

## GENETIC DIVERSITY OF THE NARROW ENDEMIC *ASTRAGALUS ONICIFORMIS* (FABACEAE)<sup>1</sup>

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*Astragalus oniciformis* Barneby is a narrow endemic xerophyte of the upper Snake River Plain of central Idaho, USA, where it inhabits stabilized, aeolian sand deposits and previously burned, sandy sites over Quaternary basalt flows. The objective of this study was to determine the levels and distribution of genetic differentiation within and among populations of *A. oniciformis*. Fifteen individuals from each of eight populations, chosen from throughout the range of the species, were selected based on accessibility, density of individuals, and large population size. Inter-simple sequence repeats were chosen as the marker to assess genetic differentiation. The two primers selected yielded 40 polymorphic loci in *A. oniciformis*. In an analysis of molecular variance, 88.69% of the variation was significantly attributed to variation within populations. High gene flow ( $N_m = 3.91\text{--}3.93$ ;  $SD = 0.01$ ) and a low percentage deviation from Hardy-Weinberg equilibrium due to population subdivision ( $G_{ST} = 0.113\text{--}0.1134$ ;  $SD = 0.0002$ ) were found among sampled populations. These results suggest that current threats to this species, including changing fire patterns and habitat loss from grazing disturbance, have not yet affected the genetic diversity of this species. Preservation of large populations and smaller, intervening, dispersed patches will help preserve the genetic integrity and the genetic diversity found in *A. oniciformis*.

**Key words:** *Astragalus*; conservation; endemic; Fabaceae; genetic diversity; Idaho, USA; ISSR.

*Astragalus oniciformis* Barneby was first collected by H. D. Ripley and R. C. Barneby in 1947 in the foothills of the Sawtooth Range on the eastern edge of the town of Picabo, Idaho, USA (Barneby, 1964). For 30 years, this single site remained the only known occurrence of this species. In the 1970s and later in the early 1980s, the Shoshone Field Office Bureau of Land Management (BLM) employees completed surveys that identified five additional populations. During a survey by Packard and Smithman in 1984, 19 new populations were discovered. Their report was the first comprehensive survey conducted for this species (Moseley and Popovich, 1995). Thirty-six populations of *A. oniciformis* were investigated in 1994. Eleven were newly discovered by Moseley and Popovich, and three from the 1984 survey were not relocated. Population sizes ranged from 10 to >10 000 individuals. Their report remains the most comprehensive inventory and natural history study of this species (Moseley and Popovich, 1995).

Known populations of *A. oniciformis* are spread throughout Lincoln, northern Minidoka, and southern Blaine Counties, Idaho. However, in the eastern portion of its range, several populations are separated from the central populations by the Minidoka Flow, an inhospitable, 12.8 km wide, basaltic lava flow. This flow has been dated at 3600 yr (Moseley and Popovich, 1995) and is too young for erosional or depositional processes to form suitable habitat for *A. oniciformis*. In the western portion of its range, two populations, located 9.3–9.6 km west of Shoshone, have not been relocated and their current status is unknown (Moseley and Popovich, 1995).

*Astragalus oniciformis* is a prostrate, caulescent perennial herb that establishes in sandy areas (often disturbed) or sandy,

aeolian pockets on basaltic lava flows (Barneby, 1964). Throughout its range, it occurs with *Artemisia tridentata* Nutt. var. *wyomingensis* (Beetle & Young) Welsh and *Hesperostipa comata* (Trin. & Rupr.) Barkworth. *Astragalus oniciformis* is a short-lived perennial with populations that can vary dramatically between droughts and cool, wet, prolonged growing seasons. Spikes of recruitment followed by population decline make managing this species difficult. It prefers open, stabilized, sandy pockets (often previously burned), but it has never been found in unstable sand dune environments (Moseley and Popovich, 1995; Popovich and Pyke, 1995). These pockets overlie extensive basaltic lava flows. In the eastern portion of its range, *A. oniciformis* populations are found in aeolian deposits on and surrounded by basalt flows ranging in age from 3600 to 12 000 yr. These flows originated in the Craters of the Moon Lava Field (Moseley and Popovich, 1995).

Habitat fragmentation and disturbance due to rangeland improvements can have major impacts on populations of *A. oniciformis*. The type and frequency of disturbance has had different effects on this taxon (Popovich and Pyke, 1995). The long-term impacts of grazing have not been investigated; however, moderate grazing levels may not be detrimental. In a study assessing mortality after heavy trampling by livestock, initial direct damage to plants was high, but a majority appeared to recover during the next growing season, perhaps due to the heavy tap roots and low caudices characteristic of *A. oniciformis*. Recruitment at trampled sites was not different from that of untrampled areas nearby (S. Popovich and D. Pyke, United States Geological Survey Biological Resources Division [USGS-BRD], unpublished data).

In general, *A. oniciformis* can persist in areas that have been revegetated after fire by drill or aerial seeding to non-native grasses. These forms of range improvements are lighter disturbances than others that heavily disturb or “plow” the soil surface using an offset-disk range-plow. When compared to adjacent untreated areas over a two- or three-year period, *A. oniciformis* density and reproduction at revegetated sites using drill or aerial seedings were similar. However, the long-term effects of these range improvement techniques on *A. onicifor-*

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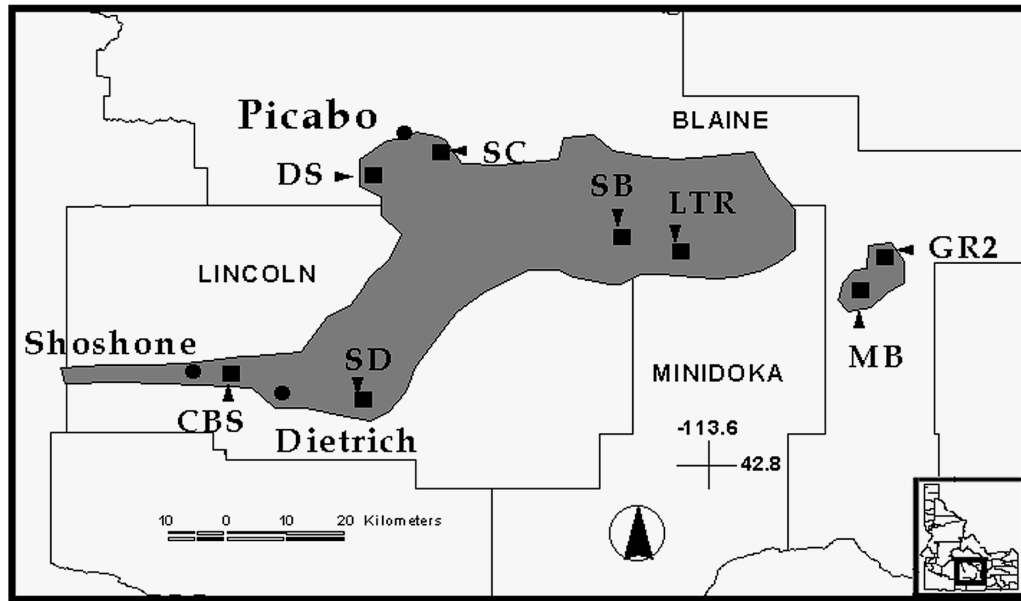


Fig. 1. A map of the known distribution of *Astragalus oniciformis* (shaded) and the locations of sampled populations (squares) with abbreviations used in this study. Extent of the range of *A. oniciformis* was based on data from Moseley and Popovich (1995). See Table 1 for explanations of population abbreviations.

*mis* recruitment and persistence have not been investigated (Popovich and Pyke, 1995; S. Popovich and D. Pyke, USGS-BRD, unpublished data).

Inter-simple sequence repeat (ISSR) markers were selected to determine the levels and distribution of genetic differentiation among populations of *A. oniciformis*. The ISSR markers have recently become widely used in population studies because they are highly variable; require less investment in time, money, and labor than other methods (Wolfe and Liston, 1998; Harris, 1999); and exhibit Mendelian inheritance (Gupta et al., 1994; Tsumura et al., 1996).

The ISSRs can generate higher percentages of polymorphic loci than other methods (Esselman et al., 1999). Differences in levels of polymorphism can exist between ISSR and allozyme data (Esselman et al., 1999), cpDNA restriction site analyses (King and Ferris, 2000), random amplified polymorphic DNA (RAPD) data (Jain et al., 1999), and amplified fragment-length polymorphism (AFLP) data (Arcade et al., 2000). These can result in different estimates of diversity. Despite this, ISSRs have been as reliable and genetically informative as RFLP analyses (Nagaoka and Ogihara, 1997; Huang and Sun, 2000).

Typically ISSRs have been used in studies of cultivated species to produce genetic linkage maps (Kojima et al., 1998; Cekic et al., 2001) and to determine the relatedness of lines of agriculturally important species (de Oliveira et al., 1996; Jain et al., 1999; Chowdhury et al., 2002; Mondal, 2002). The ISSRs have also been instrumental in determining variability and correcting misidentifications in large germplasm collections (Fang et al., 1997; Gilbert et al., 1999; Lanham and Brennan, 1999; Charters and Wilkinson, 2000).

The ISSRs have also been used to determine the genetic diversity of species of conservation concern (Esselman et al., 1999; Camacho and Liston, 2001; McGlaughlin et al., 2002; Smith and Bateman, 2002), the origin of rare endemic taxa such as *Sophora toromiro* Skottsb. (Fabaceae; Maunder et al., 1999), and the closest native species related to *Ipomoea ba-*

*tatas* (L.) Lam. (Convolvulaceae; sweet potato; Huang and Sun, 2000). In other studies, ISSRs have been successful in distinguishing between subspecies of *Plantago major* L. (Plantaginaceae), a cosmopolitan species (Wolff and Morgan-Richards, 1998), and in determining the levels of genetic variation between sympatric species of *Alnus* (Betulaceae) in Italy (King and Ferris, 2000).

Several population-level studies have also used ISSR markers. In *Calamagrostis porteri* A. Gray subsp. *insperata* (Swallen) C. W. Greene (Poaceae), moderate genetic differentiation was found among populations (Esselman et al., 1999). Comparatively, ISSR variability showed little genetic differentiation between populations of the rare variety *Eriogonum shockleyi* S. Watson var. *packardae* Reveal and the widespread var. *shockleyi* (Smith and Bateman, 2002) and among populations of the self-fertilizing species, *Botrychium pumicola* Coville (Camacho and Liston, 2001). In another study involving the rare taxon *Abronia umbellata* Lam. subsp. *breviflora* (Standl.) Munz (Nyctaginaceae), ISSR markers were useful in determining the genetic diversity of reintroduced Oregon populations (McGlaughlin et al., 2002).

In this study, populations of *A. oniciformis* and *A. mulfordae* were sampled (1) to test the reliability of ISSR markers in populations of *A. oniciformis* and their utility among closely related species, (2) to examine the levels and distribution of intra- and interpopulational genetic variation of *A. oniciformis*, (3) to determine if several thousand years of geographic isolation induced significant genetic differentiation in the populations isolated by the rift lava flow (rift populations), and (4) to recommend conservation measures, based on results of the genetic analyses, that preserve the genetic integrity and diversity of this narrow endemic *Astragalus*.

## MATERIALS AND METHODS

Eight of the 36 known populations of *A. oniciformis* were sampled in the late spring of 1999 (Fig. 1; Table 1). The two largest and most accessible

TABLE 1. Population descriptions of all sampled populations of *Astragalus oniciformis* and *A. mulfordae* (SHS). Site names, locations, and population sizes were taken from inventory data in Moseley and Popovich (1995) for *A. oniciformis* only. Coordinate data were either converted from township, range, and section coordinates listed in Moseley and Popovich (1995) or recorded in the field with a commercial global positioning system unit (\*).

Population	Site name	County, State	Latitude	Longitude	Size
CBS	Crater Butte SW	Lincoln, Idaho	42.9446	-114.3182	200
DS	Ditch Spring	Blaine, Idaho	43.2472*	-114.1197*	100
GR2	Great Rift #2	Blaine, Idaho	43.1156	-113.3318	1100
LTR	Lower Thumb Reservoir	Minidoka, Idaho	43.1208*	-113.6465*	~10 000
MB	Mule Butte	Blaine, Idaho	43.0721	-113.3500	~10 000
SB	Squaw Butte	Lincoln, Idaho	43.1426*	-113.7400*	~10 000
SC	Silver Creek	Blaine, Idaho	43.2789	-114.0206	300
SD	Sand Dunes	Lincoln, Idaho	42.8880*	-114.1329*	50
SHS	Snively Hot Springs	Malheur, Oregon	43.7189	-117.1923	~1000

populations in each geographic region were selected. Distances between population pairs range from five to 16 km. Genetic data from this study will provide a baseline for future, more intensive investigations.

A population of *A. mulfordae* in Malheur County, Oregon (SHS) was also sampled during the original survey (Table 1). This taxon is a closely related species distributed from southeastern Oregon to southwestern Idaho and is not known to be sympatric with *A. oniciformis* (Barneby, 1964).

Fifteen individuals from each population, for a total of 135 samples, were used for ISSR analyses. Individuals were randomly sampled from throughout the known geographic extent of each population. Additionally, individuals were sampled at least 10 m apart to minimize sampling of progeny. Between 20 and 80 mg of leaf material was collected from each individual. Leaf samples were air dried and then stored at -20°C until the DNA was extracted. Genomic DNA was extracted and purified using the DNeasy plant mini kit (QIAGEN, Chatsworth, California, USA). The ISSR reactions and polymerase chain reaction (PCR) protocols followed Camacho and Liston (2001). The PCR products were analyzed in 1.5% agarose gels and stained in an ethidium bromide solution on an orbital shaker.

Two samples from a single population were used for initial primer screening. Band sizes were estimated using a 100-base pair (bp) ladder (New England Biolabs, Beverly, Massachusetts, USA). Loci were named based on the primer used and estimated band size. Duplicate reactions were run for all ISSR analyses to determine the reproducibility of banding patterns. All genetic analyses were run on both primers separately and together to determine the contribution of each to the combined genetic results. Loci of band size lower than 400 bp were eliminated due to having an incomplete data set of these loci. Electrophoretic runs as long as 12 h at 70 V were needed to resolve these bands. Many of the first gels were run for only 3–3.5 h at 130 V. The longer runs were instituted so bands within 25 bp of each other would separate further to make scoring easier.

Bands were scored as present or absent (Fig. 2). The number of polymorphic loci, measures of the distribution of genetic diversity, Nei's genetic identity ( $h$ ; 1973), and Shannon index of phenotypic diversity ( $I$ ; King and Schaal, 1989) were computed with PopGene32 (Yeh et al., 2000), assuming all loci were dominant and in Hardy-Weinberg equilibrium. An unbiased genetic identity matrix (Nei, 1978) was generated by PopGene32 and used to create unweighted pair-group method arithmetic average (UPGMA) and neighbor-joining (NJ) dendrograms using NTSYSpc 2.02 (Rohlf, 1997). The deviation from Hardy-Weinberg expectations due to population subdivision ( $F_{ST}$ ) and other estimates of population-level genetic differentiation were determined using a hierarchical analysis of molecular variance (AMOVA; Excoffier et al., 1992) computed with Arlequin 1.1 (Schneider et al., 1997), which assumes these data are haplotypic. For the estimation of genetic distances between populations, a pairwise population  $F_{ST}$  matrix was generated by Arlequin 1.1, and an unbiased genetic distance matrix (Nei, 1978) was generated by PopGene32.

Geographic distances were measured based on the latitude and longitude coordinates of the populations of *A. oniciformis*. Coordinates were obtained for four populations (DS, SD, LTR, and SB) using a Magellan (San Dimas, California, USA) Trailblazer XL commercial global positioning system (GPS)

unit. Coordinates for the other populations were converted from township, range, and section (TRS) data obtained from Moseley and Popovich (1995) to latitude and longitude using TRS-data (<http://WWW.esg.montana.edu/gl/trs-data.html>). Population coordinates were then imported as a point Shapefile into ArcView GIS 3.2 (Environmental Systems Research Institute, Redlands, California, USA), and geographic distances between populations were measured within a View. The population pairwise  $F_{ST}$  matrix, Nei's unbiased genetic distance matrix, and geographic distance matrix were used in NTSYSpc 2.02 for the Mantel tests to determine if population level genetic distances and geographic distance were correlated.

Correlation between the pairwise  $F_{ST}$  and unbiased genetic distance matrices, between the pairwise  $F_{ST}$  and unbiased genetic identity matrices, and between Nei diversity ( $h$ ) and Shannon diversity ( $I$ ) were conducted in SYSTAT for Windows version 6.0 (SPSS, Chicago, Illinois, USA). All  $P$  values in statistical analyses were significant at the <0.05 level.

Estimation bias can lead to the overestimation of parameters by as much as 5% in the dominant marker data produced by RAPD and ISSR analysis (Lynch and Milligan, 1994). To reduce this bias, Lynch and Milligan proposed pruning any locus with a band frequency of higher than  $1 - (3/N)$ , where  $N$  is the number of individuals sampled. This pruning procedure was implemented with the *A. oniciformis* data set prior to the genetic analyses. Loci were tested for significant single population linkage disequilibria (Weir, 1979) and Ohta's two-locus analysis of population subdivision (1982a, b; both tests for linkage disequilibria) using PopGene32.

## RESULTS

From an initial analysis of 100 University of British Columbia (UBC) ISSR primers, the presence of multiple bands was found in 27 primers. From the subset of primers that produced multiple bands, reactions using two individuals from two populations were run to test for band reproducibility. Eight primers had bands that had a high degree of reproducibility. These eight primers were then run with a single individual from each of four populations with replicates. Two primers were then selected for the genetic analyses, UBC-818 ([CA]<sub>8</sub>G) and UBC-841 ([GA]<sub>8</sub>YC; Y = C or T). These two primers produced multiple, clear, and reproducible bands that had a degree of heterogeneity across populations (Fig. 2). These primers produced nearly identical estimates of genetic diversity despite their different banding patterns (UBC-841 had fewer total bands, many of which were nearly monomorphic; see later). Analyzing additional would be unlikely to provide discordant data. In *A. oniciformis*, UBC-818 yielded 28 putative loci, all of which were polymorphic. UBC-841 yielded 12 putative loci, all of which were polymorphic (Table 2). Twenty-three loci were present only in *A. oniciformis*, 18 from UBC-818



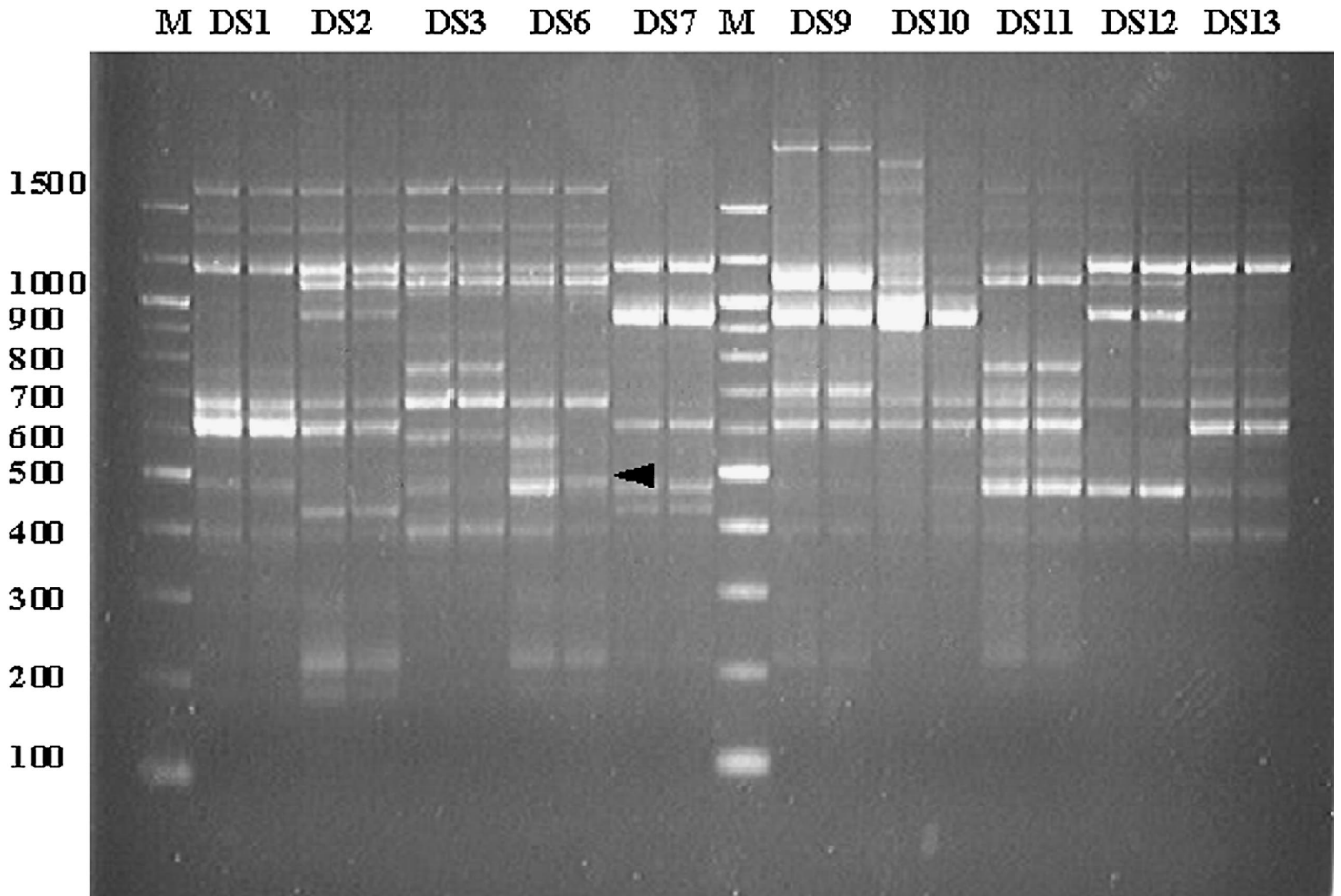


Fig. 2. Photograph of ISSR gel showing bands from DS population of *Astragalus oniciformis* from primer UBC-818. Each lane is the result of an independent PCR reaction. M is a 100-bp ladder. All scored bands were observed in at least two independent reactions. In the case of variable bands within a replicate (DS6; see arrow), at least two other independent reactions were run to test band reproducibility.

(frequency = 0.0042–0.3675), and five from UBC-841 (frequency = 0.0042–0.0710).

In *A. mulfordae*, UBC-818 yielded 11 putative loci, all of which were polymorphic except 818–400. UBC-841 yielded 12 putative loci, all of which were polymorphic. Six loci were present only in *A. mulfordae*, one from UBC-818 (frequency = 0.0339) and five from UBC-841 (frequency = 0.0339–0.2697).

Significant linkage disequilibria occurred between locus 841–775 and locus 841–475 in *A. oniciformis*. Weir’s significant single population linkage disequilibria (1979) and Ohta’s two-locus analysis of population subdivision (1982a, b) showed significant linkage between these two loci. Based on these results, locus 841–775 was deleted from all genetic anal-

yses. Loci 841–775 and 841–475 were not linked in *A. mulfordae*.

For a locus to be pruned according to Lynch and Milligan (1994) in the *A. oniciformis* ISSR data ( $N = 120$ ), the frequency of the band had to be 0.975 or higher. Because no locus was present at a frequency higher than 0.88, the Lynch and Mulligan pruning procedure was not implemented.

The number of polymorphic loci within each population and their diversity indices varied depending upon whether the primers were analyzed separately or together. In a combined analysis (Table 3), population SD had the highest number of polymorphic loci, 28 ( $h = 0.1856$ ,  $I = 0.2895$ ). Populations MB and SB were the most depauperate, with 19 and 21 polymorphic loci, respectively (MB:  $h = 0.1412$ ,  $I = 0.2182$ ; SB:

TABLE 2. Genetic diversity measures for sampled populations of *Astragalus oniciformis*. Total number of loci, number of polymorphic loci, mean expected heterozygosity within a randomly mating subpopulation ( $H_T$ ) and mean expected heterozygosity within a randomly mating population ( $H_S$ ) with their respective standard deviations (SD), mean genetic diversity among subpopulations ( $G_{ST}$ ), and mean number of individuals migrating between subpopulations per generation ( $N_m$ ) were calculated using PopGene32 (Yeh et al., 2000).

Primer	Loci	Polymorphic loci	$H_T$	SD	$H_S$	SD	$G_{ST}$	$N_m$
818	28	28	0.1899	0.03	0.1684	0.0226	0.1134	3.91
841	12	12	0.1234	0.011	0.11	0.0084	0.113	3.93
818 and 841	40	40	0.17	0.025	0.1507	0.0187	0.1133	3.91

TABLE 3. Results of single population genetic analysis of loci from sampled populations of *Astragalus oniciformis* with ISSR primers 818 and 841 using PopGene32 (Yeh et al., 2000).

Popula- tion <sup>a</sup>	No. polymorphic loci (40 loci total)	Percentage of polymorphic loci	Nei diversity ( <i>h</i> )	Shannon index ( <i>I</i> )
Rift				
GR2	23	0.575	0.1464	0.2338
MB	19	0.475	0.1412	0.2182
Western				
CBS	27	0.675	0.1689	0.2693
DS	27	0.675	0.182	0.2835
LTR	23	0.575	0.1404	0.2237
SB	21	0.525	0.1189	0.1949
SC	23	0.575	0.1222	0.2011
SD	28	0.7	0.1856	0.2895

<sup>a</sup> See Table 1 for explanations of population abbreviations.

$h = 0.1189$ ,  $I = 0.1949$ ). The analysis of primer 818 showed that DS had 23 polymorphic loci ( $h = 0.2339$ ,  $I = 0.3596$ ) and SD had 21 ( $h = 0.2043$ ,  $I = 0.3145$ ). Population LTR had the lowest, 14 polymorphic loci ( $h = 0.1396$ ,  $I = 0.2172$ ). The analysis of primer 841 showed that CBS and LTR both had nine polymorphic loci (CBS,  $h = 0.1545$ ,  $I = 0.2571$ ; LTR,  $h = 0.1421$ ,  $I = 0.239$ ). Populations DS, GR2, and MB had the lowest number of polymorphic loci, four (DS,  $h = 0.061$ ,  $I = 0.1058$ ; GR2,  $h = 0.0982$ ,  $I = 0.1512$ ; MB,  $h = 0.061$ ,  $I = 0.1058$ ).

Nei diversity ( $h$ ) and Shannon diversity ( $I$ ) were highly correlated ( $r = 0.9957$ ,  $P < 0.001$ ). Unbiased Nei distance and genetic distance as estimated by pairwise  $F_{ST}$  values were negatively correlated ( $r = -0.7751$ ,  $P < 0.001$ ) and unbiased Nei identity and the pairwise  $F_{ST}$  values were negatively correlated ( $r = -0.8275$ ,  $P < 0.001$ ). Two of the most distant populations were nearly identical: the rift population, MB, and the southwestern-most population, SD (unbiased genetic identity = 0.9902). *Astragalus mulfordae* (SHS) was the most genetically different population sampled in this study (unbiased genetic identity = 0.9544).

An NJ dendrogram of Nei's (1978) unbiased genetic identity (not shown) grouped no populations separated by less than 25 km as most similar (unbiased genetic identity = 0.9691–0.9856). The result of nearby populations not being most similar was also found in a UPGMA (not shown) dendrogram. However, the NJ and UPGMA topologies were incongruent.

AMOVA analyses of the combined 818 and 841 data (Table 4) found that 88.69% of the variation was significantly attributed to the variation within populations ( $P < 0.001$ ) and that differentiation between the rift populations and the western

populations was not significant ( $P = 0.97$ ). The results for the separate AMOVA analyses of primer 818 (88.64% of variation) and primer 841 (88.2% of variation) had nearly identical results.

The percentage deviation from Hardy-Weinberg equilibrium due to population subdivision ( $G_{ST}$ ) and estimated gene flow between subpopulations per generation of sampled *A. oniciformis* populations ( $N_m$ ) were nearly equal, whether the primers were analyzed combined or separately ( $G_{ST} = 0.1130$ – $0.1134$ ,  $N_m = 3.91$ – $3.93$ ; Table 2).

The  $G_{ST}$  values for the combined and separate analyses, are nearly identical to the  $F_{ST}$  values in the AMOVA analyses, between 0.112 and 0.118. An additional AMOVA analysis was performed on the combined *A. oniciformis* data and *A. mulfordae* data, testing whether a significant amount of variation was explained by groups of all *A. oniciformis* populations and a group of the single *A. mulfordae* population. A weakly significant ( $P = 0.1$ ) 17.34% of the variation was explained by this grouping.

A Mantel test using geographic distance and Nei's (1978) unbiased genetic distance matrix found a weakly negative correlation ( $t = -0.34809$ ,  $P = 0.0780$ ). Another Mantel test using the geographic distance matrix and pairwise  $F_{ST}$  genetic distance matrix found that they are not significantly correlated ( $t = -0.27905$ ,  $P = 0.1135$ ).

## DISCUSSION

Expression as dominant markers, homology assumptions, variable band intensity, and minor deviations in experimental protocols yielding different results are limitations shared by the RAPD and ISSR methods (Wolfe and Liston, 1998; Harris, 1999). The reliability of dominant marker techniques at detecting genetic diversity among closely related species and among populations of the same species has been criticized (Harris, 1999; Isabel et al., 1999). Dowling et al. (1996) and Harris (1999) suggest that for population genetic studies, marker characterization studies involving crossing experiments combined with Southern blotting or restriction enzyme tests of RAPD products are necessary to demonstrate the accuracy and lack of bias in dominant genetic marker data. Harris (1999) also suggested that RAPD data may also be inaccurate due to different protocols used to develop and select primers and to score and analyze data, which make comparisons between different RAPD studies difficult unless they were performed by the same researchers under identical conditions. Some of these problems can be attributed to the characteristics of RAPD primers. The typically GC-rich RAPD primers have a higher level of primer site overlap or nested priming due to lower annealing temperatures (Harris, 1999). The ISSRs are

TABLE 4. Genetic diversity estimates (total deviation from Hardy-Weinberg expectations, [ $F_{CT}$ ], among-population deviations from Hardy-Weinberg expectations [ $F_{SC}$ ], deviation from Hardy-Weinberg expectations due to population subdivision [ $F_{ST}$ ], and their respective degrees of freedom [df], sums of squares, variance components, percentages of variation, and  $P$  values) resulting from an AMOVA pairwise distance analysis of loci sampled from populations of *Astragalus oniciformis* using ISSR primers 818 and 841. Two groups, the rift populations (GR2 and MB) and the western populations (CBS, DS, LTR, SB, SC, and SD), were tested in the genetic structure analysis. Loci were analyzed with Arlequin (Schneider et al., 1997). See Table 1 for explanations of population abbreviations.

Comparison	df	Sum of squares	Variance	Variation (%)	$F$ statistics	$P$
Between groups	1	6.97	-0.16	-3.5	$F_{CT} = -0.035$	0.97
Among populations/groups	6	84.67	0.67	14.81	$F_{SC} = 0.143$	0
Within populations	112	450.93	4.03	88.69	$F_{ST} = 0.113$	0
Total	119	542.57	4.54			

more robust because the longer anchored primers have higher annealing temperatures, increasing the reproducibility of ISSR products (Tsumura et al., 1996).

Especially in studies with small sample sizes, dominant markers can lead to parameter estimation bias (Lynch and Milligan, 1994; Isabel et al., 1999). This bias cannot be corrected by increasing either the number of populations or the number of markers sampled (Isabel et al., 1999). Pruning of dominant marker loci with high frequency proved to be the most effective means of reducing parameter estimation bias (Lynch and Milligan, 1994; Isabel et al., 1999). Parameter estimation bias can be reduced by the Lynch and Milligan (1994) test, and because no bands in the *A. oniciformis* data were present at a high enough frequency to be pruned, this bias in these data is low. Also, the AMOVA procedure in Arlequin assumes the data are codominant and haplotypic. The ISSR data could cause bias in parameter estimation because ISSR markers are dominant and not haplotypic. In addition to the identical  $F_{ST}$  values and  $G_{ST}$  values, the topology of the UPGMA dendrograms of the pairwise  $F_{ST}$  and Nei's unbiased genetic distance were nearly identical, differing only in branch lengths and the grouping of the SB and SC populations. The significant correlation between the pairwise  $F_{ST}$  values (calculated by NTSYSpc) and the Nei's unbiased genetic distance values (calculated by PopGene32) is further evidence that bias was low in these data.

Violation of assumptions of software algorithms can also lead to inaccurate results. The analyses used in PopGene32 assume that all data are in Hardy-Weinberg equilibrium. Because no codominant ISSR locus was found and codominant data do not exist for *A. oniciformis*, the assumption that the data meet Hardy-Weinberg expectations could not be tested. The nearly identical  $F_{ST}$  values from the AMOVA analysis and the  $G_{ST}$  values from the PopGene32 analysis are evidence that potential problems arising from the violation of certain assumptions of the software did not occur. Inter-simple Sequence repeat studies generally use the same software algorithms and methodologies used in this study, but the ISSR data in very few of these studies are tested to determine the extent to which the potential violations bias the results. Multiple tests have been performed on our data, and bias has been found to be low.

Because RAPD and ISSR markers are sampled randomly from throughout the genome including nuclear and organellar DNA, these can produce problematic data sets due to the different population genetic histories of these regions (Harris, 1999). In this study, two different primers were analyzed. The two ISSR primers (UBC-818, which had a highly polymorphic banding pattern, and UBC-841, which had a more monomorphic banding pattern) produced, in separate and combined analyses, nearly identical results. These results were verified in the AMOVA analyses, indicating this also was not a problem in these data. Overall, the data from populations of *A. oniciformis* demonstrate the robustness of ISSR markers.

Studies of other species of *Astragalus* using genetic methods such as isozymes, AFLPs, or RAPDs have yielded similar results as ISSR markers have in *A. oniciformis* and *A. mulfordae*. In an isozyme study among populations of various species of annual *Astragalus*, Liston (1992) found that Nei's genetic identity did not fall below 0.961. The tight range of genetic identities (0.97–0.99) found in *A. oniciformis* with ISSR markers is on the high end of the range of values reported by Liston (1992).

The genetic identity of 0.95 between *A. mulfordae* and *A. oniciformis* is not unusual. Liston (1992) found a genetic identity of 0.937 between *A. breweri* A. Gray, a species native to serpentine outcrops in the Coast Range of California, and *A. tener* A. Gray var. *titi* (Eastw.) Barneby, which is found in a single population on the Monterey Peninsula of California (Liston, 1992). Like *A. mulfordae* and *A. oniciformis*, these two annual species are not sympatric. The genetic identity obtained in this study between *A. mulfordae* and *A. oniciformis* is likely to be inaccurate, because only one population was sampled. A more thorough sampling of populations of *A. mulfordae* will potentially provide additional loci in *A. mulfordae* that are currently only present in *A. oniciformis*, as well as additional loci unique to *A. mulfordae*.

Geologic features and habitat restrictions have been documented as instrumental in increasing population differentiation in species with limited distributions (Travis et al., 1996). In *Astragalus cremnophylax* Barneby, a species native to Kaibab Limestone outcrops on the North Rim and South Rim of the Grand Canyon, genetic differentiation overall among the populations is high,  $\theta$  (an equivalent of  $F_{ST}$ ) = 0.44 (Travis et al., 1996), compared to *A. oniciformis*  $G_{ST}$  = 0.113. Gene flow ( $N_m$ ) is limited for *A. cremnophylax*, between 0.2 and 0.4 migrants per generation. Gene flow has been proposed only to occur through pollinators because geographic barriers (the Grand Canyon) and habitat barriers (16 km of dense vegetation) prevent seed dispersal. The population sizes of *A. cremnophylax* ranged from two to 970 individuals, which makes this species extremely vulnerable to fluctuations in climate and habitat disturbance (Travis et al., 1996). *Astragalus oniciformis* has a much wider, continuous distribution (over 80 km), larger population sizes (10 to >10 000) individuals, and higher estimates of gene flow ( $N_m$  = 3.91–3.93). The lack of genetic differentiation among populations, especially when compared to *A. cremnophylax*, is also evidence of potential gene flow throughout the range of this species.

A wide range of  $G_{ST}$  and  $F_{ST}$  values have been obtained in studies of species of *Astragalus* (Table 5). Liston (1992) found that within annual species,  $G_{ST}$  values ranged from 0 to 0.725. The highest values were found in *A. pauperculus* Greene (0.775) and *A. clarianus* Jeps. (0.331), two species with narrow distributions in cismontane California. The widespread species had  $G_{ST}$  values between 0 and 0.254. The highest  $F_{ST}$  values in Karron et al. (1988) were found in *A. osterhouti* ( $F_{ST}$  = 0.14), which in 1988 had a total of 1500 individuals restricted to three populations. Comparatively, *A. pectinatus* Douglas ex G. Don ( $F_{ST}$  = 0.02 and 0.05) and *A. pattersoni* A. Gray ex Brandegee ( $F_{ST}$  = 0.01) are widespread species with lower levels of genetic differentiation than observed in this study (Karron et al., 1988). A  $\theta$  estimate of 0.44, found in *A. cremnophylax* (Travis et al., 1996), is additional support that in *Astragalus*, genetic differentiation and possibly speciation can occur when population size decreases, gene flow decreases, and genetic differentiation among populations increases in endemic species with narrow distributions. Although *A. oniciformis* has a relatively narrow distribution, its large population sizes, numerous occurrences, and evidently high gene flow among populations has historically resulted in a low potential for genetic differentiation.

A number of factors about the characteristics of the sampled populations could have had an impact on the genetic analyses performed in this study. The type locality, located near the eastern city limits of Picabo, was not sampled due to the de-



TABLE 5. Mean population  $F_{ST}$  (deviation from Hardy-Weinberg expectations due to population subdivision), mean population  $G_{ST}$  (deviation from Hardy-Weinberg expectations due to population subdivision for multiple alleles at a locus), and population sizes from studies of narrow endemic species of *Astragalus*. Population sizes were not reported for widespread species. Unless otherwise stated, population data were obtained from Liston (1990, 1992). Citation 2: Travis et al. (1996). Citation 3: Karron et al. (1988). Citation 4: Moseley and Popovich (1995).

Narrow species	Mean $F_{ST}/G_{ST}$	Population size	Widespread species	Mean $F_{ST}/G_{ST}$	Citation
<i>A. breweri</i>	0.000 ( $G_{ST}$ )	10–700	<i>A. nothoxys</i>	0.000 ( $G_{ST}$ )	
<i>A. clarianus</i>	0.331 ( $G_{ST}$ )	<100	<i>A. acutirostris</i>	0.254 ( $G_{ST}$ )	
<i>A. tener</i> var. <i>tener</i>	0.059 ( $G_{ST}$ )	50–250	<i>A. acutirostris</i>	0.254 ( $G_{ST}$ )	
<i>A. tener</i> var. <i>titi</i>	0.000 ( $G_{ST}$ )	<25	<i>A. gambelianus</i>	0.211 ( $G_{ST}$ )	
<i>A. rattanii</i> var. <i>jepsonianus</i>	0.053 ( $G_{ST}$ )	25–50	<i>A. nuttallianus</i>	0.000 ( $G_{ST}$ )	
<i>A. pauperculus</i>	0.725 ( $G_{ST}$ )	25			
<i>A. rattanii</i> var. <i>rattanii</i>	0.068 ( $G_{ST}$ )	100–1000			
<i>A. cremnophylax</i>	0.440 ( $F_{ST}$ )	3–970			2
<i>A. linifolius</i>	0.055 ( $F_{ST}$ )	3000	<i>A. pattersoni</i>	0.010 ( $F_{ST}$ )	3
<i>A. osterhouti</i>	0.140 ( $F_{ST}$ )	1500	<i>A. pectinatus</i>	0.035 ( $F_{ST}$ )	3
<i>A. oniciformis</i>	0.113 ( $G_{ST}$ )	100–10 000+			4

pauperate condition of the populations. Population SC was the nearest population to the type locality of sufficient size to be sampled. The observed habitat fragmentation at the type locality is likely to have some effect on the genetic differentiation of that population. Population SC was located in a small undisturbed patch of *Artemisia* between several large private farms. However, as recently as the 1960s, the entire area was typified by large expanses of sagebrush. Even though habitat fragmentation over the past 40 yr does not seem to have affected this species genetically (see discussion of populations MB and GR2 later), habitat fragmentation and low population size have the potential to significantly affect the levels of genetic differentiation among populations over longer periods (Travis et al., 1996). If decreasing population size and habitat fragmentation continue within the northern range of this species, mainly in the populations around Picabo and Silver Creek, the combination may lead to genetic differentiation among these populations and populations throughout the range of the species.

The rift populations, GR2 and MB, are separated from all other populations by the 12.8 km wide, inhospitable Minidoka Flow (Moseley and Popovich, 1995). The lack of genetic differentiation between these two populations and the western populations provides evidence that in *A. oniciformis*, either the rift populations are the result of two or more recent dispersal events or 3600 yr of separation has not caused significant genetic differentiation between the rift and western populations. Su et al. (2003) found significant genetic differentiation between subpopulations of several insect-pollinated perennial taxa after only 600 yr of isolation caused by the Great Wall of China. The rift populations of *A. oniciformis* may not have been completely genetically isolated. Two or more dispersal events have likely occurred, because MB and GR have different levels of polymorphic loci, are not grouped as being similar in the UPGMA or NJ dendrograms, and have a genetic identity of 0.9824, a value in the middle of the range for this species.

Gene flow across this inhospitable boundary has not been completely ruled out because the life histories of the pollinators of *A. oniciformis* have not been studied (S. Popovich and D. Pyke, USGS-BRD, unpublished data). Popovich and Pyke (1995) found two rare pollinators on *A. oniciformis*: *Andrena nigerrima*, a species not previously reported from west of the Rocky Mountains, and *Calliopsis barri*, a narrow endemic known only known from a few sites in the northern Great

Basin and Columbia Plateau. These pollinators nest exclusively in lava flows and are sensitive to habitat modifications that lead to the loss of nesting microsites. Many of the largest and most genetically diverse populations of *A. oniciformis* are found in the vicinity of the Minidoka basalt flow. The smallest populations of *A. oniciformis* are far from even the smallest lava flows, suggesting a correlation between the pollinators and *A. oniciformis* that warrants further investigation (Popovich and Pyke, 1995; S. Popovich and D. Pyke, USGS-BRD, unpublished data).

The lack of genetic differentiation among populations and the high level of gene flow within the range of *A. oniciformis* indicate that current threats to this species, plant community changes in the last 60 yr due to changing fire patterns, habitat alteration due to livestock grazing, and habitat loss due to past rangeland improvements (Moseley and Popovich, 1995), have not affected the genetic diversity of this species in the short term. Genetic differentiation has not occurred despite these disturbances because of the high gene flow and the numerous, large populations characteristic of *A. oniciformis*. In addition, the seed bank for *A. oniciformis* can be potentially large (D. Pyke, USGS-BRD, personal communication), so if genetic differentiation were to occur, it could be several generations before genetic drift is detectable. The techniques in this study were not sensitive enough to detect any recent genetic drift in populations of *A. oniciformis*. Conserving the numerous, large populations throughout the range of this species and the smaller intervening patches would be one strategy that would help preserve the high gene flow among populations.

The populations located at Picabo (see Fig. 1) near the type locality and the populations along Silver Creek should not be selected as a seed source for habitat restoration or enhancement projects for other populations throughout the range of *A. oniciformis*. The low levels of polymorphism, low population sizes, and the higher potential for future genetic differentiation make these populations poor candidates. Populations within the continuous central and western range of this species are the best candidates for restoration and enhancement efforts. Any reintroduction efforts will require monitoring techniques similar to those employed by McGlaughlin et al. (2002) to ensure that the new populations retain the genetic diversity of the founder populations.

Complete conversion of rangeland to non-native grass mixtures without native islands may have detrimental effects on this species by altering potential habitat and creating limits to

gene flow. In one study comparing recruitment and persistence of *A. oniciformis* between disk-plowed and adjacent native sites, preliminary monitoring data show that overall retention of *A. oniciformis* was substantially greater in the native untreated sites compared to the plowed sites. This was due in part to high rates of initial adult mortality and lower rates of seedling survivorship in the disk-plowed sites. *Astragalus oniciformis* responds negatively to range conversion practices such as offset-disk range-plowing techniques that disturb the soil horizons with the objective to destroy and replace native vegetation (Popovich and Pyke, 1995). Such techniques should be discouraged in general within the range of *A. oniciformis* and especially in the studied populations with high genetic diversity.

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