

# Genetic characterization of three varieties of *Astragalus lentiginosus* (Fabaceae)

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Knaus, B. J. (Oregon State University, Department of Botany and Plant Pathology, 2082 Cordley Hall, Corvallis, OR, 97331-2902, U.S.A.; e-mail: knausb@science.oregonstate.edu), R. C. Cronn (USDA Forest Service Pacific Northwest Research Station, 3200 SW Jefferson Way, Corvallis, OR, 97331, U.S.A.; e-mail: rcronn@fs.fed.us) & A. Liston (Oregon State University, Department of Botany and Plant Pathology, 2082 Cordley Hall, Corvallis, OR, 97331-2902, U.S.A.; e-mail: listona@science.oregonstate.edu). Genetic characterization of three varieties of *Astragalus lentiginosus* (Fabaceae). *Brittonia* 57: 334–344. 2005.—*Astragalus lentiginosus* is a polymorphic species that occurs in geologically young habitats and whose varietal circumscription implies active morphological and genetic differentiation. In this preliminary study, we evaluate the potential of amplified fragment length polymorphism (AFLP) markers to resolve infraspecific taxa in three varieties of *Astragalus lentiginosus*. Distance-based principle coordinate and neighbor-joining analyses result in clustering of individuals that is congruent with population origin and varietal circumscription. Analysis of molecular variance of two Oregon varieties demonstrates that varietal categories account for 11% of the total variance; in contrast, geographic proximity does not contribute to the total variance. AFLPs demonstrate an ability to discriminate varieties of *A. lentiginosus* despite a potentially confounding geographic pattern, and may prove effective at inferring relationships throughout the group.

**Key words:** AFLP, amplified fragment length polymorphism, *Astragalus lentiginosus*, genetic differentiation, infraspecific taxa.

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*Astragalus lentiginosus* Dougl. ex Hook. is a polymorphic species consisting of 40 varieties (Isely, 1998) distributed throughout the Intermountain and Desert Southwest regions of North America. The current varietal circumscription reflects Barneby's (1945) view that *A. lentiginosus* comprises multiple lines of evolutionary radiation, as well as occasional reticulation, both of which may explain the morphological intermediates between varieties. Many of the habitats where extant populations occur (e.g., inland dune systems, desert seeps, mountain ridges) were profoundly different during the Pleistocene (Grayson, 1993). Occurrence on these geologically young habitats suggests that the separation of current

populations and varieties may have occurred as recently as the late Pleistocene/early Holocene period. This study of *A. lentiginosus* represents an investigation of a diverse group whose circumscription reflects dramatic morphological variation and implies genetic differentiation.

Jones (1923) provided the first revision of *Astragalus* during the twentieth century. Among his innovations was the reduction of the section *Diphysi* to a single species, *Astragalus lentiginosus*. He characterized this species as the "most variable of all *Astragali*" (Jones, 1923, p. 123). As defined by Jones (1923), this species consisted of 18 varieties, many of which were originally described as species. Within his varieties Jones included "forms" (never validly published) that had originally been recognized as species or varieties.

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Applying a very different taxonomic approach, Rydberg (1929) split *Astragalus* into 28 genera (summarized by Barneby, 1964). In doing so, he divided *A. lentiginosus* between the genera *Cystium* (33 species, all currently known as *A. lentiginosus*) and *Tium* (39 species, only three of which are currently known as *A. lentiginosus*). This revision resulted in most of Jones's (1923) varieties (and forms) being raised to species (Barneby, 1945).

Barneby's treatments (1945, 1964, 1989) reduced the group back to a single species, *Astragalus lentiginosus*, with ca. 40 varieties. This classification came from the reduction of many of Rydberg's (1929) species to varieties, or similarly elevating many of Jones's (1923) "forms" to varieties. Since Barneby's 1945 treatment there has been a reduction in the original number of names accepted as varieties in part due to additional collecting that has blurred some intervarietal distinctions (Barneby, 1964, 1989). This trend has been partially offset by the description of new varieties since 1964 (e.g., Barneby, 1977; Welsh, 1981). While the currently recognized number of varieties is 40 (Isely, 1998) there is still an active debate as to what constitutes *A. lentiginosus* (Alexander, 2005) as well as to the validity of the varieties.

The varieties of *Astragalus lentiginosus* are morphologically distinct when observed at distant stations, yet when geographically proximal their distinctiveness may become obscured to the point where they are indistinguishable (Barneby, 1964, p. 922). Most of the group is characterized by an inflated bilocular pod with a false septum that intrudes from the abaxial surface and is incomplete in the beak. Barneby (1945, 1964, 1989) created major divisions within the group based on flower size (keel greater or less than 8.5 mm), raceme length (greater or less than 4 cm), and flower color (purple or white).

Attempts to evaluate taxonomic interpretations within *Astragalus* utilizing molecular methods have been complicated by low levels of divergence among the New World species. Chromosome number variation was identified as potentially useful at the infrageneric level (Barneby, 1964; Spellenberg,

1976), but phylogenetic inference was limited by the large size of the group and apparent homoplasy for this character. Molecular phylogenetic studies of nuclear ribosomal DNA internal transcribed spacers (Wojciechowski et al., 1993), chloroplast DNA (Liston, 1992; Sanderson & Doyle, 1993), and combined nuclear and chloroplast DNA datasets (Wojciechowski et al., 1999) have demonstrated the monophyly of the New World aneuploid species of *Astragalus*. However, none of these studies has resolved interspecific relationships within this clade.

Amplified fragment length polymorphism (AFLP) analysis (Vos et al., 1995) is an anonymous genetic fingerprinting technique developed for plant breeding that has been adapted to studies of natural populations (Wolfe & Liston, 1998; Mueller & Wolfenbarger, 1999). The AFLP method has been used to infer interspecific (Abdel-fattah et al., 2002; Beardsley et al., 2003) and intraspecific (Brouat et al., 2004; Juan et al., 2004; Travis et al., 1996) relationships as well as population level dynamics (He et al., 2004). This method was applied by Travis et al. (1996) to evaluate population-level differentiation of *Astragalus cremnophylax* var. *cremnophylax* occurring at the Grand Canyon, U.S.A. Results from that study showed strong differentiation of north and south rim populations, suggesting that AFLPs are sufficiently sensitive to discern genetic differences in recently diverged lineages, such as *A. lentiginosus*.

In this preliminary study we utilize AFLP analysis to test Barneby's (1945, 1964, 1989) taxonomic treatment of three *Astragalus lentiginosus* varieties. We focus on the question of whether the sampled populations of *A. lentiginosus* varieties display genetic relationships that can be attributed to geographic proximity, or whether the varieties exhibit genetic cohesiveness.

## Methods

### PLANT MATERIAL

Plants included in this study were selected to determine whether genetic differentiation could be discerned across the latitudinal extent of *Astragalus lentiginosus*.

TABLE I  
COLLECTIONS OF *Astragalus lentiginosus*

Population	N	Habitat	Latitude	Longitude	Altitude (m)	State	County
lentiginosus_1	8	Juniper woodland	42.76	-118.74	1915	OR	Harney
lentiginosus_2	8	Ponderosa pine forest	42.27	-121.30	1490	OR	Klamath
salinus_1	8	Sage shrubland	42.43	-118.08	1325	OR	Harney
salinus_2	7	Sage shrubland	43.32	-121.06	1318	OR	Lake
variabilis_1	8	Disturbed desert	34.57	-117.41	873	CA	San Bernardino
variabilis_2	7	Dune system	36.65	-116.57	744	NV	Nye

Leaves were collected during the summer of 2004 (Table I), tissue was desiccated in silica gel and stored at 4°C until DNA extraction. Collections were made from six locations (Fig. 1), with two locations per variety. Geographic distances between sample locations were calculated with ArcView GIS 3.2 (Environmental Systems Research Institute, Inc.).

Three morphologically distinct varieties were included in this study. *Astragalus lentiginosus* var. *lentiginosus* is a slender, decumbent to prostrate perennial occurring in Ponderosa pine or juniper woodland from

the Columbia Basin to northern California and the northwestern Great Basin. Barneby (1945, 1964, 1989) placed this taxon among the *A. lentiginosus* possessing short flowers (keel length < 8.5 mm), short racemes (axis < 4 cm long in fruit), and white flowers. *Astragalus lentiginosus* var. *lentiginosus* is distinctive among the varieties of *A. lentiginosus* in having moderately inflated pods with a coarse texture (thick walls), described as stiffly papery, leathery, or woody (as opposed to thinly papery or membranaceous).

*Astragalus lentiginosus* var. *salinus* (Howell) Barneby is a short-lived perennial consisting of diffuse and ascending stems occurring in sagebrush and alkaline flats from eastern Oregon to southern Idaho and throughout the northwestern Great Basin. This variety possesses short flowers (keel length < 8.5 mm long), short racemes (axis < 4 cm long in fruit), and white flowers. *Astragalus lentiginosus* var. *salinus* is geographically proximal to *A. l.* var. *lentiginosus* and can appear identical to the latter except for the thinly papery texture and inflation of the pods.

*Astragalus lentiginosus* var. *variabilis* Barneby is a monocarpic to short-lived perennial plant consisting of erect to ascending diffuse stems occurring on sandy flats, washes, desert playas, and sometimes on inland dunes throughout the Mojave Desert of southern California and southern Nevada. This variety possesses large flowers (keel length > 8.5 mm), long racemes (axis > 4 cm long in fruit) and purple flowers. This variety was chosen because it is geographically disjunct from the above varieties. In addition to these traits, *A. l.* var. *variabilis* differs from the previous two varie-

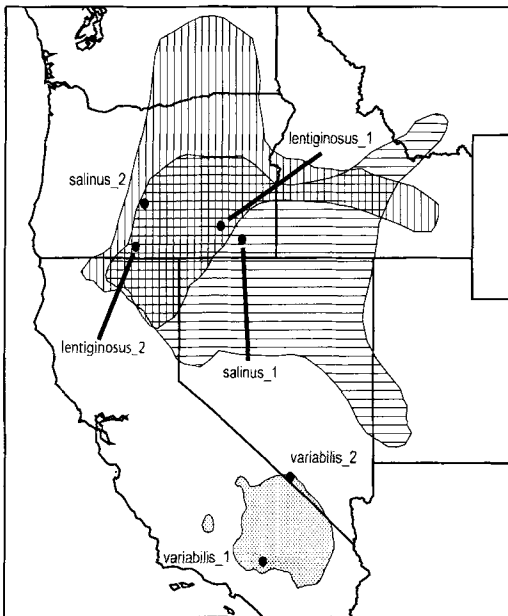


FIG. 1. Map of western North America showing ranges for three varieties of *Astragalus lentiginosus* and collections used in this study. Horizontal lines indicate the range of *A. l.* var. *salinus*, vertical lines indicate the range of *A. l.* var. *lentiginosus*, and dots indicate the range of *A. l.* var. *variabilis*.

ties in its robust and sometimes erect habit and its frequently cinereous to canescent vestiture.

#### DNA EXTRACTION

DNA extractions were based on a CTAB procedure modified from Chen and Ronald (1999). Exceptions were that leaflet tissue (ca. 50 mg) was homogenized in a bead mill (FastPrep 120; Qbiogene) using 0.5 cm<sup>3</sup> of 2.5 mm zirconia/silica beads (No. 11079125z-B; Biospec Products) and 800  $\mu$ l 2 $\times$  CTAB buffer [2% w/v CTAB, 1.4 M NaCl, 0.1 M Tris-HCl (pH 8.0), 20 mM EDTA, 2% w/v PVPP]. Samples were homogenized for 2 cycles of 20 seconds at a setting of 5. DNA was resuspended in 30  $\mu$ l of buffer [10 mM Tris-HCl, 0.1 mM EDTA (pH 8.0)] and then evaluated for quality by gel electrophoresis and quantified by fluorometry (VersaFluor; BioRad Laboratories).

#### AFLP SCREENING

Amplified fragment length polymorphism (AFLP) analysis was performed utilizing a modification of the Vos et al. (1995) method. Template DNA (~200 ng) was digested using 12 U of *EcoRI* and 8 U of *MseI* (New England Biolabs) for 2 hours at 37°C in a 20  $\mu$ l volume. Adapters were ligated to restricted DNAs by adding 20  $\mu$ l ligation mix [1 $\times$  final concentration ligase buffer, 8 U T4 DNA ligase (New England Biolabs), and 5 pmol *EcoRI* and 50 pmol *MseI* adapters] and incubating for 3 hours at 16°C. Enzymes were heat denatured at 65°C for 20 minutes after each treatment.

Pre-selective (“+1”) amplification was performed in 10  $\mu$ l [1 $\times$  PCR buffer (Fisher Scientific), 0.2 mM dNTPs, 1.5 mM MgCl<sub>2</sub>, 8 pmol each primer, 400 ng/ $\mu$ l BSA, 0.5 U *Taq* polymerase] utilizing 1  $\mu$ l of diluted (1:5) ligation product. PCR cycling conditions consisted of 2 minutes at 75°C followed by 19 cycles of 30 seconds at 94°C, 30 seconds at 56°C, and 2 minutes at 72°C; a final extension of 30 minutes was performed at 60°C.

Selective (“+3”) amplifications were performed using two primer combinations, [FAM]*EcoRI*+ACA/*MseI*+GAC and [HEX]

*EcoRI*+ACA/*MseI*+GAT, in a 10  $\mu$ l final volume [1 $\times$  PCR buffer (Fisher Scientific), 0.2 mM dNTPs, 1.5 mM MgCl<sub>2</sub>, 3.75 pmol fluorescently labeled *EcoRI* primer, 5.0 pmol unlabeled *MseI* primer, and 0.625 U *Taq* polymerase]. Selective primer pairs were chosen by screening ten primer pairs and selecting for maximum number of peaks (results not shown). Cycling conditions were 2 minutes at 94°C followed by 9 cycles of 30 seconds at 94°C, 30 seconds at 65°C (–1°C per cycle), and 2 minutes at 72°C. An additional 25 cycles were performed at 30 seconds at 94°C, 30 seconds at 56°C, and 2 minutes at 72°C, followed by a final extension at 60°C for 45 minutes.

Fragments were resolved on an Applied Biosystems Inc. 3100 capillary fragment analyzer (36 cm capillary; POP4 polymer) using a 1:2 dilution of “+3” product. Trace files were scored using ABI GeneMapper v3.0 following Rinehart (2004). To decrease the probability of including homo-plastic AFLP bands we included only bands between 200–490 bp with peak heights above 200 relative fluorescent units (Koopman & Gort, 2004).

#### DATA ANALYSIS

AFLP products were scored as the presence (1) or absence (0) of bands. Population and variety level band frequencies were calculated using GenAlEx v5.1 (Peakall & Smouse, 2001). Two distances were calculated from these data. Pairwise squared Euclidian distances ( $E^2_{ij} = \sum_k [X_{ki} - X_{kj}]^2$ , where  $i$  and  $j$  represent a pair of objects, and  $k$  denotes cases where  $y_{ki}$  and  $y_{kj}$  are both present) were calculated using GenAlEx and used as input for analysis of molecular variance (AMOVA; Excoffier et al., 1992). The Lynch shared band similarity index ( $S_{xy} = 2N_{xy}/(N_x + N_y)$  where  $N_x$  is the number of bands in sample  $x$ ,  $N_y$  is the number of bands in sample  $y$ , and  $N_{xy}$  is the number of bands shared between both samples (Lynch, 1990; Lamboy, 1994), as calculated using NTSYSpc version 2.1 (Rohlf, 2000) was used for neighbor-joining and ordination analyses. Because this index is equivalent to one minus the Dice (1945) coinci-

TABLE II  
AFLP BAND FREQUENCIES

Population	n	#loci	#bands	PLP	#private
lentiginosus_1	8	184	90	48.9	17
lentiginosus_2	8	184	67	36.4	13
salinus_1	8	184	88	47.8	14
salinus_2	7	184	70	38.0	11
variabilis_1	8	184	79	42.9	10
variabilis_2	7	184	62	33.7	8

(n), number of samples; (#loci), total number of loci; (#bands), number of bands scored as present in each population; (PLP), percent loci in each population; (#private), number of bands occurring in a population that are not present in other populations.

dence index, we refer to this measure as “Dice distance.”

Principle coordinates analysis (PCoA) of Dice distances was used to evaluate inter-individual distances in low dimension space to determine whether a priori population and varietal designations matched patterns of genetic variation. Dice distances were evaluated using the neighbor-joining algorithm (NJ; Saitou & Nei, 1987). Both PCoA and NJ were implemented in NTSYS-pc (Rohlf, 2000). Partitioning of genetic variation between populations nested within varieties was evaluated by AMOVA using the approach of Excoffier et al. (1992), as implemented with GenAlEx v5.1 (Peakall & Smouse, 2001). For variance partitioning, we looked at two groupings: at populations within varieties or regions ( $\Phi_{PR}$ ), and among varieties or regions ( $\Phi_{RT}$ ). Significance was measured with 999 permutations of individuals among hierarchical levels and recalculating null distributions of the test statistic (Excoffier et al., 1992).

## Results

Two primer pairs resulted in 184 loci scored between 200 and 490 bp with an average of 36.5 fragments per individual. Ten loci were monomorphic and 174 were polymorphic. Seventy three of the bands (40%) had a frequency of less than 0.05. Number of loci and private alleles per population are summarized in Table II.

Ordination by principle coordinates analysis (PCoA; Fig. 2) shows varieties to occupy distinct regions of coordinate space. The first axis accounted for 22.8% of the variation, the second accounted for 16.5%,

and the third accounted for 10.3% of the variation (49.6% of the total variation). Populations of *Astragalus lentiginosus* var. *variabilis* formed a single discrete cluster while most individuals of *A. l.* var. *lentiginosus* also formed a cluster. In contrast, the two populations of *A. l.* var. *salinus* clustered separately from each other. Individuals lentiginosus\_1–5, lentiginosus\_1–6, lentiginosus\_1–9, lentiginosus\_2–1, and salinus\_1–11 (A, B, C, D, E, respectively; Fig. 2) fall in a region of intermediacy between populations salinus\_1 and lentiginosus\_1. However, individual salinus\_1–11 (E) is separated from the others on the third axis by its strongly negative eigenvector (–0.0446) as opposed to the positive values on the third axis of individuals lentiginosus\_1–5, lentiginosus\_1–6, and lentiginosus\_1–9 (0.0166, 0.0176 and 0.0212 respectively). Individual lentiginosus\_2–1 (D) is notable in its intermediate third axis eigenvector (–0.0230) and its position as closer to the origin than the other members of its population. Close inspection of AFLP trace files show that these were high quality amplifications, so intermediacy is not the result of aberrant amplification.

Cluster analysis utilizing the neighbor-joining method (Fig. 3) resulted in a dendrogram that reflects ordination results with each variety forming a distinct cluster. Individual lentiginosus\_2–1 (D; Fig. 2) was the sole exception in that it neither clustered with its source population or variety. Individuals showing intermediacy on PCoA (Fig. 2; lentiginosus\_1–5, lentiginosus\_1–6, lentiginosus\_1–9 and salinus\_1–11; A, B, C and D respectively) cluster with their respective populations (Fig. 3).

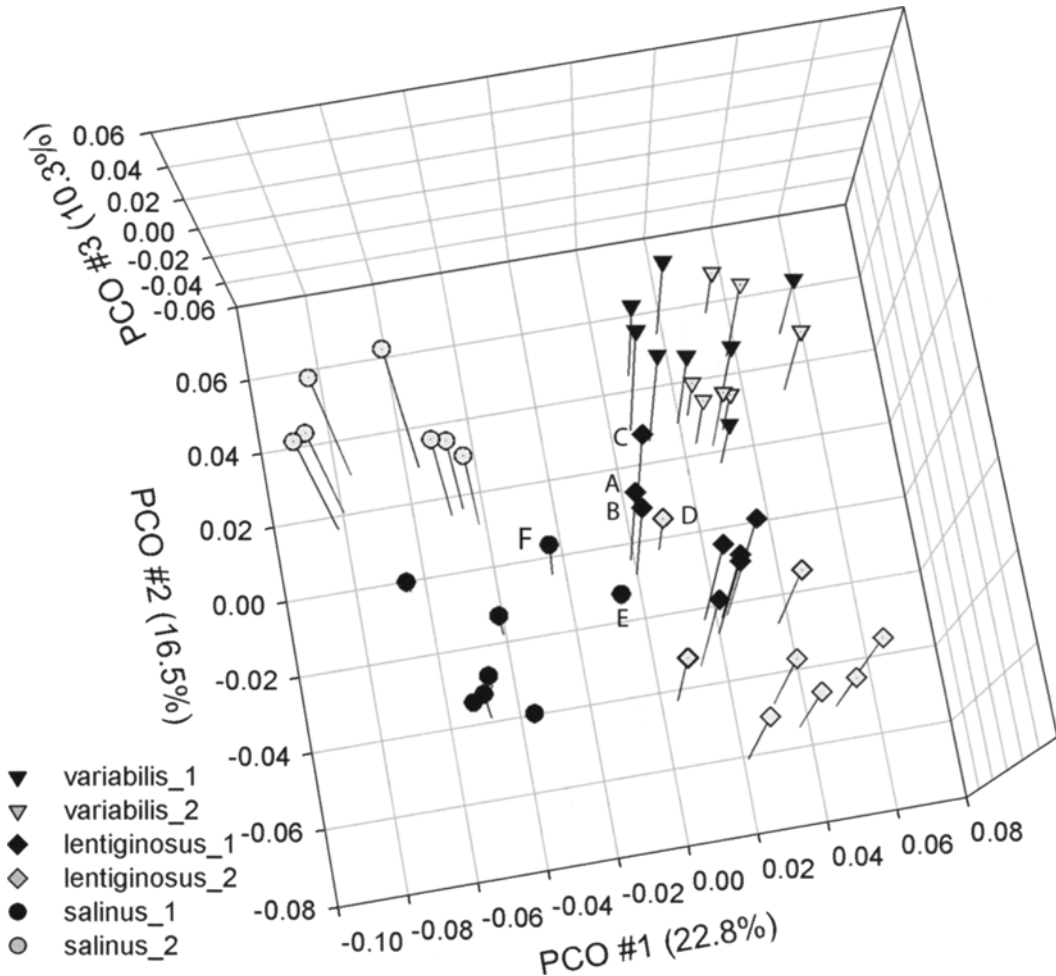


FIG. 2. Principle coordinates analysis (PCoA) based on a matrix of Dice genetic distances. A = lentiginosus.1-5. B = lentiginosus.1-6. C = lentiginosus.1-9. D = lentiginosus.2-1. E = salinus.1-11. F = salinus.1-12.

Dice genetic distances show a general pattern where within population genetic distance was lowest (Table III; mean =  $.0823 \pm 0.0202$ ,  $n = 154$ ), within variety genetic distance was slightly higher (mean =  $0.1055 \pm 0.0201$ ,  $n = 176$ ), and between variety genetic distances were greatest (mean =  $0.1216 \pm 0.0176$ ,  $n = 705$ ). Among-population genetic distances for *Astragalus lentiginosus* var. *salinus* (0.119) is comparable to the average among population divergence.

Analysis of molecular variance revealed significant structure within the sampled varieties and populations across the range of

sampled sites. Analyses were conducted three ways to evaluate diversity at different scales. Analysis #1 included all varieties and populations to address the question of whether there was structure to the sampled populations and varieties of *Astragalus lentiginosus* throughout its range. Variance decomposition showed that most of the variation in *A. lentiginosus* was contained within populations (67.3%,  $p < 0.001$ ; Table IV), due in large part to the abundance of bands restricted to one or a few individuals within a population. The remaining 32.7% of the total variance was apportioned among populations, with 13.4% of the var-

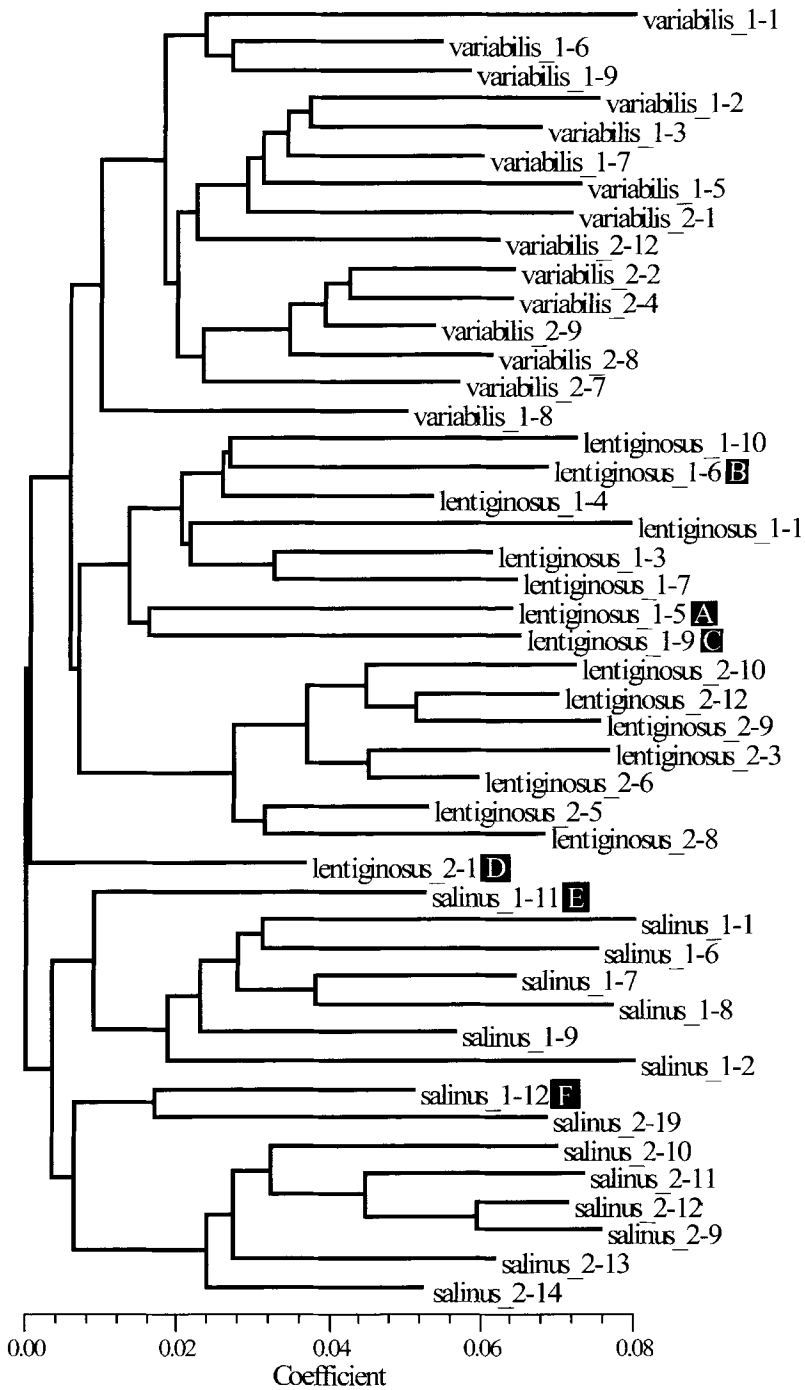


FIG. 3. Neighbor-Joining dendrogram built from a matrix of Dice genetic distances. Individuals labeled A, B, C, D, E, and F are the same as in Figure 2.

TABLE III  
GEOGRAPHIC AND GENETIC DISTANCES

	lentiginosus_1	lentiginosus_2	salinus_1	salinus_2	variabilis_1	variabilis_2
lent_1	0.092	220	64	193	917	705
lent_2	0.111	0.074	264	117	917	745
sal_1	0.124	0.122	0.095	257	877	655
sal_2	0.129	0.134	0.119	0.076	1019	837
var_1	0.113	0.113	0.127	0.122	0.085	243
var_2	0.115	0.108	0.125	0.127	0.087	0.067

Above the diagonal are geographic distances (km). Below the diagonal are Dice genetic distances. Along the diagonal are intra-population Dice genetic distances.

iation distributed among varieties ( $p < 0.001$ ) and 19.3% distributed among populations within varieties ( $p < 0.001$ ).

Because *Astragalus lentiginosus* var. *variabilis* is several hundred kilometers disjunct (Table III; Fig. 1), it may have contributed disproportionately to the varietal component of the variance. Analysis #2 was performed to determine whether geographically proximal populations retained the varietal signal exhibited in the full dataset. Removing *A. l.* var. *variabilis* populations from the analysis resulted in little change in the among-variety variance component ( $\Phi_{RT} = 10.9\%$ ,  $p < 0.001$ ; Table IV).

The third analysis was conducted to test whether geographic proximity accounted for more of the variance than varietal circumscription. The Oregon populations were grouped based on geographic proximity (region) instead of taxonomic variety. Popu-

lations *lentiginosus\_1* and *salinus\_1* were grouped into one region while populations *lentiginosus\_2* and *salinus\_2* were grouped into the second region. This grouping accounted for none of the variance at the regional level of hierarchy ( $\Phi_{RT} = 0$ ,  $p > 0.999$ ; Table IV). These results indicate that the variation revealed by AFLP shows a strong within-variety signal, and that geographic proximity does not contribute to the measured variance.

## Discussion

Barneby's (1945, 1964, 1989) circumscription of *Astragalus lentiginosus* represents an exceptionally detailed account of a group of organisms that appear too different to be considered the same taxon but also exhibit intergradation that precludes one from delimiting species; hence he chose the

TABLE IV  
ANALYSIS OF MOLECULAR VARIANCE (AMOVA)

Source	df	SS	MS	Est. var.	% total var.	Stat	Value	p-value*
Analysis 1: all populations								
Among Varieties	2	144.162	72.081	2.29	13.4	$\Phi_{RT}$	0.134	0.001
Among Pops./Varieties	3	110.532	36.844	3.312	19.3	$\Phi_{PR}$	0.223	0.001
Indiv./Within Pops.	40	460.893	11.522	11.522	67.3	$\Phi_{PT}$	0.327	0.001
Analysis 2: OR populations								
Among Varieties	1	72.532	72.532	1.962	10.9	$\Phi_{RT}$	0.109	0.001
Among Pops./Varieties	2	84.098	42.049	3.873	21.6	$\Phi_{PR}$	0.242	0.001
Indiv./Within Pops.	27	326.661	12.099	12.099	67.5	$\Phi_{PT}$	0.325	0.001
Analysis 3: OR populations/ geographic grouping								
Among Regions	1	40.549	40.549	0	0	$\Phi_{RT}$	0	0.999
Among Pops./Regions	2	116.081	58.04	5.941	32.9	$\Phi_{PR}$	0.329	0.001
Indiv./Within Pops.	27	326.661	12.099	12.099	67.1	$\Phi_{PT}$	0.284	0.001

\* p-values computed based on a simulation of 999 permutations.



taxonomic rank of variety to describe these taxonomic units. This results in a species that includes an unusually high number of varieties and has been described as unwieldy and vastly multiracial (Isely, 1998). To date there has been no test of this circumscription utilizing molecular methods, perhaps due to the complex nature of this diverse taxon.

#### GENETIC VARIATION IN *A. LENTIGINOSUS* CORRESPONDS TO VARIETAL CLASSIFICATION

Taxonomic circumscription within this group was explored with ordination by principle coordinates analysis (PCoA), which resulted in each variety occupying distinct coordinate space. Cluster analysis using the neighbor-joining method largely concurred with ordination results. A series of AMOVAs were then employed to provide statistical significance to varietal circumscription. These data suggest that interbreeding occurs preferentially within the sampled varieties as opposed to between intervarietal populations in close geographic proximity.

The comparison of Dice genetic distance with geographic distance (Table III) shows interpopulation genetic distance within *Astragalus lentiginosus* var. *variabilis* and *A. l.* var. *lentiginosus* to be relatively small. In contrast, the two populations of *A. l.* var. *salinus*, supported in both PCoA and clustering, show considerably greater divergence than the other two varieties.

One of these populations, *salinus*\_1, was collected near Whitehorse Ranch, a site mentioned by Barneby (1964, p. 922) as possessing individuals that defy classification (due to intermediate morphologies with *Astragalus lentiginosus* var. *floribundus*). Genetic divergence between the sampled populations of *A. l.* var. *salinus* may be due to factors such as introgression or hybridization with other varieties (e.g., *A. l.* var. *floribundus*; not included in this study), or it may simply represent a greater molecular diversity in this variety. The inclusion of more varieties (such as *A. l.* var. *floribundus*) in future studies may provide additional evidence for this observation.

#### INTER-VARIETAL INTERMEDIACY WITHIN *A. LENTIGINOSUS*

Four individuals of *Astragalus lentiginosus* var. *lentiginosus* and one of *A. l.* var. *salinus* were positioned near the origin in the PCoA analysis (Fig. 2). The difference between varieties is resolved on the third axis where they are clustered according to variety (except individual *lentiginosus*\_2-1) and in the neighbor-joining dendrogram, suggesting differentiation among the varieties. The position of these individuals, particularly *lentiginosus*\_2-1, near the origin, may indicate limited intergradation among the varieties. Due to the inclusion of only three varieties in this analysis, inferences about the cause of this finding cannot yet be made.

#### COMPARISON WITH *A. CREMNOPHYLAX* VAR. *CREMNOPHYLAX*

Travis et al. (1996) used AFLPs to distinguish populations of *Astragalus cremnophylax* var. *cremnophylax* on the north and south rim of the Grand Canyon. AMOVA results partitioned 63% of the variance amongst the north and south rim populations, as contrasted to 32% variance among populations of *A. lentiginosus* reported here. Principle coordinate analysis showed discrete clusters in the *A. cremnophylax* data as compared to the clusters that included intermediate individuals reported in the current study. This suggests that populations of *A. cremnophylax* var. *cremnophylax* are more structured and divergent than the varieties included in this current study despite our sampling of *A. lentiginosus* over a much larger geographic range (1000 km). Recent allozyme data (Allphin et al., in press) suggest the north and south rim populations warrant specific rank. This interpretation seems to be in accordance with the amount of AFLP divergence found between varieties in this study.

#### CONCLUDING REMARKS

In this pilot study we have demonstrated genetic differentiation between varieties of *Astragalus lentiginosus*. If populations of *A. lentiginosus* represented a simple pattern of

isolation by distance one would expect a cline of molecular diversity across the taxon's range. Populations collected at distant sites would appear distinct, yet intermediate samples would fall along a continuum, both morphologically and in terms of the molecular data. However, in the present study varietal distinction has been demonstrated in both disjunct and geographically proximal varieties suggesting that simple isolation by distance may not be responsible for all of the differentiation among varieties. While inferences about mechanisms are premature at this time, these data do not contradict Barneby's circumscription of these three varieties.

This study accompanied with that of Travis et al. (1996) show AFLPs to be a promising tool in the study of *Astragalus* species. Here we have demonstrated the ability of AFLPs to discriminate infraspecific relationships within *A. lentiginosus* despite a potentially confounding geographic pattern. This suggests their use may prove effective at inferring relationships throughout the group.

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