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Searls Prairie Clover (*Dalea searlsiae*) for Rangeland Revegetation: Phenotypic and Genetic Evaluations

Kishor Bhattarai, B. Shaun Bushman,* Douglas A. Johnson, and John G. Carman

ABSTRACT

Few North American legumes are available for use in rangeland revegetation in the western USA, but Searls prairie clover [*Dalea searlsiae* (A. Gray) Barneby] is one that holds promise. Commercial-scale seed production of this species could address the issues of unreliable seed availability and high seed costs associated with its wildland seed collection. To evaluate its utility for revegetation, we collected Searls prairie clover at 20 locations across Utah and Nevada. Amplified fragment length polymorphisms (AFLP) and morphological and phenotypic traits (measured in common-garden plots) were used to clarify the role of evolutionary forces responsible for its genetic structure. Collections were evaluated for dry-matter yield, inflorescence weight, number of inflorescences, plant height, foliage diameter, flowering date, acid-detergent fiber, neutral-detergent fiber, and crude protein at two common-garden locations in northern Utah. Collections from southern Utah and eastern Nevada exhibited high phenotypic values, whereas collections from western Nevada and northwestern Utah had low phenotypic values. Collections from northwestern Utah were genetically differentiated from those of southern Utah and Nevada via AFLP markers. Strong isolation by distance between collections suggests that genetic drift and gene flow are important factors in determining population structure in Searls prairie clover.

K. Bhattarai and J.G. Carman, Dep. of Plants, Soils, and Climate, Utah State Univ., Logan, UT 84322-4820; B.S. Bushman and D.A. Johnson, USDA-ARS Forage and Range Research Lab., Utah State Univ., Logan, UT 84322-6300. Mention of a proprietary product does not constitute a guarantee or warranty of the product by USDA, Utah State University, or the authors and does not imply its approval to the exclusion of other products. Received 1 July 2010. *Corresponding author: (Shaun.Bushman@ars.usda.gov)

Abbreviations: ADF, acid-detergent fiber; AFLP, amplified fragment length polymorphism; AMOVA, analysis of molecular variance; CP, crude protein; DMY, dry-matter yield; Dp, *Dalea purpurea*; Ds, *Dalea searlsiae*; MCMC, Markov chain Monte Carlo; NDF, neutral-detergent fiber; NJ, neighbor-joining; PC, principal component

The spread of invasive weeds in the Great Basin region has resulted in its identification as the third most endangered ecosystem in the USA (Stein et al., 2000). A large portion of rangeland in this region burns each year (4.97×10^5 ha, the average from 1998 to 2007; Mike Pellant, personal communication, 2009), and if left untreated, weed invasions will probably accelerate (Jessop and Anderson, 2007). Postfire revegetation requires large amounts of seed. For example, in 2007 the Bureau of Land Management purchased more than 3×10^6 kg of seed for revegetation. A mix of species is preferred in seed mixtures for revegetation because functionally diverse plant species are more likely to minimize the risk of weed invasion and increase ecosystem function (Pokorny et al., 2005; Sheley and Carpinelli, 2005; Walker and Shaw, 2005). North American legumes are of particular interest for inclusion in seed mixtures because they fix nitrogen, thus enhancing the nitrogen content of forage (van der Heijden et al., 2006), as well as improving wildlife forage and habitat (Madison

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and Robel, 2001). Seeds of North American legumes for use in revegetation programs are usually harvested from wildlands, a practice that results in unreliable seed availability and high seed prices, in turn limiting the use of these legumes (Walker and Shaw, 2005). Commercial seed production could reduce seed costs and make such species more readily available for rangeland revegetation.

Plant materials for rangeland revegetation are considered to be appropriate if they are adapted to and compatible with the local revegetation environment, do not pose the risk of gene transfer to related species that do not already occur naturally, are nontoxic to herbivores (because a large portion of western rangelands are used for livestock grazing), and are amenable to commercial seed production to ensure a reliable quantity and quality of seeds. Searls prairie clover [*Dalea searlsiae* (A. Gray) Barneby] is a perennial legume that naturally occurs in the southern Great Basin, the southwestern Colorado Plateau, and northern Arizona (USDA-NRCS, 2010). It is diploid ($2n = 14$, or rarely, 16) and is primarily insect pollinated (Barneby, 1977; Jim Cane, personal communication, 2009). Its natural distribution within the southern Great Basin ameliorates gene-transfer concerns in that region because hybridization with related species would have occurred naturally, if at all. Compounds that are toxic to livestock and wildlife and are found in some other legumes were below detectable levels in Searls prairie clover (unpublished data). Additionally, its relatively upright growth habit makes Searls prairie clover a potential candidate for commercial seed production.

Local germplasm has been recommended for use in revegetation to reduce the possibility of maladaption or outbreeding depression (Lesica and Allendorf, 1999; McKay et al., 2005). However, disturbances and global changes on rangelands may alter the ecosystem such that local germplasm is no longer optimal (Broadhurst et al., 2008), and maintaining and producing many local germplasm accessions for seed production can be difficult for seed growers (McKay et al., 2005; Broadhurst et al., 2008). The latter issue may lead to increased seed costs because the demand for local germplasm is unpredictable, given the sporadic nature of wildfires and subsequent variable need for local germplasm. Using phenotypic and genomic assessments to pool seed sources for native species into larger regional levels can minimize both the number of seed sources required for use in revegetation and restoration programs and the risk of maladaptation and outbreeding depression.

The suitability of regional seed sources can be determined by phenotypic and genotypic comparisons (Bhattarai et al., 2010). Common-garden plots provide a relatively uniform environment for phenotypic evaluation, such that the differences between populations are primarily due to genetic differences or to the interaction between genes and the environment. Phenotypic differences, if correlated with the climatic variables of source collection sites, suggest the possibility of local adaption (Endler, 1986). Climatic variables that correlate with common-garden phenotypes can be used to delineate regional seed sources, as has been done for tree species (Johnson et al., 2004). Neutral DNA markers (e.g., amplified fragment length polymorphisms, or AFLPs) measure overall genetic diversity due to evolutionary forces, such as gene flow, founder effects, selection, and genetic drift. As a result, genetic diversity data obtained from DNA markers indicate the evolutionary potential of populations (McKay and Latta, 2002). In addition, DNA-marker analyses provide estimations of inbreeding depression based on diversity within a collection, as well as group differentiation based on variation among collections (Ouborg et al., 2006; Bonin et al., 2007).

When the distribution of populations is beyond the gene-flow capability, isolation by distance can occur. Isolation by distance is usually detected using the Mantel test (Mantel, 1967), by a positive correlation between genetic and geographic distances among individuals. Strong isolation by distance indicates that populations are at equilibrium with respect to gene flow and genetic drift (Slatkin, 1993). However, other factors, such as precipitation, temperature, elevation, or disturbance, can influence isolation by distance (Kittlein and Gaggiotti, 2008); therefore, a partial Mantel test is often used to account for the influence of other factors when isolation by distance is estimated (Telles and Diniz-Filho, 2005).

The objective of our study was to evaluate phenotypic and DNA-marker-based variation of 20 wildland collections of Searls prairie clover. Forage production and quality, flowering date, inflorescence weight, and growth habit were assessed in two common gardens, and genetic diversity among and within collections was determined using AFLP markers. With these data, the role of genetic drift and gene flow among populations were evaluated. We also identified potentially adaptive phenotypic traits and regional seed sources that might minimize the possibility of outbreeding depression and maladaptation while providing a tractable number of seed sources for germplasm development.

MATERIALS AND METHODS

Plant Material Collection

Seed of Searls prairie clover was collected from 20 sites in Utah and Nevada during the summer of 2005 (Fig. 1). Elevation, latitude, and longitude data were obtained for each collection site (Table 1). Mean annual temperature and precipitation (average from 1961 to 1990) of the collection sites were obtained from the Moscow Forestry Sciences Laboratory (2009). A 5- to 15-g section of insecticide strip (active ingredient dochlorvos [2, 2-dichlorovinyl dimethyl phosphate] 18.6% and related compounds 1.4%, Hotshot No-Pest strip, United Industries Corp., St. Louis, MO) was placed in each bag of collected seed to reduce seed weevil damage. The seeds were air-dried in a greenhouse, threshed with a Wintersteiger seed thresher (model LD180, Wintersteiger, Des Moines, IA), and cleaned with handheld sieves and

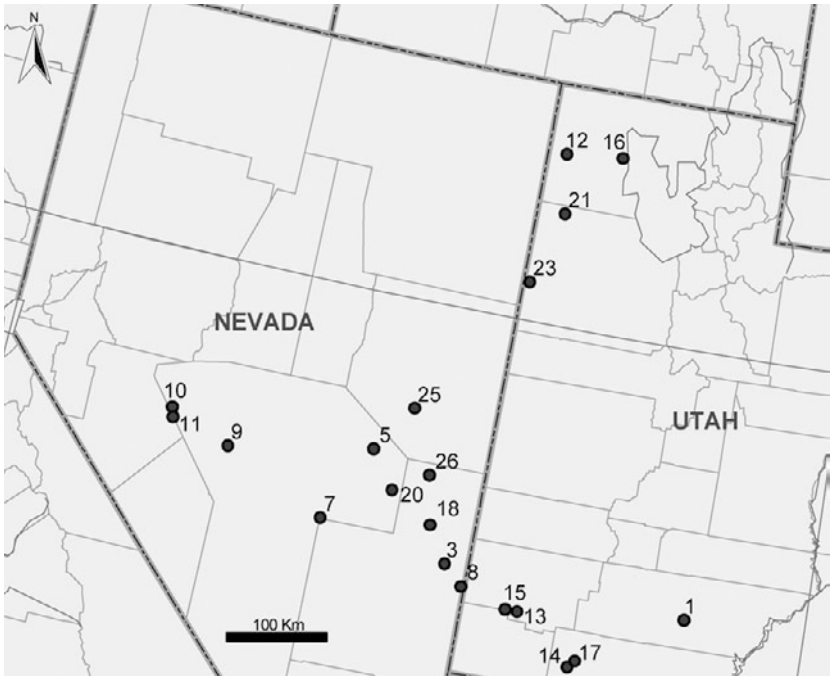

Figure 1. Map depicting collection sites of Searls prairie clover (*Dalea searlsiae*).

Table 1. Site information and number of individuals used for average similarity value and polymorphic loci for 20 collections of Searls prairie clover (*Dalea searlsiae*).

Collection	County and state	Latitude	Longitude	Elevation	Mean annual temperature	Mean annual precipitation	No. of individuals	Average similarity value	Polymorphic loci
				m	°C	mm		S	%
Ds-01	Garfield, UT	37°46′ N	111°25′ W	1604	11.3	197	8	0.70	28.0
Ds-03	Lincoln, NV	37°53′ N	114°19 ′ W	1614	10.2	255	8	0.69	33.4
Ds-05	Nye, NV	38°42′ N	115°25′ W	1695	9.0	214	8	0.64	39.0
Ds-07	Nye, NV	38°03′ N	115°52′ W	1890	9.0	238	8	0.65	37.4
Ds-08	Lincoln, NV	37°43′ N	114°05′ W	1796	8.9	291	8	0.64	38.4
Ds-09	Nye, NV	38°28′ N	117°08′ W	2000	8.2	185	6	0.66	28.6
Ds-10	Nye, NV	38°41′ N	117°53′ W	1646	9.9	152	7	0.65	32.6
Ds-11	Mineral, NV	38°36′ N	117°51′ W	1888	8.6	171	8	0.68	30.8
Ds-12	Box Elder, UT	41°25′ N	113°48′ W	1413	8.8	209	8	0.73	24.8
Ds-13	Iron, UT	37°36′ N	113°22′ W	1943	8.3	372	8	0.69	29.4
Ds-14	Kane, UT	37°13′ N	112°41′ W	1695	10.7	347	8	0.64	32.6
Ds-15	Washington, UT	37°36′ N	113°31′ W	1732	9.5	336	8	0.66	31.6
Ds-16	Box Elder, UT	41°28′ N	113°06′ W	1597	8.3	267	8	0.77	25.0
Ds-17	Kane, UT	37°17′ N	112°36′ W	1749	10.1	343	8	0.66	35.2
Ds-18	Lincoln, NV	38°10′ N	114°35′ W	1834	9.4	305	8	0.66	37.6
Ds-20	Nye, NV	38°24′ N	115°06′ W	1597	9.9	238	8	0.67	33.8
Ds-21	Toole, UT	40°56′ N	113°41′ W	1326	10.0	172	8	0.74	27.6
Ds-23	Toole, UT	40°19′ N	113°57′ W	1419	9.7	178	8	0.75	25.6
Ds-25	White Pine, NV	39°06′ N	115°02′ W	1950	7.2	249	8	0.66	32.8
Ds-26	Lincoln, NV	38°35′ N	114°42′ W	2036	7.6	321	8	0.67	32.6
Dp†	—	—	—	—	—	—	8	0.67	31.8

† Purple prairie clover (Dp) was used as an out-group.

a seed blower. Cleaned seeds were stored in a dark room maintained at 3°C with a relative humidity of 20–25%.

Common-Garden Plots

Seeds were germinated at room temperature in plastic boxes with moistened blotter paper. After germination, the seedlings were transplanted into Q-Plugs (International Horticulture Technologies, Hollister, CA), placed within Ray Leach stubby Conetainers (Stuewe and Sons, Corvallis, OR), and grown in a greenhouse at the USDA-ARS Forage and Range Research Laboratory, Logan, UT, under a 30°C-day–15°C-night temperature regime. A single seedling was grown in each Conetainer. Fertilization and watering were provided for 90 d before the seedlings were transplanted to field plots at Millville (lat 41°

39′ N, long 111° 48′ W, 1350 m.a.s.l.) and Hyde Park (lat 41° 47′ N, long. 111° 48′ W, 1370 m.a.s.l.) in northern Utah. Because of poor seedling establishment in the greenhouse, collection Ds-01 was not included at Hyde Park. The soil at Millville is a Millville silt loam (coarse-loamy over sandy or sand-skeletal, mixed, superactive mesic, Calcic Haploxerolls), and the soil at Hyde Park is a McMurdie silt loam (fine, montmorillonitic, mesic, Calcic Pachic Argixerolls). The experimental design at each location was a randomized complete block with eight replications of 5 individual plants, for a total of 40 plants of each collection at each location. Plants were spaced 0.5 m apart within rows and between rows. Plots were planted in May 2006, routinely weeded and watered during the establishment year, and regularly weeded without irrigation in subsequent years. Plants of a commercially available purple prairie clover (*Dalea purpurea* Vent.), a related species indigenous to the Great Plains of the USA, were included as checks (designated as Dp) at both locations. Seed of purple prairie clover came from transplants originating from a 40-acre commercial production field that was initiated from a 0.5-kg pool of seed harvested from five prairie-remnant sites in southern Wisconsin (Oak Prairie Farm, Pardeeville, WI). Measurements were taken during the second and third years after establishment (2007 and 2008).

Dry-matter yield (DMY), the number of inflorescences, and the flowering date were measured at both locations. Forage-quality characteristics, including acid-detergent fiber (ADF), neutral-detergent fiber (NDF), and crude protein (CP), were evaluated at Hyde Park. Plant height, the number of stems, and the foliage diameter were also measured at Hyde Park. Potential seed production was estimated at Millville by determining the weight of the inflorescences.

Collections at both locations were evaluated for DMY with two harvests taken during each growing season and combined at each location. At Hyde Park, plants were harvested at approximately 50% bloom (19 June 2007 and 15 June 2008), and then after the first frost in October (17 Oct. 2007 and 25 Oct. 2008). At Millville, DMY was obtained after mature inflorescence harvest (26 July 2007 and 4 Aug. 2008), and then after the first frost in October (17 Oct. 2007 and 26 Oct. 2008). The number of inflorescences was determined at 50% bloom at Hyde Park and immediately before inflorescence harvest at Millville. In both years, the flowering date was recorded as the number of days from 1 January until the first flower emerged on each plant and was averaged across the five plants for each replication.

At Hyde Park, the plant height, number of stems, and foliage diameter were obtained immediately before the first DMY harvest. The foliage diameter was measured at the top of the canopy. Values for NDF, ADF, and CP were determined from the first DMY harvest in 2007. Samples were ground (Cyclotec 1093 Sample Mill, Tecator AB, Hoganas, Sweden) to pass through a 1-mm-diameter screen. Ground samples were analyzed for CP using total combustion procedures (LECO TruSpec C/N analyzer, LECO Corp., St. Joseph, MI). ADF and NDF were analyzed at Agri Analysis Inc. (Leola, PA) following the procedures of the Association of Official Analytical Chemists (AOAC, 1990) and Mertens (2002), respectively, as adapted to the Ankom A200 filter-bag technique (Ankom, Macedon, NY).

Inflorescence weight was used to estimate potential seed yield at Millville. To minimize bias between early- and late-flowering collections, inflorescences were harvested sequentially from late June to late July each year. Because Searls prairie clover has racemose inflorescences and because mature seeds easily disperse from the inflorescence, plants were checked daily, and the mature seed portion was collected until the entire inflorescence was harvested. The inflorescences were bulked by replication, dried in a greenhouse, and weighed.

Analysis of variance was conducted using the MIXED procedure of SAS (SAS Institute, 2004) with collection considered a fixed factor and replication a random factor. Data were checked for normality and homoscedasticity before analysis and subsequently transformed when required. Significance was determined at $\alpha = 5\%$, and mean separations were conducted using the Tukey test. Pearson correlation and Spearman rank correlation coefficients were calculated using the CORR procedure of SAS. Principal component (PC) analysis was conducted on the standardized phenotypic data using the FACTOR procedure of SAS.

Genetic Diversity and Population Structure

Plant tissues were collected from the apical regions of the shoots of eight individual plants from each collection before field transplanting. An additional eight plants of purple prairie clover were sampled for use as an out-group. Tissues were lyophilized and stored at −20°C. Approximately 17 mg of dried plant tissue were used for each sample, and DNA was extracted using the Qiagen DNeasy 96-well procedure (Qiagen, Valencia, CA) following the manufacturer's protocol. The concentration and quality of genomic DNA were tested spectrophotometrically and by agarose gel electrophoresis. The genomic DNA concentration was adjusted to 30 ng μL^{-1}.

The AFLP procedure followed the method of Vos et al. (1995) but was modified by resolving AFLP products on an ABI3730 capillary instrument (Life Technologies, Foster City, CA). Five selective primer pairs were chosen for analysis: E(*Eco*RI).AGG/M(*Mse*I).CAT, E.AGA/M.CTG, E.AGC/M.CTA, E.AGT/M.CAC, and E.ACG/M.CTG. Amplified products were analyzed with Genescan (ABI, Foster City, CA) at the Center for Integrated Biosystems (Utah State University, Logan, UT). Amplified fragment length polymorphism bands between 50 and 500 bp were scored visually for presence or absence using Genographer software (Benham, 2001).

The percentage of polymorphic loci was estimated using the binary AFLP data. Average within-collection diversity of the collections was estimated with the Dice similarity coefficient (Dice, 1945; Leonard et al., 1999), and their variances were computed using a SAS macro reported in Leonard et al. (1999). Dice's coefficient was preferred over the simple matching coefficient because the former estimates similarity based only on the band presence in at least one of the two individuals and thus reduces the bias in similarity estimates due to band absence in both individuals (Wong et al., 2001). The similarity value of groups of collections from northwestern Utah and the remaining collections were tested for significance using the MIXED procedure in SAS. This procedure identified differences in genetic diversity between collections that were based on geographical groupings. In addition, the similarity values were correlated with PC scores for each collection using the CORR procedure in SAS.

A hierarchical analysis of molecular variance (AMOVA) was conducted using Arlequin v. 3.1 software (Excoffier et al., 2005). First, a Φ_{st} pairwise genetic-distance matrix was generated among collections using Dice's coefficient from the binary AFLP data. The Φ_{st} pairwise distance matrix was then used as an input file for the AMOVA procedure. The binary AFLP data were also used to estimate the percentage of polymorphic loci and generate a pairwise F_{st} distance matrix with 1000 bootstrap iterations in AFLP-SURV (Vekemans et al., 2002). The F_{st} matrix was used as an input file for constructing a neighbor-joining (NJ) dendrogram with node bootstrap support using the NEIGHBOR and CONSENSE programs sequentially in PHYLIP software (Felsenstein, 2009).

A model-based Bayesian clustering analysis was conducted to assess population structures, using Structure v. 2.2 (Falush et al., 2007) without advanced assignment of collections into populations. The raw binary data were analyzed with the Recessive Alleles option, with the admixture model with correlated marker frequencies and without population flags. Probabilities of the dataset for a given group from $K = 1$ through $K = 9$ were tested with three replications for each level of K. The Markov chain Monte Carlo (MCMC) procedure was used with 30,000 burn-in and 300,000 MCMC steps after burn-in to determine the probability of each structure model. The average estimated log probability of the data was plotted against the K values to observe structural fit of the data. Additionally, the second-order rate of change of log probability (ΔK) between two successively tested models was plotted against the corresponding number of groups tested as described by Evanno et al. (2005). Because the procedure described by Evanno et al. requires subtractions of former and latter structures, only groups $K = 2$ through $K = 8$ can be shown when 1–9 structures are tested. Groupings of collections found in the Structure program and NJ tree were further tested with hierarchical AMOVA using Arlequin v. 3.1.

The spatial distribution of genetic structure was tested for isolation by distance analysis using the Mantel and partial Mantel tests on the collections. A pairwise, linear, geographic-distance matrix was constructed with the Geographic Distance Matrix Generator (Ersts, 2009). The pairwise population genetic-distance matrix, the Φ_{st} matrix, was constructed using Arlequin v. 3.1. Distance-matrix correlations were also made for phenotypic, elevation, temperature, and precipitation differences. The phenotypic matrix of pairwise Euclidean distances was based on the standardized values of all phenotypic traits and was constructed using the DISTANCE procedure in SAS. Geographic distance, elevation, temperature, and precipitation distance matrices were also created using the DISTANCE procedure in SAS. The genetic- and geographic-distance matrices were used to estimate a correlation statistic between genetic and geographic distances with IBDWS v. 3.16 (Jensen et al., 2005), with and without controlling for elevation and site characteristics. Statistical significance of the Mantel test was determined by using 1000 permutations.

RESULTS

Year-by-collection interactions were not significant for the phenotypic traits measured at the Hyde Park and Millville locations, except for the number of inflorescences at the Millville location ($F_{20,271} = 2.37$, $P = 0.0011$). However, for the number of inflorescences at Millville, the Spearman rank correlation was significant ($r = 0.64$, $P = 0.003$), but the collection-by-year interactions were not significant when the Dp check and the Ds-11 collection data were removed from the analysis ($F_{18,251} = 1.58$, $P = 0.07$). Therefore, all data were combined across years. Additionally, for the flowering-date trait, the collection-by-location interactions were not significant, and the collections were combined across locations for this trait. Significant variation was detected for each trait (Table 2).

Dry-matter yield, the number of inflorescences, and the flowering date were measured at both locations. The Dp check exhibited the highest DMY at both locations (Hyde Park: 227.2 g plot^{-1}; Millville: 184.5 g plot^{-1}), but it did not differ from several Searls prairie clover collections at both locations (Table 3). Of the Searls prairie clover collections, Ds-13 had the highest DMY at Hyde Park (195.7 g plot^{-1}), and Ds-16 exhibited the lowest DMY (35.1 g plot^{-1}). At Millville, Ds-14 had the highest DMY (158.2 g. plot^{-1}), and Ds-07 exhibited the lowest DMY (38.0 g plot^{-1}). For the number of inflorescences at Hyde Park, Ds-15 had the highest number of inflorescences (45.9) and Ds-16 had the lowest number of inflorescences (8.8). At Millville, the Dp check had the highest number of inflorescences (80.5), but it did not differ from eight Searls prairie clover collections. Among the Searls prairie clover collections, Ds-15 exhibited the highest number of inflorescences (60.5), and Ds-21 had the lowest number of inflorescences (22.3). For flowering date, Ds-25 exhibited the earliest flowering at 160.1 d, while the Dp check exhibited the latest flowering date at 173.2 d. As with

Table 2. Means and standard deviations (SD) of 20 collections of Searls prairie clover (*Dalea searlsiae*) and a purple prairie clover (*Dalea purpurea*) check with the associated *F*-statistic and *P*-value for phenotypic traits as measured at Hyde Park and Millville, UT, and combined over both locations.

Location	Trait	Mean	SD	*F* statistic
Hyde Park				
	Dry-matter yield (g plot^{-1})	121	52.9	$F_{19,277} = 20.26^{***}$
	Plant height (cm)	31.7	5.5	$F_{19,277} = 11.19^{***}$
	Number of stems	10.6	1	$F_{19,277} = 7.26^{***}$
	Number of inflorescences	26.8	10.4	$F_{19,277} = 14.07^{***}$
	Foliage diameter (cm)	48.6	8.2	$F_{19,276} = 11.70^{***}$
	Acid-detergent fiber (%)	35	2.5	$F_{19,55.1} = 4.82^{***}$
	Neutral-detergent fiber (%)	46.5	2.5	$F_{19,55.1} = 2.20^{*}$
	Crude protein (%)	18.3	1.2	$F_{19,55} = 3.71^{***}$
Millville				
	Dry-matter yield (g plot^{-1})	94.8	13.7	$F_{20,292} = 13.86^{***}$
	Inflorescence weight (g plot^{-1})	46.3	28	$F_{20,289} = 14.81^{***}$
	Number of inflorescences	39.5	13.7	$F_{20,292} = 7.52^{***}$
Combined				
	Flowering date†	164.8	3.7	$F_{20,427} = 8.30^{***}$

*$P < 0.05$.

***$P < 0.001$.

†Flowering date is measured from 1 January.

Table 3. Means for various characteristics of 20 collections of Searls prairie clover (*Dalea searlsiae*) and a purple prairie clover (*Dalea purpurea*) check (Dp) at Millville, Hyde Park, and combined across both locations.

Collection	Hyde Park							Millville			Combined
	DMY†	No. stems	No. inflorescences	Plant height	Foliage diameter	ADF†	NDF†	Crude protein	DMY†	No. inflorescences	Inflorescence weight	Flowering date‡
	g plot^{-1}			cm		%			g plot^{-1}		g plot^{-1}	
Ds-01	—	—	—	—	—	—	—	—	79.8^{DEFGHI}	29.9^{DEFG}	20.7^{G}	172.9^{ABC}
Ds-03	134.3^{BCDEF§}	10.1^{BC}	32.9^{ABC}	35.6^{ABC}	50.8^{ABCD}	38.7^{A}	48.5^{AB}	17.9^{ABC}	140.0^{ABC}	44.0^{ABCDE}	57.6^{BCD}	164.0^{ABCDEFG}
Ds-05	84.3^{FGH}	8.0^{BCD}	15.3^{EFG}	35.0^{ABCD}	40.0^{DEF}	35.4^{ABC}	48.2^{AB}	16.8^{C}	70.4^{GHI}	26.1^{DEFG}	20.7^{G}	168.0^{A}
Ds-07	40.4^{HI}	6.4^{CD}	18.2^{DEFG}	21.2^{FG}	35.6^{EF}	36.1^{ABC}	46.4^{AB}	18.8^{ABC}	38.0^{J}	23.9^{FG}	22.1^{FG}	161.6^{EFG}
Ds-08	126.9^{CDEFG}	10.9^{B}	35.5^{AB}	31.4^{CDE}	59.1^{A}	36.4^{ABC}	47.8^{AB}	17.1^{ABC}	108.8^{BCDEFG}	45.2^{ABCDE}	49.1^{BCDE}	163.1^{BCDEFG}
Ds-09	74.3^{GH}	9.1^{BCD}	20.4^{BCDE}	26.7^{EFG}	49.9^{ABCD}	33.3^{BCD}	40.2^{B}	19.3^{ABC}	63.1^{HIJ}	33.8^{CDEFG}	32.6^{DEFG}	164.4^{ABCDEF}
Ds-10	108.6^{EFG}	11.5^{B}	24.6^{BCDE}	26.1^{EFG}	51.3^{ABCD}	36.6^{ABC}	45.6^{AB}	17.3^{ABC}	78.0^{DEFGHI}	43.1^{ABCDEF}	33.5^{CDEFG}	162.2^{DEFG}
Ds-11	81.2^{FGH}	9.4^{BCD}	24.1^{BCDE}	29.8^{CDEF}	45.9^{BCDEF}	35.3^{ABC}	47.6^{AB}	20.2^{ABC}	65.2^{HIJ}	35.2^{CDEFG}	27.9^{EFG}	165.7^{ABCDEF}
Ds-12	109.0^{EFG}	10.8^{B}	25.4^{BCDE}	32.4^{BCDE}	46.0^{BCDEF}	36.2^{ABC}	47.6^{AB}	17.4^{ABC}	71.4^{FGHI}	32.2^{CDEFG}	29.6^{EFG}	162.4^{DEFG}
Ds-13	195.7^{AB}	10.8^{B}	45.0^{A}	29.3^{CDEFG}	54.7^{ABC}	36.9^{AB}	48.1^{AB}	17.8^{ABC}	101.0^{CDEFGH}	52.3^{ABC}	65.4^{AB}	162.8^{CDEFG}
Ds-14	178.3^{ABCD}	11.6^{B}	25.8^{BCDE}	39.0^{AB}	58.2^{A}	36.2^{ABC}	46.5^{AB}	17.0^{BC}	158.2^{AB}	42.0^{BCDEF}	72.7^{AB}	166.5^{ABCD}
Ds-15	187.8^{ABC}	11.3^{B}	45.9^{A}	33.6^{BCDE}	54.8^{ABC}	36.0^{ABC}	45.3^{AB}	17.3^{ABC}	117.2^{ABCDEF}	60.5^{AB}	82.5^{AB}	161.9^{EFG}
Ds-16	35.1^{I}	5.9^{D}	8.8^{G}	21.3^{G}	34.2^{F}	31.2^{CD}	45.6^{AB}	19.5^{ABC}	48.5^{IJ}	26.8^{EFG}	21.7^{G}	165.1^{ABCDEF}
Ds-17	118.8^{DEFG}	10.1^{BC}	21.1^{CDEF}	33.0^{BCDE}	48.0^{ABCDE}	33.9^{ABCD}	45.1^{AB}	17.7^{ABC}	120.8^{ABCDE}	34.1^{CDEFG}	58.4^{BCD}	167.0^{ABC}
Ds-18	151.8^{ABCDE}	11.0^{B}	35.2^{AB}	35.5^{ABC}	54.5^{ABC}	36.6^{ABC}	46.3^{AB}	18.4^{ABC}	95.9^{CDEFGH}	44.6^{ABCDE}	51.3^{BCD}	161.2^{FG}
Ds-20	116.7^{DEFG}	11.7^{B}	32.6^{ABC}	35.3^{ABC}	44.8^{CDEF}	37.0^{AB}	49.3^{A}	17.1^{ABC}	73.8^{EFGHI}	33.6^{CDEFG}	29.5^{DEFG}	162.5^{DEFG}
Ds-21	58.4^{HI}	8.3^{BCD}	9.8^{FG}	27.2^{DEFG}	38.4^{EF}	27.9^{D}	41.1^{AB}	20.7^{AB}	87.9^{DEFGH}	22.3^{G}	22.7^{G}	169.4^{A}
Ds-23	79.1^{FGH}	8.6^{BCD}	16.3^{DEFG}	29.9^{CDEF}	36.3^{EF}	32.6^{BCD}	43.9^{AB}	20.7^{A}	69.9^{FGHI}	27.8^{DEFG}	30.2^{DEFG}	166.6^{ABCDE}
Ds-25	151.5^{ABCDE}	11.5^{B}	32.5^{ABC}	33.2^{BCDE}	52.7^{ABC}	34.6^{ABCD}	48.5^{AB}	18.1^{ABC}	98.5^{CDEFGH}	44.7^{ABCDE}	46.3^{BCDEF}	160.1^{G}
Ds-26	160.5^{ABCDE}	11.0^{B}	35.2^{AB}	35.3^{ABC}	56.7^{AB}	36.5^{ABC}	49.5^{A}	17.9^{ABC}	120.5^{ABCD}	46.7^{ABCD}	61.3^{ABC}	160.4^{FG}
Dp	227.2^{A}	23.1^{A}	30.8^{ABCD}	43.1^{A}	59.8^{AB}	32.7^{ABCD}	48.6^{AB}	18.7^{ABC}	184.5^{A}	80.5^{A}	137.1^{A}	173.2^{AB}

†DMY, dry-matter yield; ADF, acid-detergent fiber; NDF, neutral-detergent fiber.

‡Flowering date is measured from 1 January.

§Values followed by the same letter within a column are not significantly different at $P = 0.05$.

all traits, the high and low mean values did not differ from those of several other collections (Table 3).

At the Millville location, the Dp check had the highest inflorescence weight (137.1 g plot^{-1}), but it did not differ from that of the Ds-15, Ds-14, Ds-13, and Ds-26 collections. Collections Ds-01 and Ds-05 had the lowest inflorescence weight (20.7 g plot^{-1}), but it did not differ from that of the nine other collections.

At the Hyde Park location, the Dp check had the greatest plant height (43.1 cm), number of stems (23.1), and foliage diameter (59.8 cm), but those traits did not differ from those of several Searls prairie clover collections (Table 3). Among the Searls prairie clover collections, Ds-14 exhibited the greatest plant height (39.0 cm), one of the largest numbers of stems (11.6), and one of the largest foliage diameters (58.2 cm). Collections Ds-07 and Ds-16 had the lowest values for these three traits. As with other traits, these high and low values did not differ from several other collections. Forage quality traits exhibited significant variation among collections (ADF: $P < 0.0001$; NDF: $P = 0.01$; CP: $P = 0.0004$). Values of ADF ranged from 27.9 to 38.7%, of NDF from 40.2 to 49.5%, and of CP from 16.8 to 20.7% (Table 3). In particular, collections Ds-21 and Ds-23 (originating from northwestern Utah, Fig. 1) had comparatively higher CP values and lower-than-average ADF and NDF values.

Phenotypic Correlations and Principal Component Analysis

A majority of the field phenotypic traits were significantly correlated with each other (Table 4). A high positive correlation was observed between DMY at the two locations ($r = 0.78$, $P < 0.0001$), and DMY at Hyde Park was correlated with almost all other traits, except for flowering date and NDF. The DMY at Millville was also correlated with most traits, except for

Table 4. Pearson correlation coefficients (*r*) with their associated *P* values for 20 collections of Searls prairie clover (*Dalea searlsiae*) for dry-matter yield (DMY) and the number of inflorescences (NFL) at Hyde Park (HP) and Millville (MV), inflorescence weight (INFLW), flowering date (FLD), plant height (HT), number of stems (ST), foliage diameter (FD), ADF, NDF, and crude protein (CP).

	DMY_HP	DMY_MV	NFL_HP	NFL_MV	INFLW	FLD†	HT	ST	FD	ADF	NDF
DMY_MV	0.78***										
NFL_HP	0.88***	0.53*									
NFL_MV	0.89***	0.64**	0.92***								
INFLW	0.90***	0.86***	0.76***	0.86***							
FLD	−0.43ns‡	−0.02ns	−0.67**	−0.58**	−0.30ns						
HT	0.71***	0.74***	0.50*	0.45ns	0.58ns	−0.05ns					
ST	0.83***	0.64**	0.76***	0.74***	0.64**	−0.44ns	0.71***				
FD	0.86***	0.74***	0.80***	0.86***	0.80***	−0.46*	0.60**	0.84***			
ADF	0.62**	0.41ns	0.71***	0.58**	0.49*	−0.60**	0.52*	0.58**	0.57*		
NDF	0.36ns	0.30ns	0.39ns	0.23ns	0.24ns	−0.37ns	0.50*	0.33ns	0.31ns	0.74***	
CP	−0.59**	−0.46*	−0.52*	−0.47*	−0.45ns	0.36ns	−0.54*	−0.58**	−0.57*	−0.73***	−0.65**

*$P < 0.05$.

**$P < 0.01$.

***$P < 0.001$.

†Flowering date is measured from 1 January.

‡ns, not significant ($P > 0.05$).

flowering date, ADF, and NDF (Table 4). The number of inflorescences at both locations showed a high positive correlation with the DMY, inflorescence weight, plant diameter, and ADF and a negative correlation with the flowering date and CP (Table 4). Aside from number of inflorescences, only the flowering date was correlated with the ADF. Plant height was positively correlated with the DMY, number of stems, foliage diameter, ADF, and NDF, but negatively correlated with CP (Table 4). Among forage-quality traits, ADF and NDF were positively correlated with each other ($r = 0.74$, $P = 0.0003$), while CP was negatively correlated with ADF ($r = -0.73$, $P = 0.0003$) and NDF ($r = -0.65$, $P = 0.0028$).

Because of the high correlations between traits, principal component (PC) analysis was conducted on the phenotypic measurements to reduce the dimension of the dataset and visualize the similarities between the collections. The first three PC axes had eigenvalues greater than one, and each axis was represented by a different set of traits. The three axes explained 87% of the dataset variability (PC1 = 63%, PC2 = 13%, and PC3 = 11%; Table 5). The PC1 axis exhibited correlations with the inflorescence weight ($r = 0.95$, $P < 0.0001$), DMY (Hyde Park: $r = 0.78$, $P < 0.0001$; Millville: $r = 0.86$, $P < 0.0001$), number of inflorescences (Hyde Park: $r = 0.61$, $P = 0.0004$; Millville: $r = 0.76$, $P < 0.0001$), foliage diameter ($r = 0.69$, $P = 0.0008$), plant height ($r = 0.48$, $P = 0.032$), and number of stems ($r = 0.46$, $P = 0.041$). The PC2 axis exhibited correlations for forage-quality traits (ADF: $r = 0.75$ $P = 0.0001$; NDF: $r = 0.94$, $P < 0.0001$; CP: $r = -0.72$, $P = 0.0003$) and plant height ($r = 0.45$, $P = 0.044$). The PC3 axis exhibited correlations for the flowering date ($r = 0.92$, $P < 0.0001$), number of inflorescences (Hyde Park: $r = -0.64$, $P = 0.0024$; Millville: $r = -0.57$, $P = 0.0092$), and ADF ($r = -0.45$, $P = 0.045$) (Table 5).

The three collections with the highest PC1 scores were Ds-15, Ds-14, and Ds-13 (Fig. 2A), suggesting that these southern Utah collections had greater values for DMY, inflorescence weight, number of inflorescences, foliage diameter, plant height, and number of stems than other collections (Table 5). Other collections with PC1 scores above the mean (i.e., zero) (Ds-03, Ds-26, Ds-17, Ds-08, and Ds-18) originated from eastern Nevada and southern Utah (Fig. 1). Collections Ds-05, Ds-20, Ds-12, and Ds-07 formed a subgroup with the lowest PC1 scores as well as high PC2 scores (lower forage quality) (Fig. 2A). The scatter plot of PC1 and PC3 scores indicates that collections varied widely for flowering date across the range of PC1 (Fig. 2B; Table 3). Collections Ds-20, Ds-12, and Ds-07 remained grouped with an earlier flowering date than Ds-05 (Fig. 2B; Table 3).

A strong positive correlation ($r = 0.76$; $P = 0.0002$) was observed between PC1 scores and precipitation at the collection sites (Fig. 2C). Significant correlations were also found between PC3 scores and collection-site elevation ($r = -0.50$, $P = 0.0285$) and temperature ($r = 0.50$, $P = 0.03$). The PC2 score was not correlated with any collection site characteristic (Table 6).

Genotypic Diversity and Population Structure

The AFLP fingerprinting generated 474 polymorphic DNA bands. The correlation between band sizes and frequencies was not significant ($r = 0.03$, $P = 0.45$), suggesting the absence of size homoplasy in AFLP markers (Vekemans et al., 2002). The number of bands per collection ranged from 91 to 111, and the percentage of polymorphic loci within collections varied from 25 to 39% (Table 1). Four collections from northwestern Utah (Ds-12, Ds-16, Ds-21, and Ds-23) exhibited the lowest percentage of polymorphism. Similarly, the mean similarity index (S-value) between individuals within each collection ranged from 0.64 to 0.77, with the collections from

Table 5. Phenotypic traits of Searls prairie clover (*Dalea searlsiae*) used in principal component analysis with their respective loadings for the first three principal component axes. Eigenvalues and the cumulative proportion of the total variation of the measured trait data set are given for each principal component axis.

Traits	PC1	PC2	PC3
Eigenvalue	4.28	2.54	2.32
Cumulative proportion	0.63	0.76	0.87
Dry-matter yield, Hyde Park	0.78***	0.25ns†	−0.33ns
Dry-matter yield, Millville	0.86***	0.25ns	0.17ns
Number of inflorescences, Hyde Park	0.61**	0.26ns	−0.64**
Number of inflorescences, Millville	0.76***	0.10ns	−0.57**
Inflorescence weight	0.95***	0.16ns	−0.19ns
Flowering date‡	−0.07ns	−0.26ns	0.92***
Plant height	0.48*	0.45*	0.17ns
Number of stems	0.46*	0.20ns	−0.37ns
Foliage diameter	0.69***	0.18ns	−0.39ns
Acid-detergent fiber	0.29ns	0.75***	−0.45*
Neutral-detergent fiber	0.08ns	0.94***	−0.11ns
Crude protein	−0.24ns	−0.72***	0.20ns

*$P < 0.05$.
**$P < 0.01$.
***$P < 0.001$.
†ns, not significant ($P > 0.05$).
‡Flowering date is measured from 1 January.

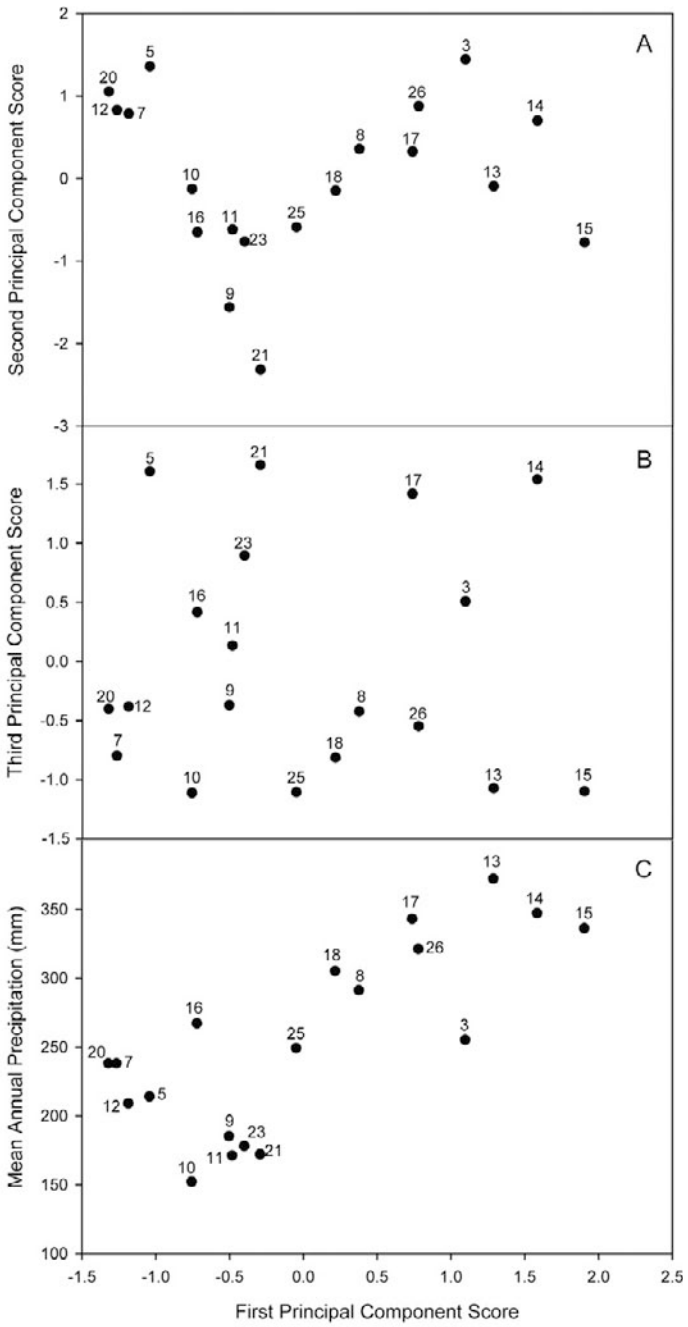

Figure 2. Scatter plots of the first principal component score with (A) second principal component score, (B) third principal component score, and (C) mean annual precipitation of the collection sites for Searls prairie clover (*Dalea searlsiae*).

northwestern Utah (Ds-12, Ds-16, DS-21, and Ds-23) having the highest within-collection similarity (or low genetic diversity) (Table 1). The analysis of variance of S-values of groups indicated significant variation ($F_{1,18}$ = 63.9, $P < 0.001$) between the four collections from northwestern Utah compared with all other collections. In addition, the amount of within-collection genetic similarity, the opposite of heterozygosity, exhibited no correlations with PC axes (Table 6).

Analysis of molecular variance apportioned 24% ($P < 0.0001$) of the genetic variation among collections and 76% ($P < 0.0001$) of the variation within collections. Genetic relationships among collections were visualized using a neighbor-joining (NJ) tree of pairwise F_{st} values. Three groups of collections were found to have bootstrap support: one from northwestern Utah (Ds-12, Ds-16, Ds-21, and Ds-23), one from western Nevada (Ds-09, Ds-10, and Ds-11), and one from southern Utah (Ds-13, Ds-15, and Ds-17) (Fig. 3). The northwestern Utah, southern Utah, and western Nevada groups were supported by bootstrap values of 100, 86, and 75%, respectively.

Bayesian clustering of collections based on AFLP data showed a smaller increase in the average log-likelihood value after the two-group test (Fig. 4A), and the second-order rate of change in the log-likelihood values showed a mode at the $K = 2$ structure (Fig. 4B). Of the two groups, the first included the four collections from northwestern Utah and the second included all other collections from southern Utah and Nevada (Fig. 1 and 4C). Little indication of admixed coancestry coefficients was detected between the two groups (Fig. 4C). When a hierarchical AMOVA was used to test the partitioning of genetic variation among these two groups, the amount of variation apportioned among the two groups was 14% ($P < 0.001$), among populations within groups was 17% ($P < 0.001$), and within populations was 69% ($P < 0.001$).

Because both the NJ dendrogram and the Bayesian clustering results indicated that geographic distances tended to separate genetic structures, isolation by distance

Table 6. Pearson correlation coefficients (*r*) with the associated *P* value for 20 collections of Searls prairie clover (*Dalea searlsiae*) for the first principal component score (PC1), second principal component score (PC2), and third principal component score (PC3) associated with collection-site elevation, temperature, and precipitation, and within-collection genetic similarity.

Component score	Elevation	Temperature	Precipitation	Similarity†
PC1	0.26ns‡	0.17ns	0.76***	−0.23ns
PC2	0.11ns	0.15ns	0.31ns	−0.39ns
PC3	−0.50*	0.50*	−0.11ns	0.24ns

*$P < 0.05$.

***$P < 0.001$.

†Similarity coefficients were estimated based on AFLP markers following Leonard et al. (1999).

‡ns, not significant ($P > 0.05$).

was examined using the genetic and linear geographic distances. The genetic-distance matrix was highly correlated with the geographic-distance matrix ($r = 0.77$, $P < 0.001$), and the correlation was still highly significant after controlling for the effects of elevation ($r = 0.76$, $P < 0.001$), precipitation ($r = 0.71$, $P < 0.001$), or temperature ($r = 0.75$, $P < 0.001$). Conversely, after controlling for the linear geographic-distance effect, the genetic-distance matrix was not correlated with precipitation ($r = -0.01$, $P = 0.49$) or temperature ($r = 0.16$, $P = 0.12$), but a low correlation ($r = 0.30$, $P = 0.011$) was found with collection-site elevation. A significant correlation ($r = 0.37$, $P = 0.005$) was also observed between the genetic- and phenotypic-distance matrices.

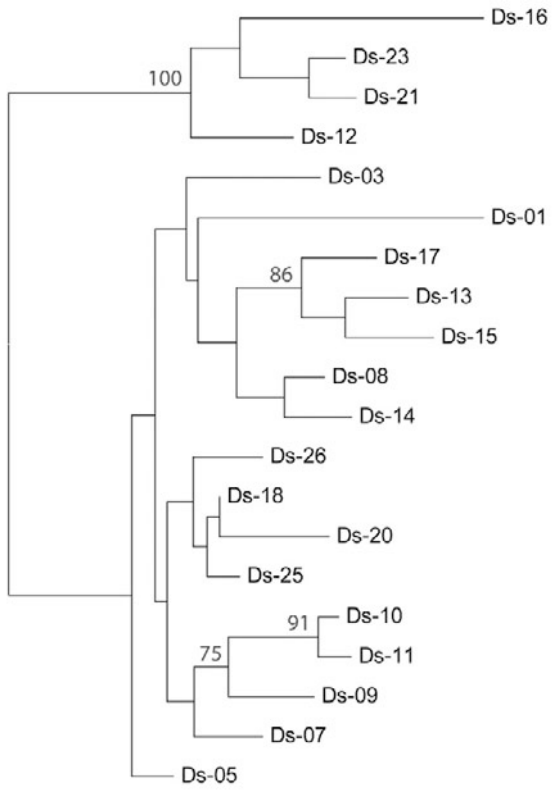

Figure 3. Neighbor-joining dendrogram obtained from a Φ_{st} genetic distance matrix among 20 collections of Searls prairie clover (*Dalea searlsiae*) with associated bootstrap values shown when greater than 75%.

DISCUSSION

Currently, few forbs from western North America are available for revegetation efforts (Walker and Shaw, 2005), especially legumes for semiarid rangelands within the Great Basin. Land managers are interested in legumes for revegetation because of their ability to fix nitrogen, their high-quality forage for wildlife and livestock, and the unique ecological niche they occupy (Pokorny et al., 2005; Walker and Shaw, 2005). One large hurdle in providing legumes for revegetation is the economical production of seed of reliable quality and quantity. Wildland-collected seed is often several-fold higher in cost than commercially produced seed. Commercial seed production also provides the ability to increase the amount of seed available and improve the consistency and quality of the seed. Our Searls prairie clover collections exhibited significant variation for each of the traits measured in the common-garden studies (Table 2), and several collections exhibited agronomic traits comparable to commercially harvested purple prairie clover (Table 3).

Taller plants and higher potential seed yield are desirable traits for commercial seed production, whereas higher DMY is desirable when the revegetation goal is to provide forage for livestock and wildlife. It is also possible that taller DMY plants could be maladapted for some regions of the species distribution. Regardless, these three traits had high loadings in PC1 and PC3, and these PC axes explained approximately 74% of the total variation in the data (Table 5). Collections with high PC1 scores, especially, could be used for germplasm release or selection and breeding when the goal is to increase seed and forage availability to livestock and wildlife. Interestingly, PC1 and PC3 also represented other highly correlated traits, such as the number of inflorescences, inflorescence weight, number of stems, and foliage diameter (Table 5). The high positive correlations of foliage diameter or number of stems with DMY and inflorescence weight suggest that foliage diameter or number of stems could be used as surrogate traits to select for DMY and seed yield potential (Table 4).

The flowering date for the collections ranged from 160 to 173 d, that is, from 8 June to 20 June (Table 3). Because most precipitation in the Great Basin comes in the form of snow or early-spring rain, early growth can be critical for utilizing available water. Two collections from eastern Nevada (Ds-25 and Ds-26) exhibited the earliest flowering dates compared with other collections and may, therefore, be good candidates for revegetation in drier areas of the Great Basin. Flowering date also showed a high loading for PC3 (Table 5), which in turn was negatively correlated with elevation ($r = -0.50$, $P = 0.0285$) and positively correlated with mean annual

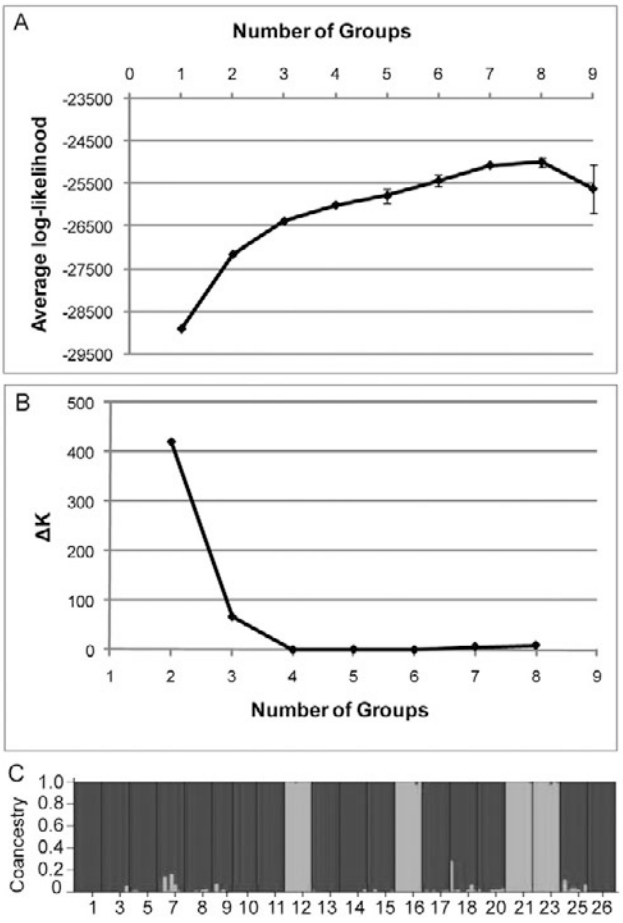

Figure 4. Bayesian clustering of Searls prairie clover (*Dalea searlsiae*) collections, testing from 1 to 9 groups. (A) average log-likelihood values of each tested group, (B) the second order of rate of change in the log-likelihood value of the subsequently tested group, and (C) the bar plot of collections grouped at $K = 2$.

temperature ($r = 0.50$, $P = 0.03$). These relationships suggest that earlier flowering generally occurred in collections that originated from sites at high elevation and low mean annual temperature. A similar trend was seen in the cold-adapted populations of whitebark pine (*Pinus albicaulis* Engelm.) in a common-garden study (Bower and Aitken, 2008).

Higher DMY is associated with lower forage quality for many plant species (White and Wight, 1984; Bhattarai et al., 2008). The CP concentration was generally negatively correlated with DMY, ADF, and NDF. As plants matured, their leaf-to-shoot ratios decreased, thus raising the DMY, ADF, and NDF but lowering CP (Romero et al., 1987). Additionally, as plants aged, the proportion of ADF in a plant increased more rapidly than NDF (Hart et al., 1983). In our study, because collections with higher DMY did not exhibit variation in ADF (15 out of 19 collections did not differ from each other), and NDF was not correlated with DMY, reductions in forage quality should not be a major concern when selecting for high DMY in Searls prairie clover.

In our study, 76% ($P < 0.0001$) of the genetic variation was apportioned within collections, highlighting that the majority of genetic variation is present within populations for this primarily outcrossing species (Jim Cane, personal communication, 2009). When a within-collection genetic-similarity index was used, the collections from northwestern Utah exhibited lower genetic diversity (higher genetic similarity) than the other collections (Table 1 and Fig. 1). Collections from northwestern Utah represented the northern distribution of Searls prairie clover, and the lower genetic diversity among them suggests a possible founder effect or environmental stochasticity (Ray, 2001).

Although a general trend of a significant positive correlation was reported between genetic diversity and population fitness in meta-analysis studies (Reed and Frankham, 2003; Leimu et al., 2006), no correlation was observed between genetic diversity and population fitness in *Clematis acerifolia* Maxim. (Lopez-Pujol et al., 2008). For the Searls prairie clover collections, correlation coefficients between PC scores obtained from phenotypic measurements and the within-collection genetic similarity (the opposite of genetic diversity) obtained from AFLP analysis were not significant. This result suggests that greater genetic diversity was not associated with larger plant size and greater fecundity (or fitness) in Searls prairie clover. However, the four collections from northwestern Utah (Ds-12, Ds-16, Ds-21, and Ds-23) exhibited the least genetic diversity and correspondingly showed lower fitness (smaller size and lower fecundity) (Tables 1 and 3).

Significant differences were also detected between populations with an overall Φ_{st} of 24% ($P < 0.0001$), indicating the presence of a population structure. Three groups of collections with bootstrap support were observed in the NJ dendrogram (Fig. 3), and the group comprising the 4 northwestern Utah collections was also detected with Bayesian clustering (Fig. 4C). The northwestern Utah group was further supported by hierarchical AMOVA, with 14% ($P < 0.0001$) of the variation apportioned among groups when compared with the remaining 16 collections in Nevada and southern Utah. Thus, our data indicate that collections from northwestern Utah formed a genetically differentiated group and that this group has lower within-collection genetic diversity (higher similarity) than the other collections.

The most diverse (lowest within-collection similarity) collections of Searls prairie clover were found across Nevada and southern Utah (Table 1 and Fig. 1). Within these collections, two groups located in western Nevada (Ds-09, Ds-10, and Ds-11) and southern Utah (Ds-13, Ds-15, and Ds-17) were identified with bootstrap support in the NJ tree (Fig. 1 and 2) but were not distinctive with the Bayesian clustering (Fig. 4C). The three southern Utah collections had some of the highest PC1 scores among all 20 collections, whereas the western Nevada collections had relatively low PC1 scores (Fig. 2). These two groups of collections were on the east-west periphery of our collection sites and showed a trend toward isolation. Indeed, the overall significant isolation by distance found among these Searls prairie clover

collections highlights this trend. Although isolation by distance can occur when a distribution of populations is greater than gene flow, it may take populations considerable time to achieve this state (Slatkin, 1993). Isolation by distance has been reported for many plant species (Raspe and Jacquemart, 1998; Stöcklin et al., 2009; Bhattarai et al., 2010; Bushman et al., 2010), although several other studies failed to find this relationship (Franceschinelli and Kesseli, 1999; Albaladejo et al., 2009). The strong genetically differentiated group in northwestern Utah and the indication of isolation by distance for east and west peripheral collections indicate that gene flow is playing an important role in shaping the existing genetic structure in these Searls prairie clover collections. The four northwestern Utah collections were within the area of prehistoric Lake Bonneville (Oviatt, 1997), and their differentiation may probably be due to recent migration.

If phenotypic traits are correlated with environmental characteristics at the collection site, these traits are considered to have potential adaptive significance (Endler, 1986). A high correlation between the PC1 scores and collection-site precipitation ($r = 0.76$, $P = 0.0002$) suggests that, if plants from high-precipitation sites are transplanted to sites with low precipitation, the traits represented by PC1 may be adversely affected. One challenge with common-garden plots is their inability to represent all the environments wherein the species is distributed, due to the inevitable need for intensive data collection and confounding affects of phenotypic plasticity. In this study, the mean annual precipitation at the collections sites varied from 152 to 372 mm, whereas precipitation at the common-garden locations was approximately 430 mm. The strongest genetically differentiated group (four collections from northwestern Utah) came from areas with low relative precipitation and had low PC1 scores (Table 1 and Fig. 2), such that a seed source developed within this area would represent a distinct genetic structure and environmental character. The larger group, from Nevada and southern Utah, included collections from a range of precipitation, and therefore further testing should be conducted to determine if gene flow within that group has constrained local adaptive potential in areas with extremely low precipitation (McKay and Latta, 2002).

In summary, a portion of collections of Searls prairie clover exhibited agronomic traits comparable with those of commercially available purple prairie clover, suggesting that Searls prairie clover has the potential for commercial seed production. From a restoration and revegetation perspective, the development of regional seed sources would attenuate concerns of outbreeding depression and maladapation. The two genetically differentiated groups identified by AFLP markers defined two seed sources: one from northwestern Utah and the other encompassing Nevada and southern Utah. However, the three westernmost Nevada collections (Ds-9, Ds-10, and Ds-11) shared low phenotypic values and low collection-site precipitation with the northwestern Utah collections, and precipitation was identified as an environmental character with possible local adaptive significance. Thus the three western Nevada collections may reside in different seed-source groups based on molecular or phenotypic information, so that further reciprocal transplant research may be necessary to place the western Nevada area into an appropriate regional seed source.

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