4 ^{efg} \pm 0.6
<i>packardae</i>	BV	5.5 ^{ab} \pm 2.4	6.5 ^{bcd} \pm 1.4	1.9 ^{bcd} \pm 0.5	1.6 ^{ab} \pm 0.6
<i>shockleyi</i>	SSI	5.5 ^{ab} \pm 3.3	6.4 ^{bc} \pm 1.2	2.2 ^{de} \pm 0.6	1.7 ^{abc} \pm 0.7
<i>packardae</i>	EH	5.6 ^{ab} \pm 2.6	5.1 ^a \pm 1.1	1.5 ^a \pm 0.4	1.3 ^a \pm 0.5
<i>packardae</i>	DW	5.7 ^{ab} \pm 2.4	7.0 ^{bcd} \pm 1.5	2.1 ^{cde} \pm 0.6	2.0 ^{bcd} \pm 0.7
<i>packardae</i>	CM	5.7 ^{ab} \pm 3.3	6.8 ^{bcd} \pm 1.3	2.1 ^{cde} \pm 0.6	1.8 ^{bcd} \pm 0.6
<i>shockleyi</i>	LSV	5.8 ^{ab} \pm 2.9	7.4 ^{ef} \pm 1.7	2.1 ^{cde} \pm 0.5	2.3 ^{defg} \pm 0.8
<i>packardae</i>	USV	5.9 ^{ab} \pm 3.2	6.8 ^{bcd} \pm 1.6	1.9 ^{bcd} \pm 0.6	1.9 ^{bcd} \pm 1.1
<i>packardae</i>	OR	6.0 ^{ab} \pm 5.5	6.2 ^b \pm 1.3	1.8 ^{abc} \pm 0.4	1.8 ^{bcd} \pm 0.7
<i>shockleyi</i>	SVB	6.3 ^{abc} \pm 2.9	7.3 ^{def} \pm 1.3	2.3 ^e \pm 0.7	2.1 ^{cdef} \pm 0.6
<i>packardae</i>	HL	7.1 ^{bcd} \pm 2.4	7.2 ^{cdef} \pm 1.6	1.9 ^{bcd} \pm 0.7	2.6 ^g \pm 1.2
<i>packardae</i>	EC	7.5 ^{bcd} \pm 2.8	7.1 ^{cdef} \pm 1.1	2.2 ^{de} \pm 0.4	2.0 ^{bcd} \pm 0.6
<i>shockleyi</i>	PB	7.5 ^{bcd} \pm 3.3	7.8 ^{fg} \pm 1.2	2.5 ^{ef} \pm 0.6	2.5 ^{fg} \pm 0.6
<i>packardae</i>	LV	8.1 ^{cd} \pm 3.3	7.8 ^{fg} \pm 1.5	2.4 ^e \pm 0.6	2.7 ^g \pm 0.9
<i>shockleyi</i>	BD	8.4 ^d \pm 3.6	7.4 ^{ef} \pm 1.4	2.4 ^e \pm 0.5	2.1 ^{cdef} \pm 0.7
<i>shockleyi</i>	SC	10.6 ^e \pm 3.6	7.1 ^{cdef} \pm 1.1	2.3 ^e \pm 0.7	2.0 ^{bcd} \pm 0.6
<i>shockleyi</i>	FG	12.7 ^f \pm 4.3	8.4 ^{gh} \pm 1.7	2.5 ^{ef} \pm 0.6	2.3 ^{defg} \pm 1.7
<i>shockleyi</i>	MG	15.1 ^g \pm 5.9	9.2 ^{hi} \pm 2.0	3.0 ^g \pm 0.6	2.5 ^{fg} \pm 1.0
<i>shockleyi</i>	TL	15.9 ^g \pm 5.6	9.5 ^f \pm 2.2	2.9 ^{fg} \pm 0.8	2.5 ^{fg} \pm 1.0

populations were considered together in these analyses. Additionally, a minimum of longitude data for each variety precluded a separate analysis for each variety separately. Precipitation data were determined from a precipitation map for the region (Fig. 3). All analyses were done using SAS (Version 8) and were conducted using the population means (Table 2) of the morphological variables.

RESULTS

Six to 12 different bands were amplified for each of the primers, with an average of 8 bands per primer (including data for primers 2 and 6) and a total of 64 loci from all 8 primers. Due to the lack of amplification for entire populations with primers 2 and 6, and limitations to the number of loci that can be analyzed using BIOSYS-1, we excluded the data from primers 2 and 6 from the analysis for a total of 51 bands examined. None of the amplified products were monomorphic across all populations examined. The least polymorphic locus had a frequency of 98.3%. Eight bands were found within a single population and are rare within each population, occurring in only 4–13 individuals from each population.

The proportion of ISSR loci polymorphic per population ranged from 25.5% (population SSI, Table 1) to 66.7% (population PB, Table 1) with the bands from primers 2 and 6 excluded, both from *Eriogonum shockleyi* var. *shockleyi*. When the data from primers 2 and 6 were added separately or combined, proportions differed only slightly and ranking was altered in terms of the highest proportion of loci polymorphic in only a few cases. With all data included, the proportion of loci polymorphic ranged from 20.3% (SSI) to 62.5% (PB and CM, the latter population *E. shockleyi* var. *packardae*).

None of the markers sampled in this analysis occurred at a frequency higher than the 0.995 recommended cutoff value of Lynch and Milligan (1994). Total genetic diversity (H_T) ranged from 0.003 to 0.499 for all 21 populations, with a mean of 0.263. Within (H_S)- and among (D_{ST})-population diversity components ranged from 0.003 to 0.391 and 0.000 to 0.381, respectively. Means for within- and among-population diversity were 0.163 and 0.100, respectively. Gene differentiation relative to total population (G_{ST}) ranged from 0.016 to 0.954, with a mean of 0.299. Means for the varieties did not differ greatly from each other

or from the overall mean for the species: $H_T = 0.268$ and 0.261 ; $H_S = 0.162$ and 0.173 ; $D_{ST} = 0.096$ and 0.089 ; $G_{ST} = 0.270$ and 0.279 for *E. shockleyi* var. *shockleyi* and *E. shockleyi* var. *packardae*, respectively.

There was little correspondence between assignment of taxonomic rank based on morphology and groupings based on ISSR data (Fig. 2). Populations from both varieties were scattered throughout the tree. There was also very little correspondence to the groupings based on UPGMA and any morphological features measured in this analysis. Genetic identity values ranged from 82.1% (FG to the remaining populations, Fig. 2) to 96.5% (MG to TL) and are within the range expected between varieties of a single species (Crawford 1983).

Populations can be segregated readily into one or the other variety on the basis of averages for morphological data (Table 2), but they do not correspond to previous classifications of these populations (Moseley and Reveal 1996). Peduncle length has been used as a key character to distinguish the 2 varieties (Reveal 1985, Moseley and Reveal 1996). Plants are assigned to *Eriogonum shockleyi* var. *shockleyi* if their peduncle length is >1 cm and to *E. shockleyi* var. *packardae* if the peduncle length is <1 cm. Populations traditionally assigned to *E.*

shockleyi var. *packardae* based on morphology (Moseley and Reveal 1996) do have a population average peduncle length <1 cm, but so do 5 other populations traditionally assigned to *E. shockleyi* var. *shockleyi* (SVB, LSV, PB, BD, and SSI; Table 2). Only 4 of the easternmost populations remain in *E. shockleyi* var. *shockleyi* based on average peduncle length alone (SC, MG, TL, and FG; Table 2, Fig. 1).

The morphological data are complex, with much of the information obscured among the averages. When the raw data are examined, it is clear that within each of these populations are individuals with characteristics of both varieties. In all but a single population of *E. shockleyi* var. *packardae* that was sampled (NC), at least 1 individual had a peduncle length that was equal to or exceeded the 1-cm boundary between the 2 varieties. Likewise, all populations of *E. shockleyi* var. *shockleyi* had a minimum of 3 individuals with a peduncle length <1 cm. Only 1 population (NC) entirely comprised individuals with a peduncle length <1 cm.

Although means for each of the morphological characters fell into distinct classes that were statistically different from each other, there were few classes that did not have one or more populations that bridged the gap (Table 2).

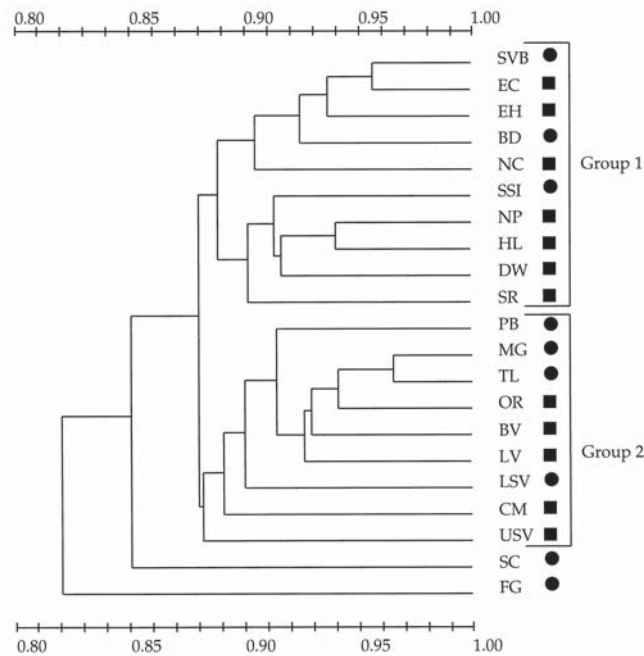


Fig. 2. UPGMA tree based on data used to calculate gene diversity statistics. Abbreviations for populations follow Table 1. *Eriogonum shockleyi* var. *packardae* are represented with squares and *E. shockleyi* var. *shockleyi* with circles.

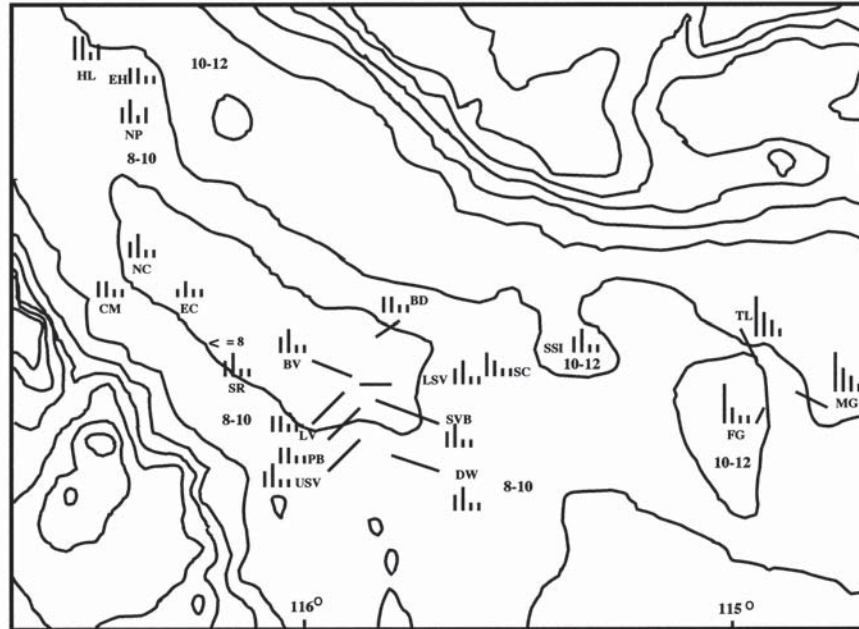


Fig. 3. Map of populations of *Eriogonum shockleyi* sampled in this study with the population average for peduncle length, leaf length, leaf width, and petiole length (Table 2) represented by bars of proportional length for each character. Isobars represent precipitation levels (inches per year), which are labeled for appropriate regions. Map is approximately the same area presented in Figure 1, although county boundaries and rivers have been removed for clarity. Population abbreviations follow Table 1.

Results of the ANOVA and regression analyses indicated that peduncle length, leaf length, and leaf width each differed significantly between the varietal designations of Moseley and Reveal (1996); $P < 0.01$ in each case. All 3 measures were larger in *E. shockleyi* var. *shockleyi* (Table 2). Petiole length did not differ significantly between varieties ($P = 0.13$). Likewise, peduncle length, leaf length, and leaf width each significantly varied with longitude ($P < 0.01$, $r^2 = 0.47$; $P < 0.01$, $r^2 = 0.33$; and $P < 0.01$, $r^2 = 0.39$, respectively), with larger measures tending to be in the more easterly populations. Petiole length did not vary significantly with longitude ($P = 0.36$). Varietal designations differed significantly in their longitude ($P < 0.01$), with *E. shockleyi* var. *packardae* being the more westerly. However, there was no significant relationship between variety and precipitation ($P = 0.20$). A significant positive relationship exists between peduncle length and precipitation ($P = 0.027$, $r^2 = 0.24$), but not for any of the leaf measurements ($P = 0.23$, 0.21 , and 0.77 , respectively).

Eriogonum shockleyi grows in areas receiving the least precipitation in Idaho. Populations

sampled in this study are found in areas receiving < 8 inches per year, 8–10 inches per year, or 10–12 inches per year. Morphological characters, expressed as averages for each population, are positioned onto a map for southwestern Idaho showing annual precipitation zones (Fig. 3).

DISCUSSION

The molecular markers sampled in this analysis do not support the taxonomic distinction of *Eriogonum shockleyi* populations into 2 varieties in Idaho (Fig. 2). The UPGMA analysis of markers from 6 ISSR primers does not group populations in accordance with previous taxonomic classifications of Moseley and Reveal (1996).

Similarly, the morphological data do not show any statistically significant gaps that separate the populations based on the characters sampled here (Table 2). However, peduncle length, leaf width, and leaf length each differed significantly between varieties based on a regression analysis, indicating that a gradient in morphology exists. While this is in agreement with the data from Moseley and Reveal (1996),

petiole length did not show a significant difference. Although this seems to provide evidence for a morphological distinctiveness between the 2 varieties, each of the characters that showed a significant difference between varietal designation also had a significant relationship with longitude. Therefore, it is not possible to state whether the morphological differences seen among these populations are related to taxonomic varieties or to longitudinal position.

A minimum of 2 groups were delimited in this analysis based on ISSR variability (Fig. 2); however, these groups do not correspond well to any morphological feature that may separate them. There is a loose correlation with peduncle length, although this mostly corresponds with the statistically significant difference between (1) SC and all other populations; (2) FG and all other populations; and (3) MG and TL together, but distinct from all other populations (Table 2, Fig. 2). Otherwise, there is only a loose correlation, as populations in group 1 (Fig. 2) have a tendency toward shorter peduncles and those in group 2 (Fig. 2) have longer peduncles (Table 2, Fig. 2). However, there is complete statistical overlap of all 4 morphological characters between all populations in these 2 groups (Table 2).

There is also little correspondence between groupings based on ISSR markers and geographic location of populations. As with the morphological data, there is a slight longitudinal trend with the westernmost populations in group 1 and the easternmost populations in group 2 (Fig. 2). However, the ISSR data do not separate the populations based on their taxonomic designations as there are populations from both varieties in both groups; most notably, FG, which is closest in proximity to MG and TL (Fig. 1), is distinct from all other populations in the analysis (Fig. 2). Likewise, SC, which is closest geographically to SSI (Fig. 1), is clearly genetically distinct from its nearest population (Fig. 2).

Although the data presented here do not demonstrate a distinct difference between the 2 varieties, based either on molecular or morphological evidence, it should be noted that *Eriogonum shockleyi* has a much broader distribution with considerable morphological variation that was not sampled as part of this study. It is possible that the lack of distinctiveness

among the Idaho populations is due to the fact that all may be members of *E. shockleyi* var. *packardae* and all may be genetically and morphologically distinct from the remainder of the species throughout its range.

Correlation with Environmental Variation

As others have noted (e.g., Moseley and Reveal 1996), there is a general trend in *Eriogonum shockleyi* toward smaller plants in the West and larger plants in the East. This same trend was also observed in this study (Fig. 3, Table 2). Thus, there is a geographic correlation with morphological variation. Statistically significant relationships between peduncle length, leaf width, leaf length, and longitude all were observed in this study, although no significant relationship between petiole length and longitude was observed. The correlation between geography and morphology and the lack of correlation between genetic markers and either of these 2 parameters imply that the differences between populations may be the result of environmental differences. Mapping the sampled populations and population averages for the 4 morphological measurements made in this study onto a map showing precipitation data gives us a rough correspondence between precipitation, geography, and morphology (Fig. 3). Populations from western Idaho comprise the smallest plants and receive the smallest amounts of precipitation. Both plant size and precipitation increase eastward across the state. Westernmost populations are found in either the <8 inch or the 10–12 inch rainfall zone. In contrast, the easternmost populations are found mostly in the 12–14 inch zone or, if in the 10–12 inch zone (SC), are close to the 12–14 inch zone boundary (Fig. 3). Thus, the easternmost populations are receiving more precipitation than those in the West.

A significant relationship between peduncle length and precipitation was detected in this analysis, although no other significant relationship between morphological characters and precipitation was observed. The relationship between morphology and precipitation is not perfect, and in a few cases smaller plants occupy sites with greater amounts of rainfall than a corresponding population with larger plants (SSI and SC). Such discrepancies may be the result of microclimatic or edaphic differences

between sites. Alternatively, seasonal differences in precipitation may result in morphological variability at different sites. Although the data provide a rough correspondence between precipitation and morphological variation in this species, further work is clearly needed to test whether this is the case. Seeds from different populations should be grown in a common garden environment and the morphological characters measured in these individuals to further verify the differences seen here.

Although the climatic data imply that morphological differences seen in *Eriogonum shockleyi* are environmental, it is possible that the 2 varieties are either in the process of becoming distinct or were formerly distinct, with gene flow now carrying both molecular markers and morphological characters across populations. This possibility also is supported by a significant relationship between longitude and varietal designation, but no significant relationship between longitude and precipitation. If the morphological variability seen among these populations could be attributed entirely to precipitation, then there should be a corresponding relationship between precipitation and longitude as there is with morphology and longitude. Although precipitation sampling areas are few and approximate, the lack of relationship between longitude and precipitation implies that the relationship between morphology and longitude could be due to isolation by distance, or it could be related to other environmental conditions that do have a significant relationship with longitude that were not sampled here.

Although peduncle length served to classify correctly only a single population (NC), there are significant trends in the morphological data. Lack of any distinctness may be the result of incomplete selection against the respective traits in the different populations. Alternatively, it could be the result of gene flow through pollen transfer. The distinctive mixture of individuals of different size classes in the different populations does imply that there may be some selection for certain sizes at different sites, but extensive gene flow from other populations continuously carries alleles with a lesser fitness into the population. To test this, further studies examining the relative fitness of plants of different size categories would be needed on plants in situ at different sites.

Progenitor-derivative Hypothesis

ISSR data do not provide evidence for a progenitor-derivative relationship (Gottlieb 1973a, 1973b, 1974) between the 2 varieties of *Eriogonum shockleyi*. If such a relationship existed, we would expect levels of genetic variation in *E. shockleyi* var. *packardae* to comprise a subset of the variation found in *E. shockleyi* var. *shockleyi*. Instead, variation was found to be approximately equal and mixed across all populations sampled in this analysis. There was only a single locus that was present in *E. shockleyi* var. *shockleyi* that was absent in *E. shockleyi* var. *packardae*. In contrast, 6 loci were unique to *E. shockleyi* var. *packardae*; however, these were restricted to single populations and were present in only 1–6 individuals within those populations. Four of the 6 loci were found within 1 population (SR). The rare alleles found in this study are most likely the result of limited sample size. Greater sampling would likely find these loci in additional populations.

Genetic Diversity Among Populations

Levels of genetic variation within rare or geographically restricted plant species are typically low (Ledig and Conkle 1983, Waller et al. 1987, Lesica et al. 1988, Hickey et al. 1991), although several recent studies have indicated high levels of genetic variability in some rare species (Gottlieb et al. 1985, Karron 1987, Ranker 1994, Lewis and Crawford 1995, Smith and Pham 1996, Hickerson and Wolf 1998, Archibald et al. 2001). Within this study, the level of variation as estimated by proportion of ISSR loci polymorphic is approximately equal for populations of both *Eriogonum shockleyi* vars. *shockleyi* and *packardae*. Populations of *E. shockleyi* var. *shockleyi* have both the highest (PB) and lowest (SSI) levels. Because of this, the 2 varieties cannot be separated based on their levels of ISSR loci polymorphic per population.

Conservation Implications

Results of this study indicate that the varieties of *Eriogonum shockleyi* in Idaho are not distinct from each other based on genetic or morphological data. Morphological variation that exists may be the result of environmental

differences or, potentially, could be selection in process. Prior to any changes in conservation status of these populations, important information needs to be gathered. First, it is imperative that these populations be compared to others of *E. shockleyi* from throughout the range of the species. It may be that although all Idaho populations are similar, they are distinct from the remainder of the species, and these limited populations in Idaho merit attention. Likewise, even if the Idaho populations do not merit any taxonomic status, their geographic location at the northern limits of the species' range implies that unique loci or alleles may be present and should be accounted for in conservation programs. Second, common garden experiments should be performed on the Idaho populations to determine if, and which, environmental parameters may be affecting morphological variability. If populations remain distinct under common environmental conditions, then it is clear that there is selection occurring among these populations and that they merit conservation status.

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