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Population Structure and Genetic Diversity in North American *Hedysarum boreale* Nutt.

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ABSTRACT

Hedysarum boreale Nutt. is a perennial legume native to western North America, with robust foliage in the late spring season. Due to its wide native range, forage value, and N_2 fixation, *H. boreale* is of interest for rangeland revegetation and production. Seed cost is a major obstacle for utilization of *H. boreale*, primarily due to seed shattering and unreliable seed production, such that a need for improved germplasm exists. This study characterized the genetic relationships of *H. boreale* accessions, so plant breeders and geneticists will have the information necessary to maintain a broad genetic base within selected germplasm populations. Amplified fragment length polymorphism markers were used on 17 available accessions from Utah, Idaho, Colorado, and Alaska. Seventy percent of the total genetic variation was found within all 17 accessions, yet each accession showed significant isolation by distance. Genetic diversity within accessions was greatest in sites located in eastern Utah. The sole cultivar, Timp, had slightly greater genetic diversity than a collection made from the same site approximately 20 yr later. Two groups of metapopulations were identified in Utah, separated longitudinally approximately along the Wasatch mountain range.

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Abbreviations: AFLP, amplified fragment length polymorphism; AMOVA, analysis of molecular variance.

The use of legumes on rangelands is of interest to land managers in the western USA. Revegetation efforts on rangelands after damaging disturbance have emphasized species diversity or richness to fill complementary niches (Jacobs and Sheley, 1999; Strauss et al., 2006), and legumes have been shown to deter invasion of broadleaf weeds (Sheley and Carpinelli, 2005). Legume production on rangelands, along with other forbs, was shown to enhance sage grouse habitat (Bunnell et al., 2004), and legumes have the potential to increase carrying capacity for wildlife and livestock (Plummer et al., 1968; Rumbaugh, 1983). One of the critical considerations to rangeland legume revegetation is the availability of adapted and productive species.

Hedysarum boreale Nutt. is a perennial legume native to western North America (Northstrom and Welsh, 1970), with flowering and robust foliage occurring in the spring season. The most recent taxonomic treatment of North American *Hedysarum* defined four species: *H. boreale* Nutt., *H. occidentale* Greene, *H. sulphurescens* Rybd., and *H. alpinum* L. (Welsh, 1995). Within these four species are many synonyms, epithets, and subspecies, due to the paucity of intraspecific diagnostic characters (Northstrom and Welsh, 1970; Welsh, 1995). Welsh (1995) reported that *H. boreale* was diploid with

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$2n = 2x = 16$ chromosomes. Often referred to as northern sweetvetch, boreale sweetvetch, or Utah sweetvetch, *H. boreale* is common to foothills and lower mountain elevations (Johnson et al., 1989). Primarily due to its wide native range, N_2 fixation, and value as forage, *H. boreale* has been of interest for rangeland production and revegetation (Plummer et al., 1968; Rumbaugh, 1983; Johnson et al., 1989).

The major obstacle in the utilization of *H. boreale* is the cost of seed. Initially, *H. boreale* seed comprised wildland collections, with corresponding erratic quantities and unknown quality. A previous study in *H. boreale* productivity (Johnson et al., 1989) yielded the sole cultivar, Timp, originating from a wildland collection in north-central Utah (Stevens et al., 1994). Timp was a combination of the original collected seed and extant selections for superior N_2 fixation and seed production (Stevens et al., 1994). However, Timp is still prone to poor seed set and seed shattering, such that prices range from US\$88 to US\$198 kg^{-1}. As part of an interest in developing improved germplasm, public and private land management agencies that use *H. boreale* germplasm are interested in the relationships among existing germplasm.

The objective of this study was to characterize the genetic diversity and relationships among available *H. boreale* accessions before initiating a breeding program. Neutral amplified fragment length polymorphism (AFLP) markers were used to genotype collections that were available on initiation of the study, with samples occurring mainly in Utah. Our results provide genetic relatedness among and within the collections, and can be used to maximize genetic diversity and broaden the genetic base for use in germplasm and cultivar development.

MATERIALS AND METHODS

Seventeen *H. boreale* accessions were used, representing the available germplasm: 12 wild-land collections provided by the Great Basin Research Center (Ephraim, UT), four accessions from the National Plant Germplasm System (Pullman, WA), and 2002 certified seed from the cultivar Timp (Table 1). Included in these accessions was an accession from Alaska, and an accession from the Wasatch Front, Utah. The Alaskan accession had no source location information, and is reported as the subspecies *mackenziei* (Table 1). The Wasatch Front accession putatively comprised collections throughout north-central Utah, with no single or specific site information. Approximately 20 plants per accession were analyzed, but for several accessions fewer individuals germinated (Table 1). The map of the collection sites was made using ArcGis Version 9.0 (ESRI, Redlands, CA). Herbarium voucher specimens for most accessions are available at the Intermountain Herbarium, Utah State University, Logan (Table 1). An additional two populations of *H. occidentale* were included as an out-group for phylogenetic reconstructions.

Seeds were planted in conetainers (Stuewe and Sons, Corvallis, OR) in a greenhouse in Logan, UT. Fresh seedling tissue

Table 1. Source information of 17 *Hedysarum boreale* Nutt. and two *H. occidentale* Greene accessions used in this study, with the percent polymorphic loci and average similarity index.

Species	Seed source†	Site name	Herbarium voucher	Plants	Polymorphic Loci	Similarity ± SE‡
				no.	%	
H. boreale Nutt.	Stevenson Seed	cv. Timp	n/a§	19	33	0.809 ± 0.005
H. boreale Nutt.	GBRC-UH5	Orem water tank	UTC 238053	20	29	0.825 ± 0.004
H. boreale Nutt.	GBRC-UH7	Payson	UTC 238062	20	27	0.839 ± 0.005
H. boreale Nutt.	GBRC-UH11	Echo Reservoir	UTC 238050	20	29	0.819 ± 0.005
H. boreale Nutt.	GBRC-UH9	Twelve-Mile Canyon	UTC 238061	20	32	0.802 ± 0.007
H. boreale Nutt.	NPGS-DLEG 900004	Wasatch Front	n/a	13	29	0.802 ± 0.012
H. boreale Nutt.	GBRC-UH12	Cutoff	UTC 238052	20	36	0.791 ± 0.006
H. boreale Nutt.	GBRC-UH6	Dry Fork	UTC 238057	20	37	0.789 ± 0.007
H. boreale Nutt.	GBRC-UH8	Rabbit Gulch	UTC 238054	20	30	0.807 ± 0.007
H. boreale Nutt.	GBRC-UH13	Willow Creek	UTC 238060	20	36	0.797 ± 0.008
H. boreale Nutt.	GBRC-UH10	Nine Mile Lower	UTC 238055	20	32	0.808 ± 0.007
H. boreale Nutt.	GBRC-UH18	San Rafael Swell	UTC 238056	20	30	0.816 ± 0.005
H. boreale Nutt.	GBRC-UH15	Escalante	UTC 238051	18	26	0.838 ± 0.004
H. boreale Nutt.	NPGS-DLEG 900300	Jefferson County, CO	n/a	6	17	0.828 ± 0.009
H. boreale Nutt.	NPGS-W6 17266	Custer County, ID	n/a	20	22	0.848 ± 0.006
H. boreale Nutt.	GBRC-UH14	Antelope Butte, ID	UTC 238058	19	21	0.871 ± 0.010
H. boreale Nutt. ssp. *mackenziei* (Richardson) S.L. Welsh	NPGS-AG 255	Alaska	n/a	19	23	0.859 ± 0.009
H. occidentale Greene	GBRC-UH19	Nine Mile Lower	UTC 238049	18	11	0.927 ± 0.005
H. occidentale Greene	GBRC-UH16	Joes Valley	n/a	20	13	0.918 ± 0.005

†GBRC = Great Basin Research Center, Ephraim, UT; NPGS = National Plant Germplasm Service.

‡Mean similarity index calculated according to Leonard et al. (1999).

§Not available.

was harvested and DNA extracted using the DNeasy 96-well extraction kit (QIAGEN, Valencia, CA). The 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.ACA/M.CTC, E.AGA/M.CTG, E.AGC/M.CTA, E.AGG/M.CAT, E.AGG/M.CTC, E.AGT/M.CAC, and E.AGT/M.CTT. Primer sets were chosen and genotyped with *H. occidentale* included, and all bands were scored. Amplicons were separated on a capillary ABI 3700 instrument with the GS-500 LIZ size standard and GeneScan software (Applied Biosystems, Foster City, CA). Individual profiles were visualized and manually scored for the presence or absence of fragments with Genographer software (Benham, 2001).

Diversity of the 19 accessions was estimated with the similarity index (S) (Leonard et al., 1999). Average within-accession S values and their variances were computed, and t tests on all pairwise comparisons were then generated according to Leonard et al. (1999), with the null hypothesis that the two average within-accession S values were equal. Vector geographic distances between sites were computed with ArcGIS Version 9.1 (ESRI, Redlands, CA), and the Pearson correlation coefficient between S values and vector distances was determined with the CORR procedure of SAS (SAS Institute, 2003).

Population subdivision of the 17 *H. boreale* accessions was tested using two methods: analysis of molecular variance (AMOVA) and Bayesian clustering. In the former test, raw binary data was converted to Euclidean distances. The resulting distance matrix constituted the input file for AMOVA using the Arlequin 2.0 software (Schneider et al., 2000), with and without the two *H. occidentale* accessions. The average number of pairwise differences between populations, resulting from the AMOVA procedure, comprised the user-defined input matrix for the construction of a neighbor-joining dendrogram using PAUP* Version 4.0b (Swofford, 2002). A further hierarchical AMOVA test was conducted to test the main Utah clades of the dendrogram (Schneider et al., 2000), withholding the Idaho and Alaska accessions. Matrix correlation between geographic and genetic distances was estimated with Mantel's test statistic Z (Mantel, 1967), using the MXCOMP procedure of NTSYS (Rohlf, 2000). The genetic distances were represented as the average number of pairwise differences between populations from the AMOVA procedure.

Bayesian clustering without a priori assignment of individuals to geographic areas or populations was performed with Structure Version 2.1 (Pritchard et al., 2000). Raw binary data was used as input by inserting the alternate alleles as missing values. The AFLP data were analyzed using an admixture model with correlated marker frequencies. Population sizes for the number of populations (K) = 2 through K = 9 structures were tested with four replications per analysis. The Markov chain Monte Carlo (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 structure selected was at the K value where the log-likelihood reached an asymptote.

RESULTS

The geographic distribution of *H. boreale* accessions tested in this study is shown in Fig. 1. A total of 1629 markers were scored on 333 individuals. The average total number of AFLP bands in all the *H. boreale* accessions was 375 ± 15, indicating similar genome complexity and ploidy levels

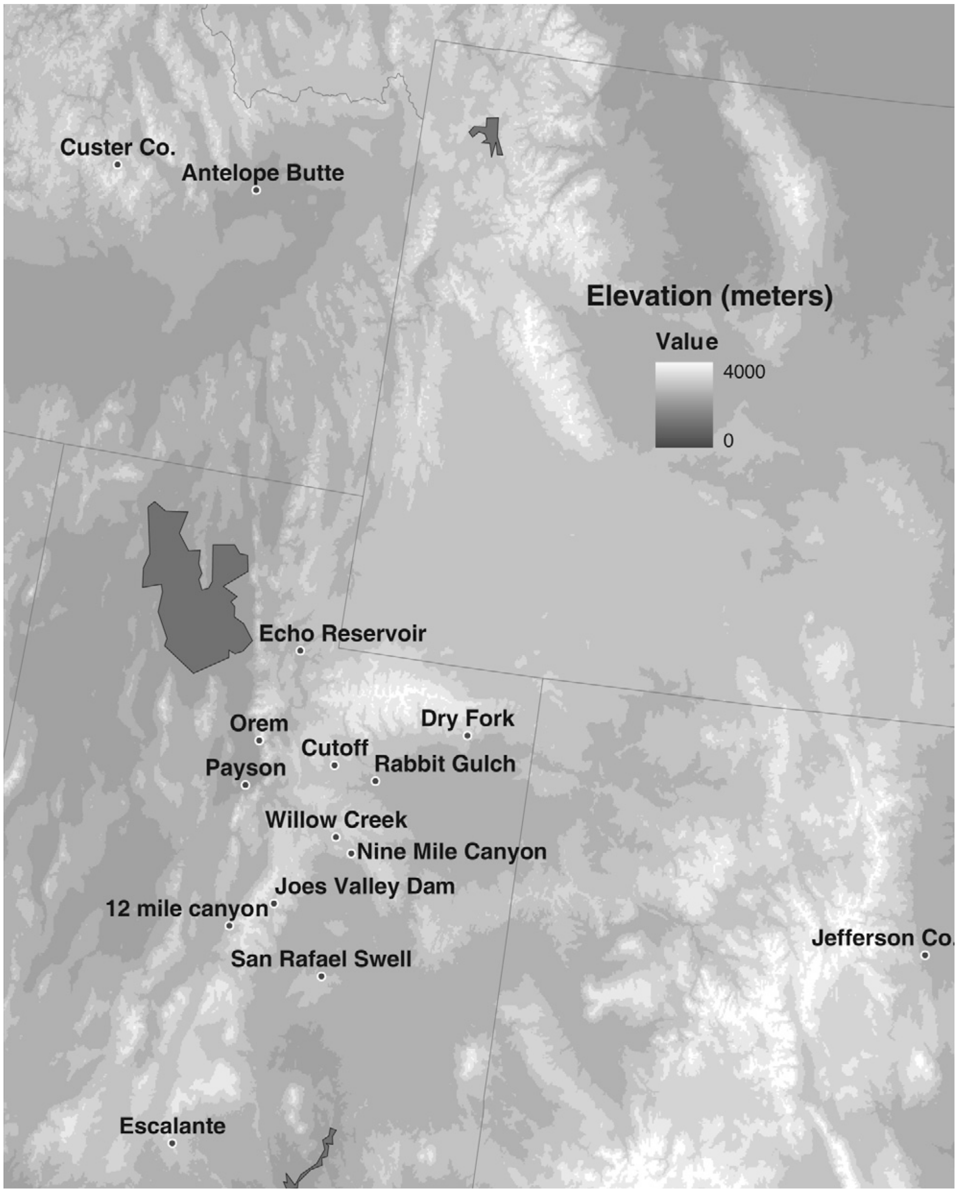

Figure 1. Map of collection sites (except Alaska) for *Hedysarum boreale* Nutt. and *H. occidentale* Greene, with elevation and large bodies of water.

Table 2. Matrix of the average similarity indices (*S*) within 17 *Hedysarum boreale* Nutt. accessions (diagonal), and *t* tests for significant difference of *S* values for pairwise comparisons among accessions. Approximately 20 plants per accession were tested with 1629 amplified fragment length polymorphism markers.

Site	'Timp'	Orem	Payson	Echo Reservoir	Twelve-Mile Canyon	Wasatch Front	Cutoff	Dry Fork	Rabbit Gulch	Willow Creek	Nine-Mile Canyon	San Rafael	Escalante	Jefferson, CO	Custer, ID	Antelope, ID	Alaska
'Timp'	0.809																
Orem	*	0.825															
Payson	***	*	0.839														
Echo Reservoir	NS†	NS	**	0.819													
Twelve-Mile Canyon	NS	**	***	NS	0.802												
Wasatch Front	NS	NS	**	NS	NS	0.802											
Cutoff	*	***	***	***	NS	NS	0.791										
Dry Fork	*	***	***	***	NS	NS	NS	0.789									
Rabbit Gulch	NS	*	***	NS	NS	NS	NS	NS	0.807								
Willow Creek	NS	**	***	*	NS	NS	NS	NS	NS	0.797							
Nine-Mile Canyon	NS	*	***	NS	NS	NS	NS	*	NS	NS	0.808						
San Rafael	NS	NS	**	NS	NS	NS	**	**	NS	*	NS	0.816					
Escalante	***	*	NS	**	***	**	***	***	***	***	***	**	0.838				
Jefferson, CO	NS	NS	NS	NS	NS	NS	**	**	NS	NS	NS	NS	NS	0.828			
Custer, ID	***	**	NS	***	***	**	***	***	***	***	***	***	NS	NS	0.848		
Antelope, ID	***	***	**	***	***	***	***	***	***	***	***	***	**	**	NS	0.871	
Alaska	***	**	NS	***	***	***	***	***	***	***	***	***	*	*	NS	NS	0.859

* Significant at the 0.05 level.

** Significant at the 0.01 level.

*** Significant at the 0.001 level.

†NS = not significant at 0.05.

($2n = 2x = 16$; Welsh, 1995). When the total set of markers was considered, the percentage of polymorphic AFLP loci ranged from 37% in the Dry Fork site to 17% in the Colorado site (Table 1).

The mean *S* value within the *H. boreale* accessions ranged from 0.87 to 0.79 (Table 1), with the highest *S* at the Antelope Butte site and the lowest at the Dry Fork site. Paired *t* tests on all two-accession comparisons, except Alaska and Wasatch Front, showed that the sites outside of Utah had higher intraaccession *S* values than those in north-central Utah (Table 2). As the distance from other accession sites to the Dry Fork site increased, the intraaccession *S* values increased (diversity decreased; $r = 0.81$, $P = 0.0004$). The Orem accession and the Timp cultivar, collected from the same site, had unequal mean *S* values and the Timp cultivar was more diverse (Table 2). Both *H. occidentale* accessions exhibited substantially lower percentages of polymorphic loci and genetic diversity within accessions than the *H. boreale* accessions (Table 1).

Using AMOVA to test for population subdivision in *H. boreale* accessions, approximately 70% of the variation was apportioned within the accessions, and the remaining 30% was apportioned among accessions (Φst). Pairwise Φst values between each pair of accessions were calculated, representing the apportioned genetic variation between the two accessions relative to within the two. All pairwise Φst were significant at $P < 0.001$ (Table 3), indicating distinct population structure for each accession. The smallest pairwise Φst was 0.04, corresponding to the comparison of the Echo Reservoir and Twelve-Mile Canyon accessions (Table 3). All other small Φst values relative to the overall mean were between accessions in close geographic proximity: Timp and Orem, Willow Creek and Nine-Mile Canyon, and Cutoff and Rabbit Gulch (Table 3). The largest Φst value was 0.53, contrasting the Alaska and Escalante accessions. Mantel's *Z* statistic was used to compare a genetic distance matrix of the Φst values with a geographical distance matrix.

The resulting correlation of genetic and geographic distances was $r = 0.80$ ($P < 0.01$) without the Alaska site, and $r = 0.76$ ($P < 0.01$) with the Alaska accession, indicating isolation by distance.

Four main genetic clades were distinguished with a neighbor joining tree: the Alaska accession, two Idaho accessions, eastern Utah accessions, and the western Utah accessions (Fig. 2). The Colorado site grouped with the eastern Utah accessions. Both Idaho sites formed a separate clade from the Utah accessions. Hierarchical AMOVA revealed that 11% of the variation was apportioned between the eastern and western Utah clades, and 15% of the variation was apportioned among the accessions within the two clades ($P < 0.001$, 1000 permutations). The remaining 74% of variation was apportioned within accessions.

The Bayesian clustering model with the highest statistical support consisted of seven groups, but a trend separating eastern and western Utah accessions persisted in tests from three to nine groups. The seven groups of accessions from the clustering model corresponded to clades in the phylogenetic tree (Fig. 2), with additional separation of the western Utah clade into two groups and the eastern Utah clade into three groups (Fig. 2). Also within the model of seven structures, partial admixture was detected in the Willow Creek and Wasatch Front accessions. The Willow Creek plants contained 63% similarity to other eastern Utah accessions and 34% similarity to western Utah accessions. The Wasatch Front accession is of mixed, putatively western Utah origin, and showed admixture solely among western Utah accessions.

DISCUSSION

In this study, 70% of the genetic variation was apportioned within *H. boreale* accessions, showing high amounts of diversity commonly observed in out-crossing plant species. Considering the amount of within-population variation, much of the DNA polymorphism present in the species of this region are contained in any of the natural source populations, and could be used to develop improved germplasm. Accession sites in northeastern Utah had the highest levels of genetic diversity, while those sites in Colorado, Idaho, and Alaska had the lowest (Table 1). Although these differences in diversity were marginal and in some instances insignificant (Table 2), the trend indicates that the most genetically diverse *H. boreale* germplasm within the geographic scope of our study exists in eastern Utah.

In an attempt to revise the treatment of North American *Hedysarum* taxa and address their phylogeny, Northstrom and Welsh (1970) proposed a pre-Pleistocene divergence of *H. boreale* and its subspecies *mackenziei* imposed by glacial ice sheets. This geographic isolation would have been accompanied by less gene flow and

Table 3. Pairwise matrix of the percentage of variation apportioned among accessions (Φst) of 17 *Hedysarum boreale* Nutt. accessions. Approximately 20 plants per accession were tested with 1629 amplified fragment length polymorphism markers. All pairwise comparisons were significantly different at *P* < 0.001.

Site ID	'Timp'	Orem	Payson	Echo Reservoir	Twelve-Mile Canyon	Wasatch Front	Cutoff	Dry Fork	Rabbit Gulch	Willow Creek	Nine-Mile Canyon	San Rafael	Escalante	Jefferson, CO	Custer, ID	Antelope, ID	Alaska
'Timp'	0																
Orem	0.061	0															
Payson	0.122	0.157	0														
Echo Reservoir	0.120	0.189	0.216	0													
Twelve-Mile Canyon	0.119	0.173	0.203	0.036	0												
Wasatch Front	0.095	0.167	0.219	0.117	0.108	0											
Cutoff	0.195	0.220	0.259	0.211	0.170	0.220	0										
Dry Fork	0.234	0.245	0.284	0.283	0.255	0.293	0.098	0									
Rabbit Gulch	0.250	0.262	0.313	0.283	0.244	0.286	0.060	0.104	0								
Willow Creek	0.191	0.222	0.261	0.188	0.152	0.200	0.124	0.168	0.171	0							
Nine-Mile Canyon	0.218	0.263	0.289	0.219	0.192	0.252	0.157	0.179	0.195	0.054	0						
San Rafael	0.261	0.291	0.321	0.292	0.270	0.310	0.165	0.165	0.187	0.172	0.176	0					
Escalante	0.315	0.326	0.363	0.338	0.284	0.338	0.218	0.241	0.246	0.235	0.276	0.253	0				
Jefferson, CO	0.292	0.324	0.364	0.331	0.287	0.301	0.232	0.245	0.276	0.258	0.293	0.297	0.310	0			
Custer, ID	0.321	0.364	0.390	0.342	0.325	0.322	0.382	0.419	0.431	0.402	0.436	0.441	0.460	0.455	0		
Antelope, ID	0.333	0.356	0.384	0.348	0.330	0.342	0.400	0.432	0.450	0.417	0.456	0.456	0.482	0.502	0.272	0	
Alaska	0.449	0.479	0.501	0.477	0.452	0.464	0.454	0.471	0.489	0.461	0.490	0.497	0.532	0.513	0.499	0.526	0

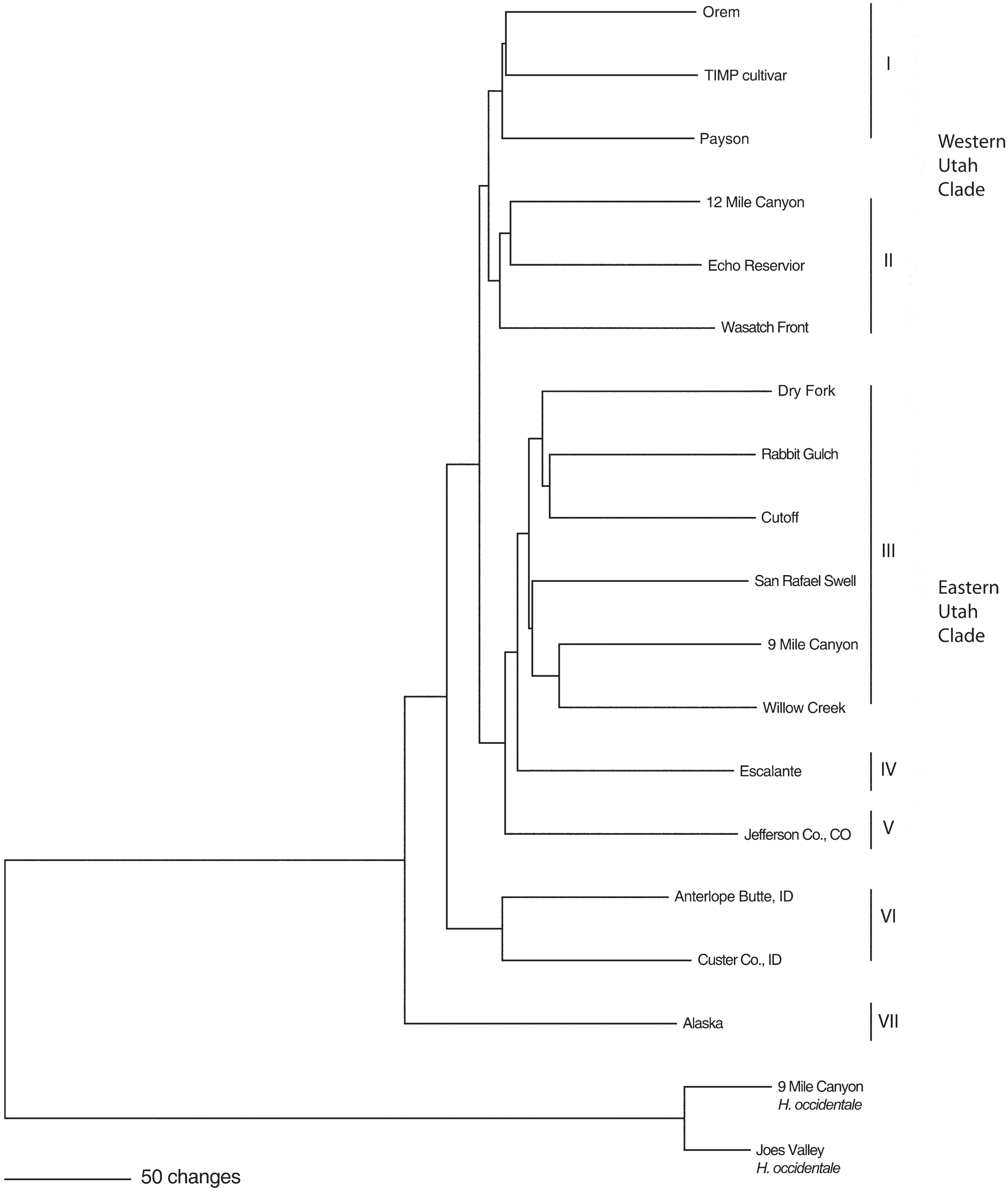

Figure 2. Neighbor joining dendrogram of 17 *Hedysarum boreale* Nutt. accessions and two *H. occidentale* Greene accessions. Roman numerals designate seven groups detected by Bayesian clustering without a priori defining populations.

phenotypic variation within the subspecies *mackenziei* ancestors than those of *H. boreale* due to a smaller geographic range and more uniform, harsher environmental conditions at the time. In our data, the sole Alaskan accession was not significantly less diverse than several accessions in Idaho and Utah (Table 2). The slight diversity differences between subspecies *mackenziei* and *H. boreale* appear unsubstantial for recognition as a subspecies, but a larger sampling across the continuum of the geographic ranges would be necessary to more adequately address the differences in diversity.

Analysis of molecular variance found 30% of the total variation apportioned among accessions. Similar apportionment of variation estimates among populations for a variety of other plant species reported values from 12 to 45% for allogamous species (Huh and Huh, 2001; Jorgensen et al., 2002; Kolliker et al., 1999; Larson et al., 2003), and >80% for predominantly autogamous grass species (Larson et al., 2001, 2003). The *H. boreale* Φst was within the range of values from the allogamous species, and indicates significant population structure among the accessions. Each accession was distinct from all others (Table 3), but no AFLP band was completely diagnostic for an accession. This population isolation could reflect drift or partial barriers to gene flow, but does not completely preclude gene flow.

Larger groups of accessions were detected by the neighbor-joining tree, hierarchical AMOVA, and Bayesian clustering. The Bayesian clustering identified seven groups without a priori assignment based on geographic locale, which corresponded to clades in the neighbor-joining tree. As the Idaho and Alaska accession sites were separated from Utah collections by large geographic distances, however, wherein no sample locations were tested, caution should be used in assuming their independent groupings. Prominent among the groups of accessions was the longitudinal separation of Utah accessions. The eastern and western Utah accessions are separated by the Wasatch mountain range, extending north–south through central Utah (Fig. 1). The range probably provides a geographic barrier around which isolation could occur. As the significant apportionment of variation among the two Utah groups was smaller than the other two sources of AMOVA variation, and genetic diversity was only marginally less in western Utah accessions, this separation into metapopulations is probably immature.

The Timp cultivar is derived from a 1:1 ratio of seed from selected and nonselected plants originating from the same geographic site as Orem, but collected approximately 20 yr previous (Johnson et al., 1989; Stevens et al., 1994). The Φst between Timp and the Orem accession was significantly different, and the difference was larger than pairwise comparisons of several other accessions where geographic separation was larger (Table 3). The test for diversity between Timp and the Orem accession also indicated a significant difference, but the cultivar, which underwent artificial selection before release, was more genetically diverse (Table 2). Assuming approximately equal sampling, these differences between Timp and the Orem accession may be explained by a genetic bottleneck of the original site that occurred in the time frame between collections of the Timp and Orem seed, or possibly by seed contamination during the years of Timp cultivar propagation. It is encouraging, however, not to detect diminished genetic diversity in the cultivar.

Although *H. occidentale* was intended to be included only as an out-group for phylogenetic reconstructions, its diversity estimates suggest a different reproductive system from that of *H. boreale*. Both *H. occidentale* accessions had a substantially lower percentage of polymorphic loci and less genetic diversity than the *H. boreale* accessions (Table 1). A similar difference in genetic diversity was found for a pair of Mediterranean *Hedysarum* species, *H. spinosissimum* L. ssp. *capitatum* and ssp. *euspinosissimum* (Baatout et al., 1991). The former is allogamous and the latter is autogamous. The autogamous subspecies *euspinosissimum* had significantly less diversity than the allogamous subspecies *capitatum*, similar in magnitude to the differences between *H. boreale* and *H. occidentale* in the present study. Additionally, in the present study, genetic similarity within accessions of *H. occidentale* was >90%, similar in magnitude to other autogamous grass species (Larson et al., 2001; Pakniyat et al., 1997). Although we have found no record of the mode of pollination of *H. occidentale*, based on the lower diversity values we predict a larger portion of autogamy than that in *H. boreale*.

Buyers of seed for range and forest lands often consider conservation perspectives in addition to plant production, including interest in the source of improved germplasm, how improved germplasm relates to the local plant material, and its likelihood of fitness success once planted and left alone. Results from this study can aid germplasm development efforts in addressing those perspectives. Plant breeding efforts have the option of incorporating the population structure data to create a broad-based improved germplasm, or of placing improved germplasm in geographic and genetic context with populations in this study. Genetic diversity and fitness have a positive correlation in plants (Reed and Frankham, 2003), especially in allogamous perennials (Leimu et al., 2006), and maximizing genetic diversity would increase the likelihood of fitness of the resulting improved germplasm.

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