Population structures of *Astragalus filipes* collections from western North America

B. Shaun Bushman, Kishor Bhattarai, and Douglas A. Johnson

Abstract: The majority of species used for revegetation in semi-arid western rangelands of North America are grasses, with few forbs and nearly no legumes. *Astragalus filipes* (Torr. Ex A. Gray) is a western North American legume and a promising candidate for use in rangeland revegetation, but assessments of plant species diversity and structure are necessary to determine which collections should be used to constitute a conservation unit or regional seed source. To address this issue, we characterized within-collection genetic diversity, identified genetically differentiated groups, and tested genetic correlations with environmental variables on 67 collections of *A. filipes*. Within-population genetic diversity was greatest for collections in Oregon and lowest for collections in British Columbia and central Nevada. Five genetically differentiated groups were detected: one with strong support from central Nevada, one with strong support from British Columbia, and three with weak support comprising all other collections throughout Oregon, Washington, California, Idaho, and Nevada. Although there was significant correlation between genetic and linear geographic distance matrices, there was no correlation between genetic and phenotypic, elevation, temperature, or precipitation distance matrices. These results show that geographic distance contributes to genetic differentiation, and that structured populations have occurred in northernmost and southernmost groups of collections of *A. filipes*.

Key words: Astragalus filipes, basalt milkvetch, restoration legumes, population structure, AFLP.

Résumé : La majorité des espèces utilisées pour la revégétation des prairies montagnardes semi-arides de l'Ouest nord-américain comporte des herbacées, avec quelques arbustes et pratiquement aucune légumineuse. L'*Astragalus filipes* (Torr. ex A. Gray) constitue une espèce de légumineuse de l'Ouest nord-américain prometteuse pour assurer la revégétation des prairie semi-arides, mais on doit d'abord évaluer la diversité et la structure des espèces de plantes afin de déterminer quelles récoltes devrait-on utiliser pour constituer une unité de conservation ou source régionale de semences. Pour examiner cette question, les auteurs ont caractérisé la variation génétique intra récolte ont identifié des groupes génétiquement différenciés, et ont testé des corrélations génétiques avec des variables environnementales de 67 récoltes de l'*A. filipes*. Ils ont observé une plus grande diversité génétique dans les populations de l'Oregon et plus petite dans celles de la Colombie-Britannique et du Nevada. Ils ont décelé cinq groupes génétiquement distincts : un fortement supporté venant du centre du Nevada, un fortement supporté de la Colombie-Britannique, et trois faiblement supportés distribué dans les autres collections venant de l'ensemble de l'Oregon, Washington, Californie, Idaho et Nevada. Bien qu'on trouve une corrélation significative entre la génétique et les matrices de distances géographiques linéaires, il n'a y a pas de corrélation entre les matrices de distances génétiques et phénotypiques, l'élévation, la température ou la précipitation. Ces résultats montrent que la distance géographique contribue à la différentiation génétique et qu'il existe des populations structurées dans les groupes de collections venant des régions les plus nordiques et les plus méridionales de l'*A. filipes*.

Mots-clés : Astragalus filipes, astragale du basalte, légumineuses pour revégétation, structure des populations, AFLP.

Introduction

Semi-arid rangelands of western North America are subject to degradations imposed by wildfires, exotic weed invasion, overgrazing, and recreational use. Reseeding of disturbed rangelands with suitable plants serves to reduce weed invasion, stabilize soils, provide wildlife habitat, and provide forage for livestock and wildlife (Pellant et al. 2004; Sheley and Carpinelli 2005). The vast majority of species used for revegetation of western rangelands are grasses, with nearly no legumes currently available. Legumes, however, are known to complement grasses by their capacity to biologically fix nitrogen and provide forage with a high protein content (Cherney and Allen 1995; Posler et al. 1993), and their ability to inhibit weed colonization following disturbance (Liao et al. 2008). Consequently, there is a need to include additional legume species in reseeding mixtures for semi-arid rangelands of western North America.

A candidate legume species for revegetation should have potential for economic seed production, must be widely distributed such that any hybridization with related species would have already occurred naturally, if at all, and must

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be non-toxic to livestock, as much of the western lands are permitted for grazing. Agronomic and economic production of rangeland plant seed is ideal. Hand-collected seed is often expensive, of variable quality and quantity, and may be prone to over-collection from some sites. If seed can be agronomically produced, its quantity and quality is consistent and its cost is usually low.

Astragalus filipes (Torr. ex A. Gray), or basalt milkvetch, is a possible candidate for rangeland reseeding. It is a member of the Papilionoideae subfamily of Fabaceae (Wojciechowski et al. 1999). Plants are perennial, upright at 20–90 cm tall, and form large clumps of stems (Welsh et al. 1993). *Astragalus filipes* is distributed throughout the western USA and Canada, mainly in the northern Great Basin and the Columbia River Plateau (Barneby 1989). It has high seed production potential and is prevalent in rangeland areas that have been recently burned (Bhattarai et al. 2008). Previously, seed collections of *A. filipes* were evaluated for plant biomass, morphometric measurements, seed production, and toxic secondary metabolites. Collections varied significantly for nearly all phenotypic traits and contained negligible nitrotoxins, selenium, or indolizidine alkaloids (Bhattarai et al. 2008).

To preserve adaptive traits and co-adaptive gene complexes of seeded species, McKay et al. (2005) argued that revegetation of disturbed rangelands using local germplasm is ideal. Although ideal, in practice it can be intractable to collect, maintain, and produce seed of many local sources of germplasm for the myriad of species used in rangeland revegetation (Broadhurst et al. 2008). Additionally, with the drastic disturbances that have occurred on some rangeland sites, widespread exotic weed invasions, and possible climatic changes, the revegetation site may have been altered such that erstwhile locally adapted germplasm may no longer be such (D'Antonio and Vitousek 1992; Liao et al. 2008). To circumvent some of the constraints of local-origin plant materials, it may be optimal to develop regional seed sources such as those used in forest regeneration (e.g., Rehfeldt et al. 1999), or consider broad-based cultivars with the genetic potential to adapt to a wide variety of sites (Broadhurst et al. 2008; Hufford and Mazer 2003). Broad-based cultivars may have greater adaptive potential than that of local-origin materials, but they may also have the potential to cause out-breeding depression with local germplasm (Hufford and Mazer 2003). Regional seed sources could be composed of meta-populations across large geographic areas, which may have a lower probability of out-breeding depression than broad-based cultivars. Regional seed sources would require fewer seed production fields and storage facilities than highly localized seed sources, and have the benefit of allowing predictable seed production and thus high quality seed for planting.

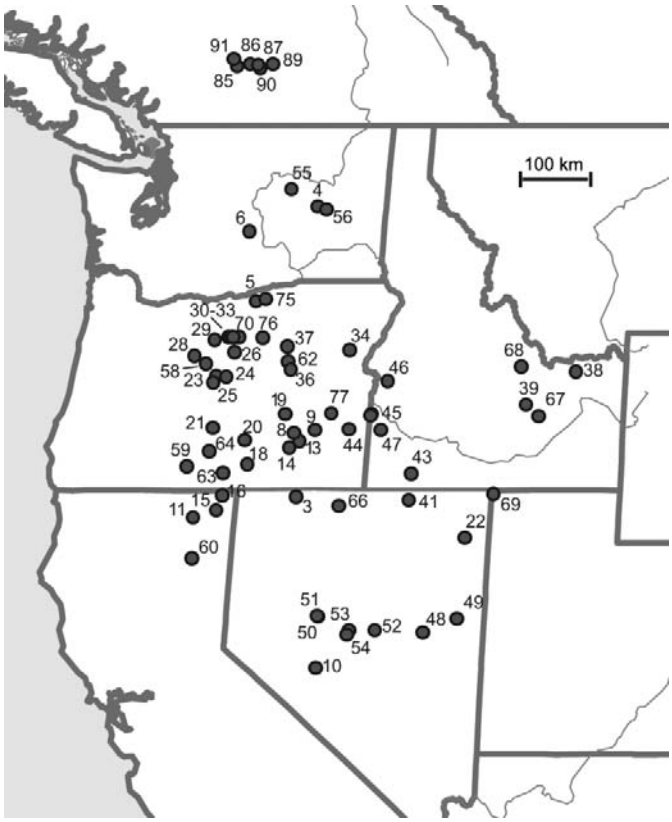
Designation of groups of populations for regional seed production, however, can rely on any number of plant characteristics, often unknown, and which likely differ for each species under consideration (Broadhurst et al. 2008; Hufford and Mazer 2003). Phenotypic characterizations in a common garden setting are used to determine whether there is variation for a set of phenotypic traits in a species under consideration, and whether any trait variation is correlated with collection site environmental parameters. The resulting phenotypic traits are considered to have adaptive potential. However, the common garden analysis is limited by the number and type of traits measured, and by the locations of the common garden(s). The use of molecular genetic diversity and structure for plant species characterization provides another tool in the decision paradigm concerning which collections should be used to constitute a regional seed source (Hufford and Mazer 2003; Rivera-Ocasio et al. 2006). The assessment of genetic diversity and structure utilizes neutral DNA markers (e.g., AFLPs or SSRs) to evaluate relationships within and among collections (Meudt and Clarke 2007). It allows for prediction of inbreeding depression potential based on within-collection diversity, and identification of genetically differentiated groups of populations based on among-collection variation (Bonin et al. 2007; Ouborg et al. 2006). Genetically differentiated groups indicate a cumulative effect of gene-flow barriers, including effects of isolation, drift, and selection.

Because *A. filipes* is a promising native legume for revegetation in western North America, this study was designed to assist in designating regional seed sources for *A. filipes* germplasm development. We characterize within-collection genetic diversity, identify genetically differentiated group structures, and test isolation by distance for 67 *A. filipes* collections. Additionally, we investigate possible relationships between genetic distances and phenotypic (Bhattarai et al. 2008), environmental, and topographical differences through matrix correlations.

Materials and methods

Sixty-seven wildland site collections were used (Fig. 1), with 9.2 ± 1.1 (mean ± SD) plants per collection (Table 1), for a total of 629 individuals. Seeds were planted in conetainers (Stuewe and Sons, Inc., Corvallis, Oregon) in a greenhouse in Logan, Utah. Fresh seedling tissue was harvested, and DNA extracted using the DNeasy 96-well extraction kit (QIAGEN, Valencia, California). Quantity and quality of genomic DNA were assessed by spectrophotometry and agarose gel electrophoresis. The AFLP procedure followed the protocol of Vos et al. (1995), using the selective primers E(*Eco*RI).ACC/M(MseI).CAC, E.ACT/M.CAC, E.AGG/M.CAA, E.AGG/M.CAG, and E.AGG/M.CTC. Amplicons were separated on a capillary ABI 3700 instrument with the GS-500 LIZ size standard and GeneScan software (Applied Biosystems, Foster City, Calif.). Individual profiles were visualized and manually scored for the presence or absence of fragments between 50–500 bp with Genographer software (Benham 2001). There was a significant correlation between band size and band frequency ($r = 0.20$, $P < 0.001$), such that there was a slight bias toward large-sized bands. For a subset of 24 randomly chosen samples, a second DNA extraction was conducted and the samples were assayed concurrently with the total set. These 24 duplicates were used to score AFLP band consistency and obtain an error rate (2.7%) by dividing the number of markers with differences within the duplicates by the total number of markers. Markers were also removed from analysis if they were present or absent in greater than 99% (<11) of the individuals. None of the removed markers showed frequency bias to any collection. A final total of 1194 AFLP bands were used for analysis.

Fig. 1. Map of locations of 67 *Astragalus filipes* collections in western North America.



The within-accession intraspecific genetic diversity values were estimated using the similarity index (Dice 1945; Lynch 1990; Nei and Li 1979). The similarity index is defined as: $S_{xy} = 2N_m / N_x + N_y$, where N_m is the number of AFLP bands that are shared between individuals x and y, and N_x and N_y are the total number of bands for each respective individual (Leonard et al. 1999). Standard errors (SE) of the mean S values were computed as per Leonard et al. (1999). Significances of state boundaries on S-values were computed with the general linear model procedure of SAS version 9.2 (SAS Institute Inc. 1999), using the LSD command to obtain pairwise tests.

Population subdivision of the *A. filipes* accessions was estimated using F_{ST} genetic distances and Bayesian clustering, and analysis of molecular variance (AMOVA). Raw binary AFLP data were converted to Euclidean distances and the resulting distance matrix constituted the input file for AMOVA and hierarchical AMOVA, using Arlequin version 3.11 (Schneider et al. 2000). A neighbor-joining dendrogram with bootstrap support was constructed from F_{ST} distances generated in AFLP-SURV (Vekemans et al. 2002), and a majority-rule consensus tree constructed in Phylip (Felsenstein 1989). Only nodes with bootstrap support (1000 replicates) greater than 0.70 (70) were denoted. The tree was midpoint-rooted using FigTree version 1.3.1 (Rambaut Research Group 2009). Population structure was tested with Bayesian clustering using the STRUCTURE 2.2.3 program (Falush et al. 2007). Raw binary data were used with the RECESSIVE ALLELES option, the admixture model with correlated marker frequencies, but without population flags. Population sizes of $K = 1$ through $K = 9$ groups were tested with three replications per analysis. The MCMC procedure within Structure was used to determine the strength of each structure model, with 20 000 burn-in and 200 000 MCMC steps after burn-in. The average (three replications) estimated log-likelihood of the data, and the second order difference between average log-likelihood values of the tested structure, denoted as ΔK (Evanno et al. 2005), were graphed against the tested groups to visualize how the structures fit the data. As the ΔK procedure requires subtractions of former and latter structures, only groups $K = 2$ through $K = 8$ are shown for that graph. Plants were assigned to a group for hierarchical AMOVA based on the majority of their coancestry coefficient.

Matrix correlations between genetic distances and geographic, phenotypic, elevation, mean annual precipitation, and mean maximum/minimum temperature distances were estimated with the Mantel's test statistic Z (Mantel 1967), using IBD version 3.16 (Bohonak 2002). For the genetic distance matrix, the Φst pairwise differences between populations were used from the AMOVA procedure. The geographic distance matrix was constructed from the latitude/longitude decimal form. Phenotypic data, previously reported in Bhattarai et al. (2008), were collected from common garden studies, and included dry matter yield, seed yield, winter mortality, plant height, number of stems, number of inflorescences, crude protein, and seed weight. Collections from British Columbia, Canada, were not included in that common garden study, so the matrix correlation between phenotypic and genetic data also did not include data for the British Columbia collections. Environmental and topographic matrices included collection site elevation, mean annual maximum temperature, mean annual minimum temperature, and mean annual precipitation. The latter three were determined from models obtained from 1971–2000 data using the USDA–ARS/Utah State University Data Extraction Tool for the United States collections (earth.gis.usu.edu/ars) and the ClimateBC database for British Columbia collections (genetics.forestry.ubc.ca/cfgc/ClimateBC/Default.aspx). For variables that were significant in initial Mantel tests, partial Mantel tests were conducted using IBD version 3.16. Geographic locations of populations were mapped using ArcGIS version 9 (ESRI, Redlands, Calif.).

Results

The *A. filipes* collections had between 21% and 39% polymorphic markers within collections. Of the 67 collection sites tested (Fig. 1 and Bhattarai et al. 2008), those with the lowest similarity (S-value), or highest average within-collection genetic diversity, were located within the state of Oregon (Table 1). The Oregon collections had mean similarity index values between 0.62 and 0.65. Collections with the highest values were located in British Columbia, while collections from Nevada, Idaho, Washington, and California were intermediate between Oregon and British Columbia (Table 1). Because of this trend, analysis of variance was used with state boundaries as class variables, and showed significant differences in average similarity index values across states ($F = 30.04$; $P < 0.0001$). Collections from British Columbia and Oregon had significantly higher and lower

Table 1. *Astragalus filipes* collections used for DNA analysis, sorted in descending order of similarity index (*S*) values.

Accession ID	State/Province	County	No. of plants	Mean *S*-value	SE of *S*-value
Af-86	BC	n/a	8	0.789	0.010
Af-90	BC	n/a	8	0.787	0.013
Af-85	BC	n/a	8	0.780	0.013
Af-10	NV	Nye/Mineral	7	0.758	0.013
Af-87	BC	n/a	8	0.754	0.013
Af-53	NV	Lander	10	0.752	0.013
Af-89	BC	n/a	7	0.742	0.022
Af-54	NV	Lander	10	0.739	0.009
Af-91	BC	n/a	7	0.738	0.010
Af-50	NV	Churchill	5	0.734	0.012
Af-52	NV	Eureka	8	0.732	0.006
Af-68	ID	Custer	10	0.730	0.016
Af-48	NV	White Pine	7	0.729	0.008
Af-38	ID	Clark	10	0.724	0.010
Af-46	ID	Payette	10	0.716	0.014
Af-39	ID	Butte	10	0.716	0.028
Af-51	NV	Churchill	10	0.715	0.007
Af-59	OR	Klamath	10	0.713	0.012
Af-60	CA	Lassen	9	0.708	0.012
Af-6	WA	Kittitas	8	0.706	0.035
Af-4	WA	Lincoln	8	0.702	0.016
Af-3	NV	Humboldt	9	0.693	0.011
Af-22	NV	Elko	10	0.693	0.010
Af-16	CA	Modoc	10	0.692	0.007
Af-11	CA	Modoc	10	0.683	0.004
Af-49	NV	White Pine	10	0.681	0.018
Af-55	WA	Douglas	10	0.677	0.031
Af-67	ID	Butte	9	0.673	0.010
Af-5	OR	Morrow	9	0.672	0.010
Af-66	NV	Humboldt	9	0.668	0.006
Af-45	ID	Owyhee	10	0.667	0.008
Af-36	OR	Grant	9	0.667	0.008
Af-41	NV	Elko	10	0.667	0.007
Af-69	UT	Box Elder	9	0.666	0.011
Af-64	OR	Lake	10	0.665	0.012
Af-34	OR	Baker	10	0.664	0.012
Af-18	OR	Lake	10	0.664	0.026
Af-15	CA	Modoc	8	0.664	0.011
Af-25	OR	Crook	10	0.663	0.010
Af-56	WA	Lincoln	10	0.662	0.012
Af-43	ID	Owyhee	10	0.662	0.006
Af-29	OR	Wasco	10	0.661	0.015
Af-19	OR	Harney	10	0.659	0.012
Af-63	OR	Lake	10	0.658	0.018
Af-75	OR	Morrow	8	0.658	0.009
Af-76	OR	Wheeler	8	0.653	0.011
Af-20	OR	Harney	8	0.652	0.013
Af-30	OR	Wheeler	10	0.650	0.008
Af-70	OR	Wheeler	10	0.648	0.014
Af-47	ID	Owyhee	8	0.647	0.012
Af-8	OR	Harney	10	0.647	0.010
Af-42	OR	Malheur	10	0.646	0.011
Af-9	OR	Harney	10	0.645	0.006
Af-62	OR	Grant	10	0.642	0.011
Af-37	OR	Grant	10	0.641	0.011
Af-26	OR	Wheeler	9	0.640	0.009

Table 1 (*concluded*).

Accession ID	State/Province	County	No. of plants	Mean *S*-value	SE of *S*-value
Af-44	OR	Malheur	10	0.640	0.014
Af-33	OR	Wheeler	10	0.640	0.008
Af-23	OR	Crook	10	0.640	0.007
Af-77	OR	Malheur	10	0.639	0.008
Af-58	OR	Crook	10	0.639	0.008
Af-21	OR	Lake	9	0.637	0.017
Af-13	OR	Harney	9	0.633	0.012
Af-28	OR	Jefferson	10	0.633	0.017
Af-32	OR	Wheeler	10	0.632	0.007
Af-24	OR	Crook	10	0.626	0.010
Af-14	OR	Harney	10	0.622	0.011

Table 2. LSD pairwise comparisions of the average similarity index values (by state/province) for *Astragalus filipes* collections.

	British Columbia	Nevada	Idaho	Washington	California	Oregon
British Columbia	—					
Nevada	*	—				
Idaho	*	ns	—			
Washington	*	ns	ns	—		
California	*	ns	ns	ns	—	
Oregon	*	*	*	*	*	—

Note: ns, non-significant.
*Statistically significant ($P < 0.05$).

values, respectively, than those of all others, (Table 2), indicating that maximum genetic diversity was found within central Oregon.

The AMOVA partitioned 73% of the variation within collections and 27% among collections, thus an F_{ST} value of 0.27 across all collections. When a F_{ST}-based genetic distance neighbor-joining dendrogram was used to explore collection relationships, several groups of collections had high bootstrap support (Fig. 2). Most of these groups of collections grouped geographically: collections 85–91 from British Columbia, collections 10 and 50–54 from central Nevada, collections 38–39 and 67–68 from eastern Idaho, four collections from California, and several two- or three-collection groups within Oregon and Idaho. To test for population structures, Bayesian clustering was used to test a priori 1–9 groupings. For these tests the average log-likelihood values increased gradually (Fig. 3*a*) and the second-order difference in log-likelihood values showed a clear mode beginning at the five group test (Fig. 3*b*), indicating that little relative improvement in model fit was obtained above that structure. At the five group structure, the British Columbia and central Nevada collections were clearly separated from the remainder of the collections (Fig. 4), and in all high group tests thereafter both groups of collections remained separated and had homogeneous coancestry coefficients (data not shown). The third group included 18 collections from Idaho, eastern Oregon, and eastern Nevada (collections 3, 9, 22, 38–45, 47–49, 66–69, 77). Within this group, collections 38, 39, 68, and 69 from eastern Idaho had relatively homogeneous coancestry coefficients, while other collections close to the Oregon border (e.g., 43, 45, 46) had admixed coancestry with other groups (Fig. 4 and Fig. 1). The remaining two groups contained 37 collections from California, Oregon, and Washington and had large portions of collections with admixed coancestry. Each of the collections within the three latter groups showed increasing levels of admixture in large group tests thereafter (data not shown).

The five groups identified in Bayesian clustering were tested for significance and apportionment of variation with hierarchical AMOVA; wherein 14% of the variation was apportioned among the five groups, 16% among populations within groups, and 70% was apportioned within populations (Table 3). 3). When each of the five groups was subjected to two-group hierarchical AMOVA testing, the percentages of variations apportioned among groups were 21% for British Columbia vs. the remaining collections, and 17% for central Nevada vs. the remaining collections. The remaining three groups had between 3%–6.5% of variation apportioned among groups when compared with the remaining collections (Table 3).

As genetic structuring tended to separate groups geographically, the relationship between genetic and geographic distances was investigated using Mantel's correlation. The correlation between genetic and geographic distance matrices was significant ($P < 0.001$) with an $r = 0.79$ (Table 4), indicating isolation by distance for genetic diversity distribution. Because the collections from British Columbia were absent in phenotypic analyses (Bhattarai et al. 2008), the genetic distance matrix was also tested without them. Without the British Columbia group of collections, the correlation was still highly significant ($P < 0.001$) and the coefficient reduced to $r = 0.69$. When correlated to a phenotypic distance matrix presented in Bhattarai et al. (2008), there was no significant correlation with the genetic distance matrix (Table 4). Additionally, to investigate whether genetic dis-

Fig. 2. Neighbor-joining dendrogram of *Astragalus filipes* collections using F_{ST} genetic distances, with bootstrap support from 1000 iterations.

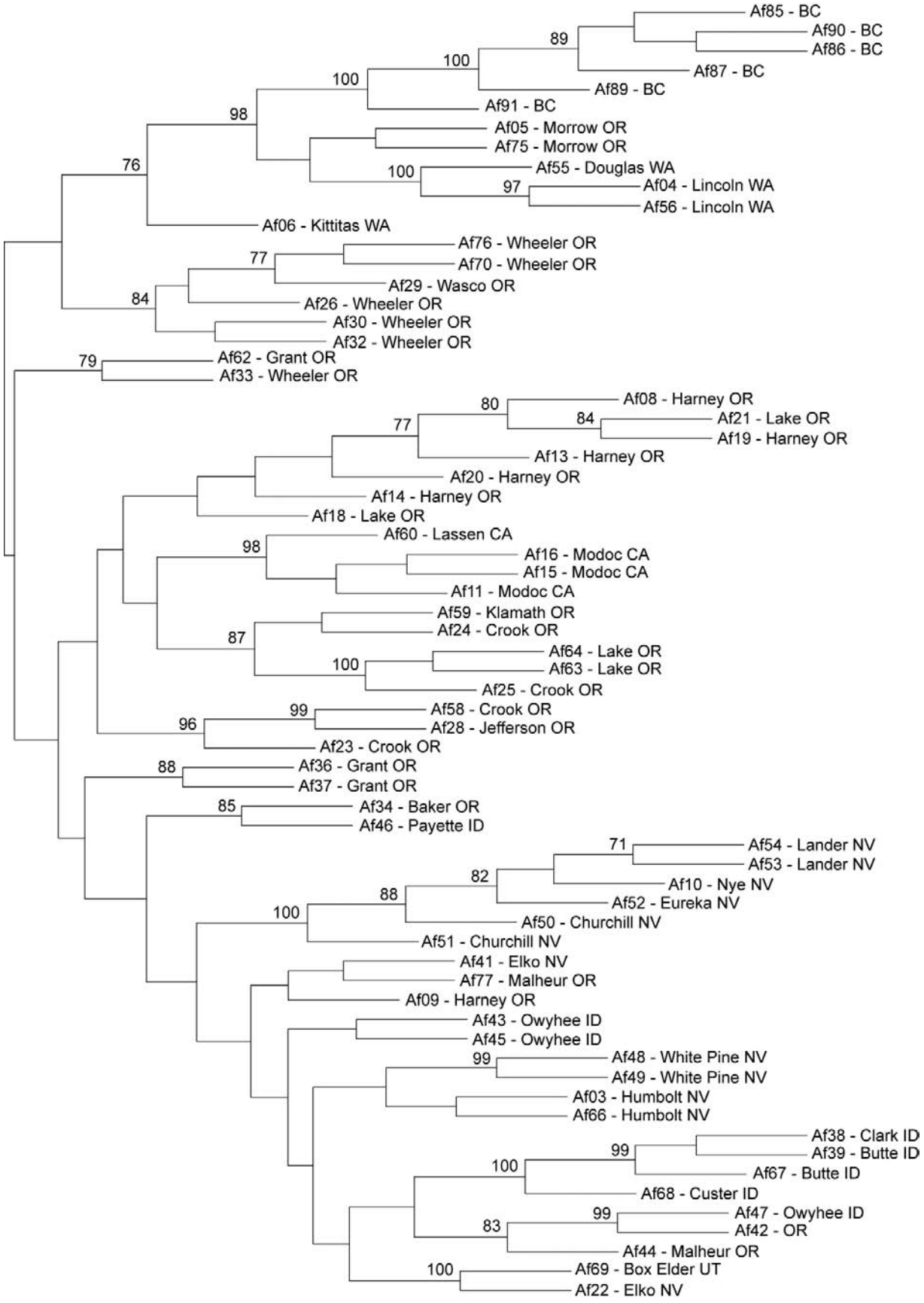
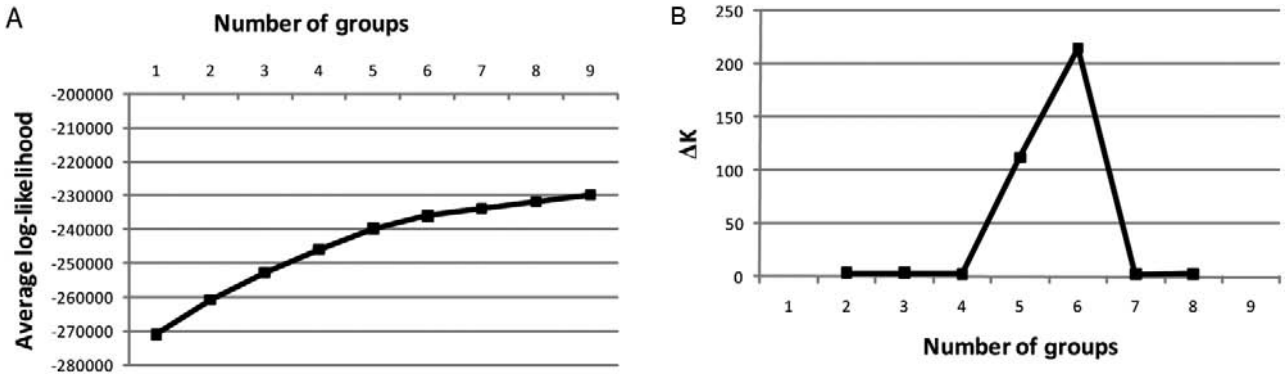


Fig. 3. (A) The average log-likelihood scores from three replications of Bayesian structure analysis for each structure, and (B) the second-order difference (ΔK) between the average log-likelihood scores for 1–9 a priori group tests.



tances were correlated with other environmental or topographic distances, matrix correlations were obtained for elevation, mean maximum and minimum temperatures, and mean annual precipitation (Table 4). Genetic distance was moderately correlated with the elevation difference matrix ($r = 0.38$, $P = 0.001$) and the mean annual temperature difference matrix ($r = 0.27$, $P = 0.002$). However, when corrected for geographic distance, elevation was no longer significant and maximum temperature was significant only to a small extent ($r = 0.18$, $P = 0.01$) (Table 4).

Discussion

Within-population genetic similarity (e.g., diversity) estimates are critical when population sizes are small or if plants are otherwise in danger of genetic bottlenecks. With a broadly distributed, out-crossing and perennial legume such as *A. filipes*, the within population similarity index values were all sufficiently low to preclude concern for adaptive potential. Yet the similarity index values were different among the collections, with a geographical trend toward increasing diversity in central Oregon (Tables 1 and 2). Plant collections are often made based on existing herbarium records, and for *A. filipes* we collected from all known areas except the Sierra Nevada Mountains in central California and Mexico. As the trend in similarity index values increased (thus decreasing diversity) for collections in California compared with Oregon, it would be worthwhile to sample populations in the Sierra Nevada Mountain Range. Assuming the sampling was otherwise comprehensive, our similarity index data indicate a center of diversity of this species in central Oregon. Correspondingly, Oregon collections also showed the greatest levels of admixed co-ancestry in population structure tests (Fig. 4). This pattern of *A. filipes* distribution is consistent with early Holocene glacial declines, wherein warm and dry conditions in the northern Great Basin allowed steppe conditions to develop (Minckley et al. 2007).

The group of collections with the highest similarity values, from southern British Columbia, also showed strong support for genetic differentiation in the bootstrap, Bayesian clustering, and hierarchical AMOVA analyses (Figs. 2 and 4; Table 3). These British Columbia collections were found only in localities with reported mean annual rainfall less than 500 mm (Eflora BC 2010), a classification area comprising the lowest precipitation for that province. Their structure and higher similarity support a model of "northern purity" (Hewitt and Ibrahim 2000), which could have derived from a central Oregon population as glacial maxima declined (Licciardi et al. 2004). Although surrounded by high mountains and wet climates, there is a continuum of low precipitation along the Okanogan River from Washington State north to the *A. filipes* collection sites in British Columbia.

The group of collections from central Nevada also showed high similarity index values and strong evidence of genetic differentiation (Figs. 2 and Fig. 4; Table 3). This group of collections was obtained from high-elevation mountain ranges (>1800 m a.s.l.) in central Nevada, which are longitudinally arranged throughout that portion of the Great Basin with intervening low-elevation, low-precipitation valleys. No *A. filipes* were found in the low-elevation valleys, providing a possible mechanism for genetic isolation between the high-elevation ranges. Interestingly, although each Nevada high-elevation collection could have the potential for this genetic isolation, only the region between collections 52 and 48 showed indications of a gene-flow barrier, in that six central Nevada collections (10, 50–54) with homogeneous coancestry coefficients were distinct from collections 48 and 49 in eastern Nevada. These central Nevada collections represented the southern extremity of the collections, further supporting the trend of a center of diversity in central Oregon and more genetic homogeneity and isolation in peripheral populations.

The remaining three structured groups include a group of 18 Nevada and Idaho collections, a small group of 7 collections in southern Oregon, and a final group of 30 throughout Oregon, California, and Washington (Fig. 4). All of these three latter groups showed substantial levels of admixture, wherein collections contained plants with proportions of admixed coancestry with very few homogeneous or "pure-type" collections (Fig. 4). The Nevada and Idaho group had homogeneous collections in eastern Idaho (38, 39, 68, 69; Fig. 4) and admixed collections in western Idaho, again reflecting the trend of increased genetic purity in distal collections from central Oregon. The most homogeneous collections in the two remaining groups were found in south-central Oregon, yet were still surrounded by admixed collections. The admixed coancestry prevalent in these three groups indicates a lack of strong genetic isolation. Additionally, these three groups each explained a low relative percentage of variation apportioned among groups with hierarchical AMOVA (Table 3). This

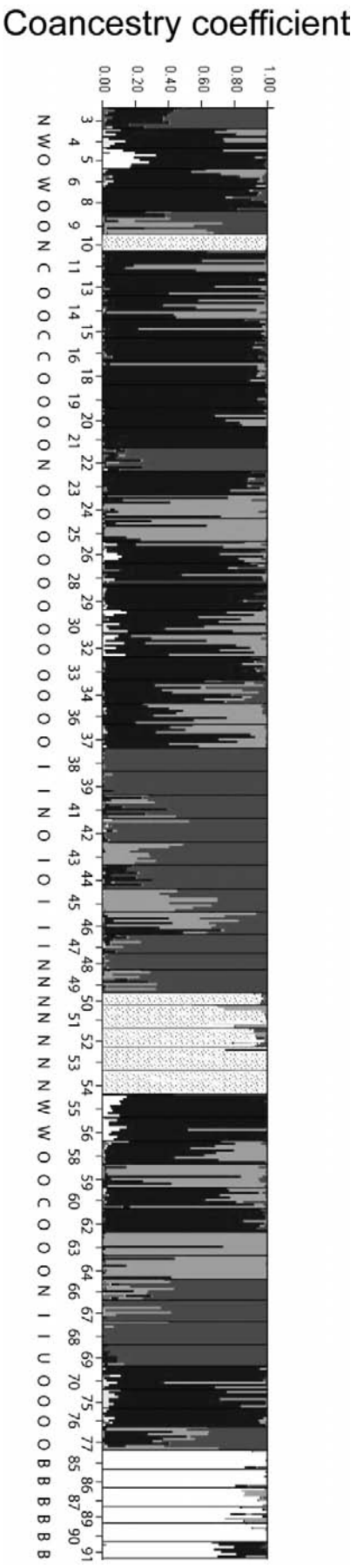


Fig. 4. Bayesian clustering bar plot of *Astragalus filipes* collections into five groups. X-axis numbers correspond to collection numbers in Table 1, Fig. 1, and Fig. 2. The capital letter designations correspond to the state/province of origin: B, British Columbia; C, California; I, Idaho; O, Oregon; N, Nevada; U, Utah; W, Washington.

prevalence of admixture and relatively low apportionment of variation suggests that these three groups could be combined into one group with a low risk of out-breeding depression when considering regional seed sources.

A principle component clustering of phenotypic variables reported by Bhattarai et al. (2008) showed no strong distinctness in grouping of collections; however, a first principal component comprising biomass, seed yield, plant height, winter mortality, crude protein content, and the number of inflorescences was correlated with elevation ($r = –0.71$, $P < 0.01$). Collections with the highest values of biomass and seed yield, and lowest mortality and crude protein, were found from low elevation sites. The effect of elevation was not detected for the genetic groups in this study when corrected for linear geographic distance (Table 4). Together these data suggest that within genetic structures, elevation differences could affect phenotypic performance. Collection sites of the two genetic structures with strong support and a lack of admixed coancestry, were from relatively homogeneous elevations. British Columbia collections were from low elevations (<1000 m a.s.l.), while central Nevada collections were from high elevations (approximately 2000 m a.s.l.). Interestingly, the common garden plots reported in Bhattarai et al. (2008) were at elevations of 1350 m a.s.l. and 1370 m a.s.l., yet the collection sites from low elevations still performed the best with regards to winter mortality, biomass, seed production, and several other traits. This phenotypic plasticity suggests a broad ability of low-elevation *A. filipes* collections to adjust to environmental variables, but further studies are needed to evaluate the magnitude of effects of extremes in elevation on revegetation success in this species.

A high matrix correlation between genetic and linear geographic distance showed isolation by distance for *A. filipes* in the western United States and Canada, with an apparent center of diversity in central Oregon and structured populations in the northernmost (British Columbia, Canada) and southernmost (central Nevada, USA) collections. From a conservation perspective, our data suggest that regional seed sources could be made from the British Columbia group of collections and the central Nevada group of collections. The remaining large number of collections throughout Oregon, Idaho, northern Nevada, California, and Washington, could feasibly be combined into a third seed source, owing to the admixture present among the collections. These three sources of germplasm would be more tractable to produce and maintain than many highly localized populations, yet would minimize the risk of out-breeding depression that may occur from a single germplasm source. An intriguing future direction would test for out-breeding depression in site-transfer experiments.

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Table 3. Heirarchical AMOVA comparisons of the five groups of *Astragalus filipes* with the remaining collections.

Source of variation	df	Sums of squares	Variance components	Percent total	*P*
Five groups					
Among 5 groups	4	6910.3	13.4	13.5	<0.001
Among collections within groups	63	13,884.0	16.3	16.4	<0.001
Within collections	561	38,952.3	69.4	70.1	<0.001
British Columbia group (6) vs. other collections					
Among 2 groups	1	2355.5	24.7	21.2	<0.001
Among collections within groups	66	18438.9	22.6	19.4	<0.001
Within collections	561	38952.3	69.4	59.4	<0.001
Central Nevada group (6) vs. other collections					
Among 2 groups	1	2025.6	19.1	17.0	<0.001
Among collections within groups	66	18768.7	23.2	20.8	<0.001
Within collections	561	38952.3	69.4	62.2	<0.001
NV/UT/ID group (18) vs. other collections					
Among 2 groups	1	1953.7	6.4	6.5	<0.001
Among collections within groups	66	18840.6	23.8	23.5	<0.001
Within collections	561	38952.3	69.4	70.0	<0.001
OR/WA group (30) vs. other collections					
Among 2 groups	1	1315.5	3.3	3.4	<0.001
Among collections within groups	66	19478.9	24.4	25.1	<0.001
Within collections	561	38952.3	69.4	71.5	<0.001
Small Oregon group (7) vs. other collections					
Among 2 groups	1	920.6	4.9	5.0	<0.001
Among collections within groups	66	19873.8	25.1	25.2	<0.001
Within collections	561	38952.3	69.4	69.8	<0.001

Note: NV, Nevada; UT, Utah; ID, Idaho; OR, Oregon; WA, Washington.

Table 4. Matrix correlations of genetic distances with phenotypic, geographic, environmental, and topographic variable distances of *Astragalus filipes*.

Comparison	Matrix correlation (*r*)	*P* (random $Z \geq$ observed Z)*,†
Genetic vs. geographic	0.80	<0.001
Without BC	0.69	<0.001
Genetic vs. phenotypic*	0.08	0.198
Genetic vs. elevation	0.38	0.001
Genetic vs. mean annual precipitation	0.10	0.104
Genetic vs. mean max. temp.	0.26	0.002
Genetic vs. mean min. temp.	0.09	0.149
Genetic vs. geographic, controlling elevation	0.76	<0.001
Genetic vs. elevation, controlling geographic	–0.14	0.976
Genetic vs. geographic, controlling max temp.	0.79	<0.001
Genetic vs. max. temp. controlling geographic	0.18	0.012

*Probabilities based on 1000 permutations.

†The comparison involving the phenotypic matrix did not include British Columbia (BC) collections.

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