GENETIC DIVERSITY OF THE NARROW ENDEMIC 
Astragalus oniciformis (Fabaceae)1

J. ANDREW ALEXANDER, AARON LISTON, AND STEVE J. POPOVICH2

Department of Botany and Plant Pathology, Oregon State University, Corvallis, Oregon 97331 USA

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-
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 Ogiwara, 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 Skottsbg. (Fabaceae; Maunder et al., 1999), and the closest native species related to Ipomoea batatas (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
populations in each geographic region were selected. Distances between popu-
lation 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
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 sam-

cles 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 screen-
ing. Band sizes were estimated using a 100-base pair (bp) ladder (New Eng-
land Biolabs, Beverly, Massachusetts, USA). Loci were named based on the

city, and state coordinates of the populations of *Astragalus oniciformis* (SHS).

**TABLE 1.** Population descriptions of all sampled populations of *Astragalus oniciformis* and *A. mulfordae* (SHS). Site names, locations, and
distance coordinates listed in Moseley and Popovich (1995) or recorded in the field with a commercial global
positioning system unit (*). Coordinates were obtained
from township, range, and section coordinates listed in Moseley and Popovich (1995) or recorded in the field with a commercial global
positioning system unit (*).

<table>
<thead>
<tr>
<th>Population</th>
<th>Site name</th>
<th>County, State</th>
<th>Latitude</th>
<th>Longitude</th>
<th>Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBS</td>
<td>Crater Butte SW</td>
<td>Lincoln, Idaho</td>
<td>42.9446</td>
<td>−114.3182</td>
<td>200</td>
</tr>
<tr>
<td>DS</td>
<td>Ditch Spring</td>
<td>Blaine, Idaho</td>
<td>43.2472*</td>
<td>−114.197*</td>
<td>100</td>
</tr>
<tr>
<td>GR2</td>
<td>Great Rift #2</td>
<td>Blaine, Idaho</td>
<td>43.1156</td>
<td>−113.3318</td>
<td>1100</td>
</tr>
<tr>
<td>LTR</td>
<td>Lower Thumb Reservoir</td>
<td>Minidoka, Idaho</td>
<td>43.1208*</td>
<td>−113.6465*</td>
<td>10 000</td>
</tr>
<tr>
<td>MB</td>
<td>Mule Butte</td>
<td>Blaine, Idaho</td>
<td>43.0721</td>
<td>−113.3500</td>
<td>10 000</td>
</tr>
<tr>
<td>SB</td>
<td>Squaw Butte</td>
<td>Lincoln, Idaho</td>
<td>43.1426*</td>
<td>−113.7400*</td>
<td>10 000</td>
</tr>
<tr>
<td>SC</td>
<td>Silver Creek</td>
<td>Blaine, Idaho</td>
<td>43.2789</td>
<td>−114.0206</td>
<td>300</td>
</tr>
<tr>
<td>SD</td>
<td>Sand Dunes</td>
<td>Lincoln, Idaho</td>
<td>42.8880*</td>
<td>−114.1329*</td>
<td>50</td>
</tr>
<tr>
<td>SHS</td>
<td>Snively Hot Springs</td>
<td>Malheur, Oregon</td>
<td>43.7189</td>
<td>−117.1923*</td>
<td>1000</td>
</tr>
</tbody>
</table>

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 pop-
ulations were run to test for band reproducibility. Eight prim-
ers had bands that had a high degree of reproducibility. These eight prim-
ers 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 (ICA49G) and
UBC-841 ([GA]4YC; Y = C or T). These two primers produ-
ced 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 analyses. 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 Milligan 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>
<thead>
<tr>
<th>Primer</th>
<th>Loci</th>
<th>Polymorphic loci</th>
<th><em>H</em>&lt;sub&gt;1&lt;/sub&gt;</th>
<th>SD</th>
<th><em>H</em>&lt;sub&gt;1&lt;/sub&gt;</th>
<th>SD</th>
<th><em>G</em>&lt;sub&gt;ST&lt;/sub&gt;</th>
<th><em>N</em>&lt;sub&gt;a&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>818</td>
<td>28</td>
<td>28</td>
<td>0.1899</td>
<td>0.03</td>
<td>0.1684</td>
<td>0.0226</td>
<td>0.1134</td>
<td>3.91</td>
</tr>
<tr>
<td>841</td>
<td>12</td>
<td>12</td>
<td>0.1234</td>
<td>0.011</td>
<td>0.11</td>
<td>0.0084</td>
<td>0.113</td>
<td>3.93</td>
</tr>
<tr>
<td>818 and 841</td>
<td>40</td>
<td>40</td>
<td>0.17</td>
<td>0.025</td>
<td>0.1507</td>
<td>0.0187</td>
<td>0.1133</td>
<td>3.91</td>
</tr>
</tbody>
</table>

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*<sub>1</sub>) and mean expected heterozygosity within a randomly mating population (*H*<sub>1</sub>) with their respective standard deviations (SD), mean genetic diversity among subpopulations (*G*<sub>ST</sub>), and mean number of individuals migrating between subpopulations per generation (*N*<sub>a</sub>) were calculated using PopGene32 (Yeh et al., 2000).
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.

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

* 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 (23.39, $I = 0.3596$) and SD had 21 (20.43, $I = 0.3145$). Population LTR had the lowest, 14 polymorphic loci ($I = 0.1936, I = 0.2172$). The analysis of primer 841 showed that CBS and LTR both had nine polymorphic loci (20.1545, $I = 0.2571$; 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 ($P = 0.17$) 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 (Wolfle 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...
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 organelar 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. mulfordiae*. 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. mulfordiae* 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. mulfordiae* and *A. oniciformis*, these two annual species are not sympatric. The genetic identity obtained in this study between *A. mulfordiae* and *A. oniciformis* is likely to be inaccurate, because only one population was sampled. A more thorough sampling of populations of *A. mulfordiae* will potentially provide additional loci in *A. mulfordiae* that are currently only present in *A. oniciformis*, as well as additional loci unique to *A. mulfordiae*.

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 cremnophyllum* 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. cremnophyllum*, 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. cremnophyllum* 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 >10000) 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. cremnophyllum*, 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. osterhoutii* ($F_{ST}$ = 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. pattersonii* 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. cremnophyllum* (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 Picacho, was not sampled due to the de-
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

<table>
<thead>
<tr>
<th>Narrow species</th>
<th>Mean FST/ST</th>
<th>Population size</th>
<th>Widespread species</th>
<th>Mean FST/ST</th>
<th>Citation</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. breweri</em></td>
<td>0.000</td>
<td>10–700</td>
<td><em>A. nothoxys</em></td>
<td>0.000</td>
<td>2</td>
</tr>
<tr>
<td><em>A. clarianus</em></td>
<td>0.331</td>
<td>&lt;100</td>
<td><em>A. acutirostris</em></td>
<td>0.254</td>
<td>3</td>
</tr>
<tr>
<td><em>A. tener</em></td>
<td>0.059</td>
<td>50–250</td>
<td><em>A. acutirostris</em></td>
<td>0.254</td>
<td>4</td>
</tr>
<tr>
<td><em>A. tener</em></td>
<td>0.000</td>
<td>&lt;25</td>
<td><em>A. gambelianus</em></td>
<td>0.211</td>
<td>2</td>
</tr>
<tr>
<td><em>A. rattanii</em></td>
<td>0.053</td>
<td>25–50</td>
<td><em>A. nuttallianus</em></td>
<td>0.000</td>
<td>2</td>
</tr>
<tr>
<td><em>A. pauperulus</em></td>
<td>0.752</td>
<td>100–1000</td>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td><em>A. rattanii</em></td>
<td>0.068</td>
<td></td>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td><em>A. crenophylax</em></td>
<td>0.440</td>
<td>3–970</td>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td><em>A. linifolius</em></td>
<td>0.055</td>
<td>3000</td>
<td><em>A. pattersoni</em></td>
<td>0.010</td>
<td>2</td>
</tr>
<tr>
<td><em>A. osterhouti</em></td>
<td>0.140</td>
<td>1500</td>
<td><em>A. pectinatus</em></td>
<td>0.035</td>
<td>2</td>
</tr>
<tr>
<td><em>A. oniciformis</em></td>
<td>0.113</td>
<td>100–10 000+</td>
<td></td>
<td></td>
<td>2</td>
</tr>
</tbody>
</table>
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 higher 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.

**LITERATURE CITED**


HUANG, J. C., AND M. SUN. 2000. Genetic diversity and relationships of sweetpotato and its wild relatives in *Ipomoea* series Batatas (Convolvulaceae) as revealed by inter-simple sequence repeat (ISSR) and restriction site analysis of chloroplast DNA. *Theoretical and Applied Genetics* 100: 1050–1060.


SMITH, J. F., AND J. A. BATEMAN. 2002. Genetic differentiation of rare and
common varieties of Eriogonum shockleyi (Polygonaceae) in Idaho using
Travis, S. E., J. Maschinski, and P. Keim. 1996. An analysis of genetic
variation in Astragalus cremnophylax var. cremnophylax, a critically en-
of inter-simple sequence repeat polymorphisms in Douglas-fir (Pseudotsuga menziesii) and sugi (Cryptomeria japonica). Theoretical and Ap-
plied Genetics 92: 40–45.
Weir, B. S. 1979. Inferences about linkage disequilibrium. Biometrics 35:
235–254.
to plant systematics and evolutionary biology. In P. S. Soltis, D. E. Soltis,
and J. J. Doyle [eds.], Molecular systematics of plants: DNA sequencing,
Wolff, K., and M. Morgan-Richards. 1998. PCR markers distinguish
Plantago major subspecies. Theoretical and Applied Genetics 96: 282–
286.
Yeh, F. C., R. Yang, T. J. Boyle, Z. Ye, and J. M. Xian. 2000. Pop-
gene32, Microsoft Windows-based freeware for population genetic anal-
ysis, version 1.32. Molecular Biology and Biotechnology Centre, Uni-
versity of Alberta, Edmonton, Alberta, Canada.