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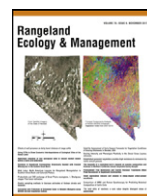
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Original Research

Utah Lotus: North American Legume for Rangeland Revegetation in the Southern Great Basin and Colorado Plateau^{☆,☆☆}



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ABSTRACT

Utah lotus (*Lotus utahensis* Ottley) is a North American leguminous forb that may hold promise for rangeland revegetation in the western United States for diversifying planting mixtures, attracting pollinators, providing high-quality forage, and expanding habitats for insects needed by sage-grouse chicks. Fourteen wildland seed collections of Utah lotus originating from Nevada and Utah were assessed for genetic variation of a wide range of phenotypic traits and genetic relationships. Population structure estimates defined by 552 amplified fragment length polymorphism (AFLP) markers identified three primary subgroups within the Utah lotus collections, which corresponded to their geographic origin. Two collections of Utah lotus (LU-5 and LU-20) were among the top-performing collections for the phenotypic traits examined, including dry-matter yield, pod production, number of stems, canopy height, and persistence. No significant Pearson's correlations or canonical correlations were observed among the phenotypic traits and environmental characteristics at the collection sites. Significant correlations were detected between genetic and geographic matrices, and phenotypic and geographic distance matrices ($r = 0.89$, $P = 0.001$ and $r = 0.24$, $P = 0.04$, respectively). Condensed tannin (CT) contents of Utah lotus were between 146 and 199 g kg⁻¹ dry matter, which was nearly 10 times higher than CT content of birdsfoot trefoil (*Lotus corniculatus* L.) with 17.3 g kg⁻¹ dry matter. Because of our phenotypic and genotypic evaluations, one pooled germplasm source of Utah lotus comprising collections LU-5 and LU-20 could be developed for use in rangeland revegetation in the southern Great Basin and Colorado Plateau.

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Introduction

Mismanagement of rangelands in the Intermountain Region of the western United States during the late 1800s and early 1900s led to changes in vegetation composition (Fleischner, 1994; Harrison et al., 2003). Changes in species composition and reduced biodiversity contributed to the spread of invasive species such as cheatgrass (*Bromus tectorum* L.),

prickly Russian thistle (*Salsola tragus* L.), burningbush (*Bassia scoparia* (L.) A. J. Scott), and tall tumble mustard (*Sisymbrium altissimum* L.) (Chambers et al., 2007). These invasive, fire-adapted species outcompeted native plant species, increased fuel loads, and exacerbated the frequency of fires (Whisenant, 1990; Brooks, 1999), which in turn encouraged increasingly more frequent devastating wildfires. Revegetation of these degraded rangelands with a biologically diverse suite of plant species (including forbs) is necessary to increase the intervals between fires and stabilize rangeland resources.

Trends in the use of plant materials for rangeland revegetation among public land-management agencies have transitioned from a focus on planting introduced species to planting species native to the western United States (Shaw et al., 2005). Although a number of native grass species are currently available for revegetation uses, there is increased interest in the development of native forb plant materials, particularly for enhancing habitat for pollinators and sage-grouse. North American forbs have the potential to enhance biodiversity, provide important forage, and contribute to soil stabilization. In addition, native

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leguminous forbs can increase soil fertility through biological nitrogen fixation (Rumbaugh, 1990). Germplasm is available for several North American leguminous forb species for revegetation purposes in the western United States, including Utah sweetvetch (*Hedysarum boreale* Nutt.) (Stevens et al., 1994), basalt milkvetch (*Astragalus filipes* Torr. ex A. Gray) (Johnson et al., 2008), western prairie clover (*Dalea ornata* [Douglas ex Hook.] Eaton & J. Wright) (Johnson et al., 2011), and Searls prairie clover (*Dalea searlsiae* [A. Gray] Barneby) (Bhattarai et al., 2011; Johnson et al., 2015). However, a broader range of forb species is necessary for revegetation of diverse rangeland environments of the Intermountain West United States.

Forage legumes sustain animal-based agriculture by providing high-quality forage throughout the growing season (Rumbaugh, 1990). Members of the genus *Lotus* L. are recognized as an important pasture species (Belesky, 1999; Greene, 1999) because of their high digestibility and protein content (Escaray et al., 2012). They also have condensed tannins (CTs), which are of economic interest because they reduce the risk of bloat, improve nutrient absorption, reduce the rate of protein degradation in the rumen (Undersander et al., 1993; Smith and Kelman, 1997), and control intestinal parasites (Novobilsky et al., 2011). The genus *Lotus* has a worldwide distribution of > 100 species that include diploid, tetraploid, and hexaploid taxa (Ito et al., 2000). The two centers with the highest diversity of species within the genus are the Mediterranean Basin and Intermountain Region of the western United States, with 39 species endemic to the continental United States (Kirkbride, 1999). Of the *Lotus* species in the United States, big deervetch (*L. crassifolius* [Benth.] Green), a native to California, Oregon, and Washington, is the only North American *Lotus* species currently used for wildlife and wildland restoration in the western United States (Young-Matthews and Darris, 2011).

Birdsfoot trefoil (*Lotus corniculatus* L.) is an important forage legume from Eurasia and North Africa (Grant and Small, 1996; Kirkbride, 1999; Steiner, 1999). This species is used extensively in pastures of the mid-western and northeastern United States and adjacent portions of Canada, alone or with various forage grasses (Undersander et al., 1993). Utah lotus (*L. utahensis* Ottley) is a North American legume with a native range that includes southern Utah, southeastern Nevada, and the mountains of central Arizona. Because of the successful use of birdsfoot trefoil in mesic areas of North America, the distribution of Utah lotus in semiarid areas of the Intermountain West, and its apparent drought and heat tolerance, Utah lotus has the potential to be a valuable revegetation species in rangelands of the western United States. Other plant traits that have been considered important for species used in revegetation include adaptation to the environmental conditions of the restoration site (Broadhurst et al., 2008; Johnson et al., 2010), excellent seedling establishment and high seed production (Pywell et al., 2003), and favorable biomass and plant height characteristics (Fischer et al., 2013).

The objectives of this study were to 1) make seed collections of Utah lotus throughout its distribution; 2) evaluate these collections for important phenotypic traits (forage and seed production, plant morphology, phenology, survival, forage quality, and CT content) at three common garden sites in northern Utah; and 3) use DNA fingerprinting techniques to evaluate genetic relationships among the collections. This information is critical to determine the potential for possible development of plant materials of Utah lotus for revegetation in the Intermountain West.

Materials and Methods

Plant Materials

Seed collections of Utah lotus were made in 2012 from 14 sites in eastern Nevada and central to southern Utah (Table 1; Fig. 1). Initial potential collection sites were identified using site data from herbarium specimens for Utah lotus contained in the online database of the Southwest Environmental Information Network (SEINet, 2016). Some of these potential collection sites had only general information about the specific site where the specimen was collected, no longer had Utah

lotus plants at the site, did not have an adequate number of plants to make a collection, or plants had dispersed their seed before visiting the site. Herbarium vouchers for the 14 Utah lotus collections were obtained from the Blue Creek common garden in 2014 for species verification. The Intermountain Herbarium at Utah State University in Logan, Utah confirmed the 14 collections to be Utah lotus.

The birdsfoot trefoil cultivar “Norcen” (Miller et al., 1983) was included in our study for comparison with the Utah lotus collections because Norcen has been studied widely for its agronomic and forage quality characteristics. In addition, five collections of scrub lotus (*Lotus wrightii* [A. Gray] Greene) were included in our study to compare Utah lotus with another *Lotus* species native to the western United States.

Common Garden Studies

In January 2013, seeds from each of the 14 Utah lotus collection sites, plus seed of Norcen birdsfoot trefoil and five scrub lotus collections, were germinated and grown in Q-Plugs (International Horticulture Technologies, Hollister, CA) placed within Ray Leach stubby containers (Stuewe and Sons, Corvallis, OR) in a greenhouse at the US Department of Agriculture – Agricultural Research Service Forage and Range Research Laboratory at Logan, Utah, under a 30°C-day–15°C-night temperature regime. A single seedling was grown in each container. Seedlings were treated with a *Lotus*-specific, peat-based rhizobial inoculant mixed with water (Tom Wacek, Lafayette, IN). During May 2013, seedlings were transplanted into cultivated soils at three common gardens in northern Utah (Millville, North Park, and Blue Creek) (see Table 1) in weed barrier fabric. Common garden plots did not receive any supplemental irrigation and were weeded on a weekly basis as needed.

The experimental design at each common garden site was a randomized complete block with five replications. Individual plots consisted of six plants of a single collection within each replication (block). Rows were spaced 0.31 m apart, and plants within rows were spaced 0.31 m apart. A total of 600 plants were transplanted into each common garden site. In addition, Norcen plants were planted around the outside periphery of the common garden plots so that all *Lotus* plants in our study were exposed to competition from other *Lotus* plants. Norcen plants were more vigorous than our native *Lotus* collections. As a result, we found it necessary to cut back the Norcen plants on the periphery of our plots weekly during the 2015 growing season to prevent them from overtaking plants of the native *Lotus* collections.

Plants at each of the three common garden sites established and grew during the 2013 growing season. During the 2014 and 2015 growing seasons, plants at each site were evaluated for survival, date of spring emergence, flowering date, vegetative period (days from emergence to flowering), canopy height, canopy width, longest stem length (LSL), dry-matter yield (DMY), number of stems, pod mass, crude protein (CP), neutral detergent fiber (NDF), and CT content. Pod mass was chosen as a surrogate measure for the number of flowers or seeds per plant because the prolific number of flowers produced by Utah lotus made it impractical to count the number of flowers on each plant. In addition, not all the various collections of Utah lotus matured at the same time, and seeds of Utah lotus can be dispersed considerable distances when pods mature and dehisce. As a result, pod mass was used as a practical indicator of seed production for each plant and reported as pod production.

Spring emergence and flowering dates were recorded weekly beginning the first week of April in 2014 and mid-March in 2015. Spring emergence date was defined as the day at least one stem of an observed plant emerged from the ground. Flowering date was defined as the day the first flower bud opened on any plant in a plot. Survival (persistence) was recorded if a plant was actively growing on the date of DMY harvest. Canopy height, canopy width, and longest stem length were determined at full bloom (Blue Creek on 2 July 2014 and 3 July 2015, Millville on 30 June 2014 and 2 July 2015, and North Park on 1 July 2014 and 1 July 2015). The DMY was harvested when plants reached full pod

Table 1

Geographic and environmental data for 14 Utah lotus (*Lotus utahensis*, LU) and five scrub lotus (*Lotus wrightii*, LW) seed collection sites and three common garden sites. Mean average temperature (MAT), mean diurnal temperature range (MDTR), maximum temperature of warmest month (MTWM), minimum temperature of coldest month (MTCM), and annual precipitation (AP) were extracted from BIOCLIM (Hijmans et al., 2005).

Collection	Site name	County and state	Latitude	Longitude	Elevation (m)	MAT (°C)	MDTR (°C)	MTWM (°C)	MTCM (°C)	AP (mm)
LU-2	Panaca Summit	Lincoln, Nevada	37.764	−114.189	2 048	8.3	15.6	29.5	−8.5	363
LU-4	Muleshoe	Lincoln, Nevada	38.470	−114.690	1 907	8.1	17.7	30.7	−10.7	293
LU-5	Clear Creek	Kane, Utah	37.260	−112.844	1 926	9.1	16.7	30.3	−8.6	401
LU-6	Kanosh	Millard, Utah	38.735	−112.352	1 800	8.0	17.1	30.6	−10.9	322
LU-7	Indian Creek	Beaver, Utah	38.433	−112.565	2 146	5.4	17.7	27.8	−13.0	437
LU-8	City Creek	Piute, Utah	38.261	−112.306	2 269	5.5	18.1	27.8	−13.5	376
LU-9	Koosharem	Sevier, Utah	38.476	−111.957	2 516	4.0	17.1	25.4	−14.4	372
LW-10	Mt. Ord	Gila, Arizona	33.927	−111.401	1 779	12.0	15.9	30.6	−3.7	679
LW-12	Munds Park	Coconino, Arizona	34.939	−111.620	2 095	8.7	16.2	28.0	−7.7	597
LW-13	Stoneman Lake	Coconino, Arizona	34.784	−111.538	2 038	8.9	16.0	28.0	−7.3	618
LW-14	Milk Point Ranch	Coconino, Arizona	34.407	−111.411	2 198	8.6	15.3	27.0	−7.1	711
LU-15	Pine Valley	Washington, Utah	37.373	−113.456	2 140	7.4	15.6	28.0	−9.3	450
LU-16	Navajo Lake	Kane, Utah	37.517	−112.729	2 780	3.3	15.8	23.6	−13.3	535
LU-17	Boulder Mountain	Garfield, Utah	37.965	−111.554	2 510	5.0	15.4	25.4	−13.0	342
LU-18	Fish Lake	Sevier, Utah	38.529	−111.740	2 706	3.3	16.0	24.0	−14.5	405
LU-19	Torrey	Wayne, Utah	38.119	−111.326	2 689	3.8	14.4	23.6	−13.4	407
LU-20	Kolob Reservoir	Washington, Utah	37.389	−113.043	2 408	5.7	15.9	26.1	−11.0	488
LW-22	Jacob Lake	Coconino, Arizona	36.737	−112.228	2 356	6.8	15.1	26.7	−9.6	466
LU-23	Cove Fort	Millard, Utah	38.616	−112.614	1 810	8.3	17.3	31.2	−10.6	315
Common garden										
Blue Creek		Box Elder, Utah	41.935	−112.438	1 568	6.9	15.8	30.2	−11.9	400
Millville		Cache, Utah	41.657	−111.813	1 435	8.5	13.8	31.2	−9.4	490
North Park		Cache, Utah	41.786	−111.815	1 386	8.7	14.0	31.8	−9.3	457

maturity and first pod dehiscence. Utah lotus accessions were harvested at Blue Creek on 22 July 2014 and 14 July 2015, Millville on 11 July 2014 and 13 July 2015, and North Park on 22 July 2014 and 13 July 2015. Because Utah lotus and scrub lotus flowered and matured at different times, DMY for scrub lotus was harvested 3 weeks after Utah lotus on 11 August 2014 and 13 July 2015 at Blue Creek, 25 July 2014 and 31 July 2015 at Millville, and 11 August 2014 and 31 July 2015 at North Park.

DMY was a result of whole plant harvest above 5 cm of all plants in each plot (up to six). The DMY samples were dried at 70°C to a constant weight (a minimum of 24 h) and then immediately weighed. Primary stems were counted, and pods were separated and weighed. The DMY samples were ground to pass through a 1-mm screen in a Wiley mill (Thomas Scientific, Swedesboro, NJ) and scanned with a near infrared reflectance spectroscopy (NIRS) instrument (Model 6500, Pacific Scientific Instruments, Silver Spring, MD). Software from NIRS (IS-O122FS, Infrasoft International LLC, Port Matilda, PA) was used to calibrate forage equations to predict crude protein (CP) and neutral detergent fiber (NDF) for each sample. Representative samples were selected from each collection, common garden, and year and used as a calibration data set for total N. Total N was determined on 90 samples using total combustion procedures (LECO TruSpec C/N analyzer, LECO Corp., St. Joseph, MI). The NDF was determined on 67 samples using the Ankom-200 filter-bag technique (Ankom, Macedon, NY). The r^2 value between predicted and actual CP was 0.95, with a corrected standard error of prediction (SEP) of 0.62. The r^2 value between predicted and actual NDF was 0.94 with a SEP of 2.02.

Samples for determination of CT content were harvested separately from those for measuring DMY. Harvest for CT entailed clipping 3–5 shoots (including leaves) from the base of each plant, immediately placing the samples on dry ice, and storing the tissue in a −80°C freezer. Following lyophilization, tissues were ground through a 1-mm screen using an electric coffee grinder to minimize sample loss. Triplicate ground samples were analyzed for CT content using the acetone-butanol-HCl-iron assay described by Grabber et al. (2013). Separate standards of purified CT were prepared for each species (Utah lotus, birdsfoot trefoil, and scrub lotus) to accurately predict CT concentration in tissues of the respective species.

Statistical Procedures

Phenotypic data were statistically evaluated using the MIXED procedure of SAS 9.4 (SAS, 2011). Year and site combinations were assumed to represent separate environments. Collection (accession) was considered a fixed effect, whereas common garden sites, years, and replications within each common garden site were considered random effects. Sites and their interactions were treated as random effects in the mixed model, as suggested by St-Pierre (2001). Least square (LS) means were calculated for collections by averaging across the six common garden site-year combinations and for common garden sites by averaging across the 14 seed accessions. Mean separations were calculated using Tukey's HSD test at a significance level of $\alpha = 0.05$.

Pearson's correlation coefficients were computed among standardized phenotypic traits, and Spearman's rank coefficients were used to assess magnitude changes of each collection at the common garden locations for Utah lotus using the CORR procedure in SAS (SAS, 2011). Highly correlated traits for Utah lotus that were biologically similar (Parsons et al., 2011) were consolidated into three composite variables with principal-component analysis (PCA) using the R base foundational software 3.2.2 (R Foundation for Statistical Computing, Vienna, Austria). Using the FACTOR procedure in SAS (Hair et al., 2006; SAS, 2011), common-factor analysis (CFA) was used to identify relationships among composite data and remaining original data for the Utah lotus seed collections. Three factors were included in the model, and orthogonal rotations were applied to generate a more interpretable result with relatively few high factor loadings for several variables and low factor loadings for the majority of variables (Parsons et al., 2011).

Relationships among factor loadings and the collection site geographic and environmental characteristics (longitude, latitude, elevation, annual mean temperature, mean diurnal temperature range, maximum temperature of the warmest month, minimum temperature of the coldest month, and annual precipitation [see Table 1]) for Utah lotus were evaluated by Pearson's correlation coefficients using the CORR procedure in SAS (SAS, 2011). In addition, canonical correlation analysis (CCA) using the CANCELL procedure in SAS (SAS, 2011) was used to identify traits with possible adaptive significance.

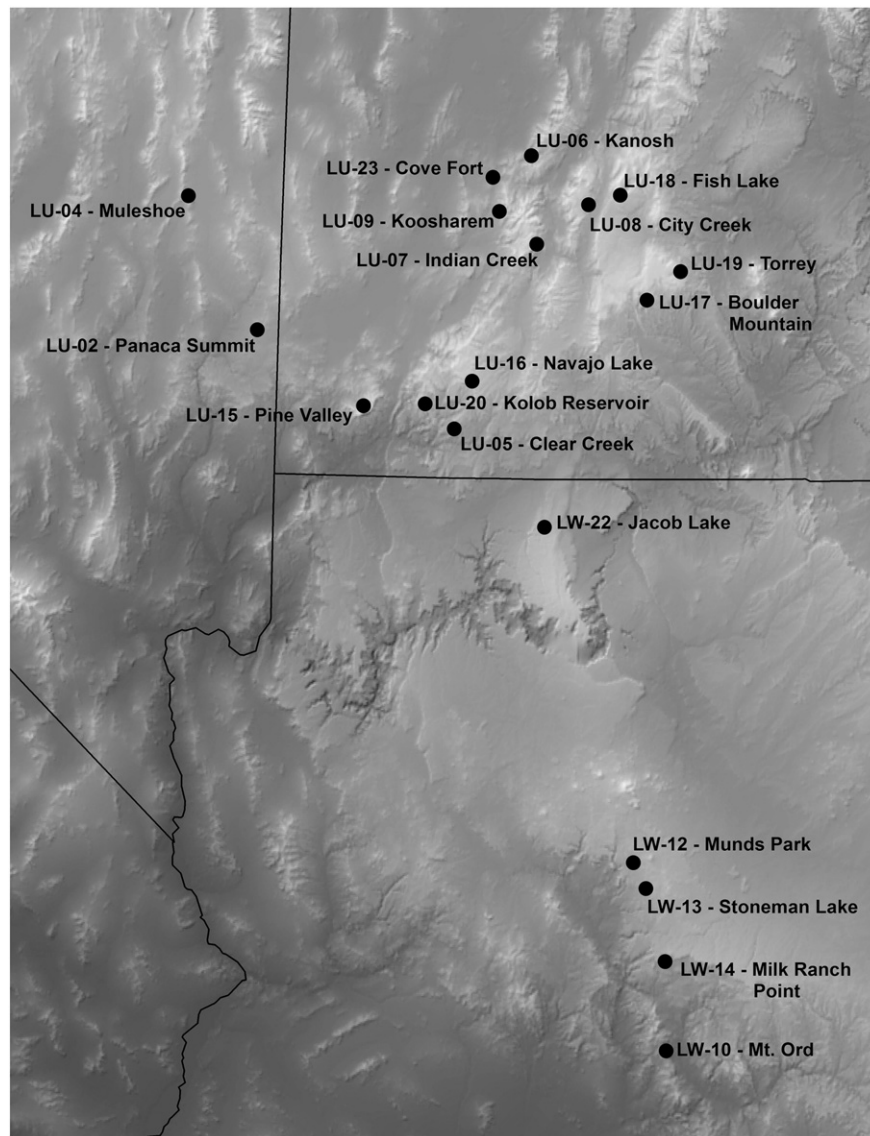


Figure 1. Seed collection sites of Utah lotus (LU) in eastern Nevada and southern Utah and scrub lotus (LW) in Arizona.

Molecular Evaluations

Seedling shoot apical regions of 8–12 individual plants from each accession were sampled from greenhouse seedlings before field transplanting. Supplemental samples from the Millville common garden site were obtained as needed in 2014 for extra DNA extractions. Tissues were freeze dried and stored at -20°C . DNA was extracted using the Qiagen DNeasy 96-well procedure (Qiagen, Valencia, CA) according to the manufacturer's protocol. Concentration and quality of genomic DNA were assessed spectrophotometrically and by gel electrophoresis, and the genomic DNA concentration was then adjusted to approximately $30\text{ ng}/\mu\text{L}^{-1}$. The AFLP procedure of Vos et al. (1995) was used with six selective primer pairs chosen for analysis: E.AGT/M.CAC, E.ACT/M.CAG, A.AGA/M.CTG, E.AGG/M.CAG, E.ACA/M.CTC, and E.ACC/M.CAC. The AFLP bands were scored visually for presence and absence using Genographer software (Benham, 2001). Duplicates of 12 plants (7% of total samples) provided an estimated error rate of 0.032.

Similarity within collections was calculated using the Dice coefficient implemented as per Leonard et al. (1999). GenAlEx software v6.5 (Peakall and Smouse, 2006, 2012) was employed to generate a matrix of pairwise genetic distances (ϕ_{ST}), which were used to construct a neighbor-joining (NJ) dendrogram with PHYLIP software

v3.695 (Felsenstein, 2009). Strength of nodes within the dendrogram was evaluated using a bootstrap analysis with 5 000 permutations. Analysis of molecular variation (AMOVA) and principal coordinate analysis (PCoA) were conducted using GenAlEx software to show variation among collections. Population structure was evaluated using the Bayesian clustering analysis feature in STRUCTURE v2.3.4 (Falush et al., 2007). Probabilities of $K = 1$ through $K = 9$ groups were tested for each level of K . The Delta K was plotted against the K values to observe the structural fit of the data. Discrete groupings of collections identified with STRUCTURE and NJ tree were further analyzed using PCoA, which does not assume hierarchical order in determining groupings.

Mantel's Z statistic (Mantel, 1967) was used to determine if marker-based genetic distances were correlated to geographic, phenotypic, and environmental distance matrices using the Isolation by Distance web service 3.23 (Jensen et al., 2005). A genetic distance matrix (a ϕ_{ST} matrix) was obtained using GenAlEx. A geographic distance matrix was constructed in GenAlEx using the geographic coordinates (latitude and longitude) at each collection site. Phenotypic and environmental distance matrices were constructed with the DISTANCE procedure in SAS (SAS, 2011), using phenotypic traits (Table 2) and environmental characteristics (see Table 1), respectively.

Results

Common Garden Studies

Site, year, and the interaction between site and year were significant for all traits measured ($P \leq 0.05$), with the exception of pod mass with site and year, and survival and emergence date with the interaction between site and year. The Spearman's rank coefficients across sites and years were > 0.7 ($P < 0.05$) for all traits, which indicated that changes between sites and years were primarily due to magnitude changes rather than rank changes between accessions. Therefore, data were combined and averaged across sites and years for each collection.

For Utah lotus collections, high positive correlations were observed between DMY and number of stems ($r = 0.71$, $P < 0.001$), pod mass ($r = 0.78$, $P < 0.001$), canopy height ($r = 0.81$, $P < 0.001$), canopy width ($r = 0.79$, $P < 0.001$), and longest stem length ($r = 0.84$, $P < 0.001$) (Table 4). A high negative correlation was observed between NDF and CP ($r = -0.67$, $P < 0.001$). A high positive correlation was observed between spring emergence date and flowering date ($r = 0.74$, $P < 0.001$), and a negative correlation was observed between spring emergence date and vegetative period ($r = -0.73$, $P < 0.001$).

For the three composite variables created for Utah lotus using PCA data reduction, Composite1_{LU} consisted of DMY, number of stems, pod mass, canopy height, canopy width, and longest stem length with 79.5% of their total variation described by the first principal component. Composite2_{LU} consisted of NDF and CP with 83.7% of their total variation described by the first principal component. Composite3_{LU} consisted of spring emergence date, flowering date, and vegetative period with 72.9% of their total variation described by the first principal component. Survival and CT were not combined into composite variables and were considered separate variables.

Eigenvalues of the first two factor loadings for Utah lotus were > 1 , and the first three factor loadings described 96% of the total variation (Table 5). Factor1_{LU} described 67% of the total phenotypic variation and had highly positive factor loadings with survival ($r = 0.97$) and Composite3_{LU} ($r = 0.84$). Factor2_{LU} described 21% of total phenotypic variation and had high positive factor loadings for Composite1_{LU} ($r = 0.79$) and Composite2_{LU} ($r = 0.89$). Factor3_{LU} described 8% of total variation and had a high positive factor loading for CT ($r = 0.93$).

Collections with the highest Factor1_{LU} loadings, which represented survival and phenology, were LU-6, LU-16, and LU-20 (Table 6). These collections had the highest 2-yr survival rates and exhibited relatively early emergence. Also, LU-16 flowered earlier than all other collections (see Table 2). Collections with the highest Factor2_{LU} loadings, which represented morphology and forage quality, were LU-2, LU-4, and LU-7 (see Table 6). LU-2 trended to the lowest NDF but was not different from LU-7 for that trait (see Table 2). Collections LU-2, LU-4, and LU-7 also had the highest CP. However, for most of the traits combined within the first composite variable (Composite1_{LU}) (DMY, number of stems, pod mass, canopy height, canopy width, and longest stem), these three collections were among the lowest. Collections with the highest Factor3_{LU} loadings, which represented CT, were LU-8, LU-9, and LU-7 (see Table 6). LU-8 trended to the highest CT levels (199 g/kg⁻¹ dry matter) but was not different from LU-9 and LU-7 for that trait (see Table 2).

Genetic Relationships Among Collections

Genetic diversity and population structure for Utah lotus was determined using 552 AFLP markers (Meudt and Clarke, 2007). The average similarity among individuals within each collection ranged from 0.599 (LU-15) to 0.727 (Norcen) with a mean of 0.671. AMOVA apportioned 27% of the variation among Utah lotus collections and 73% of the variation within collections. Based on pairwise ϕ_{ST} distances of all collections, an NJ dendrogram rooted with Norcen was used to investigate genetic structures (Fig. 2). Principal coordinate analysis showed that LU-2 and LU-4 grouped together and closer to Norcen than all other collections. LU-15 was intermediate between the LU-2/LU-4 group and the LU-5/LU-16/LU-20 group.

Bayesian clustering showed that the increase in average log probability values diminished after the $K = 3$ test (Fig. 3). The second order rate of change reached its peak value at the $K = 3$ group, indicating that the most parsimonious and strongest structure fit occurred with three genetically differentiated groups. The three primary populations consisted of LU-2, LU-4, and a portion of LU-15; LU-5, LU-16, LU-20, and a portion of LU-15; and the remaining eight Utah lotus collections (Fig. 4). PCoA also separated groups within Utah lotus on the second axis. LU-2 and LU-4 collections grouped together and closer to Norcen than all other collections. LU-15 was equidistant between the LU-2

Table 2

Means of phenotypic characteristics of 14 accessions of Utah lotus (*Lotus utahensis*, LU) and five accessions of scrub lotus (*L. wrightii*, LW) with Tukey mean separations. Data are averaged across six site-year combinations.

Entry	DMY ¹ (g)	No. of stems	Pod mass (g)	Canopy height	Canopy width	LSL	NDF	CP	CT	Survival	Emergence date ²	Flower date	Vegetative period
					cm			g kg ⁻¹					
LU-2	4.4 ¹ 3	7.8 ^{GH}	0.7 ^A	7.8 ^I	19.1 ^H	12.5 ^I	244 ^F	148 ^A	185 ^{ABC}	4.8 ^{BCDE}	134 ^{ABC}	158 ^B	28 ^G
LU-4	4.9 ^I	8.6 ^{FGH}	1.3 ^{AB}	12.4 ^H	20.6 ^{GH}	15.8 ^H	297 ^{CDE}	137 ^{ABC}	154 ^{DEFGH}	4.9 ^{BCDE}	133 ^{ABC}	159 ^B	29 ^{FG}
LU-5	20.5 ^{AB}	23.8 ^{AB}	4.1 ^D	27.0 ^A	31.1 ^{ABCD}	28.9 ^{AB}	357 ^{AB}	120 ^{DEFG}	152 ^{EF}	5.4 ^{AB}	124 ^F	162 ^B	41 ^{ABC}
LU-6	11.6 ^{DEFG}	16.2 ^{BCDE}	2.1 ^{BC}	17.7 ^{CDEF}	24.9 ^{DEFGH}	20.3 ^{EFG}	276 ^{DEF}	131 ^{ABCD}	172 ^{BCD}	5.7 ^A	126 ^{DEF}	160 ^B	38 ^{BCD}
LU-7	8.3 ^{FGH}	12.7 ^{DEFG}	1.5 ^{AB}	13.8 ^{GH}	21.7 ^{FGH}	16.6 ^{GH}	270 ^{DEF}	137 ^{ABC}	184 ^{ABC}	5.4 ^{ABC}	131 ^{BCDE}	159 ^B	32 ^{DEFG}
LU-8	7.3 ^{GHI}	10.9 ^{EFG}	1.3 ^{AB}	14.0 ^{GH}	19.0 ^H	15.9 ^H	267 ^{DEF}	138 ^{ABC}	199 ^A	4.7 ^{DEF}	132 ^{BCD}	162 ^B	34 ^{CDEFG}
LU-9	10.3 ^{EF}	14.4 ^{CDEF}	1.6 ^B	16.2 ^{EFG}	23.4 ^{EF}	18.6 ^{FGH}	260 ^{EF}	141 ^{AB}	192 ^{AB}	4.4 ^{EF}	132 ^{BCD}	162 ^B	34 ^{CDEFG}
LW-10	11.9 ^{CDEFG}	8.6 ^{FGH}	1.1 ^{AB}	19.7 ^{CDE}	37.2 ^A	28.4 ^{ABC}	376 ^A	121 ^{CDEFG}	143 ^{GH}	3.7 ^G	131 ^{BCDEF}	176 ^{CD}	48 ^A
LW-12	12.2 ^{CDEFG}	13.2 ^{DEFG}	1.2 ^{AB}	19.1 ^{CDE}	32.1 ^{ABCD}	25.7 ^{ABCD}	357 ^{AB}	125 ^{BCDEF}	142 ^H	4.3 ^{EF}	135 ^{ABC}	176 ^{CD}	46 ^B
LW-13	19.2 ^{ABC}	19.0 ^{BCD}	1.8 ^{BC}	21.2 ^{BC}	33.6 ^{ABC}	25.6 ^{ABCD}	374 ^A	117 ^{EF}	153 ^{DEFGH}	4.7 ^{CDEF}	134 ^{ABC}	178 ^D	47 ^A
LW-14	14.4 ^{BCDE}	9.0 ^{FGH}	1.2 ^{AB}	24.6 ^{AB}	33.3 ^{ABC}	29.3 ^{AB}	393 ^A	113 ^{FG}	161 ^{DEFGH}	4.7 ^{DEF}	139 ^A	174 ^{CD}	39 ^{BCD}
LU-15	14.6 ^{BCDE}	14.1 ^{DEF}	4.7 ^D	21.1 ^{BC}	28.2 ^{BCDEF}	24.2 ^{BCDE}	321 ^{BC}	109 ^G	167 ^{CDE}	5.2 ^{ABCD}	125 ^{EF}	158 ^B	37 ^{CDEF}
LU-16	16.3 ^{BCDE}	21.8 ^{ABC}	1.7 ^B	19.9 ^{CD}	35.4 ^{AB}	25.2 ^{BCD}	267 ^{DEF}	138 ^{ABC}	164 ^{CDEF}	5.6 ^A	125 ^{EF}	151 ^A	31 ^{DEFG}
LU-17	12.8 ^{CDEF}	15.9 ^{BCDE}	1.8 ^{BC}	20.6 ^{BCD}	26.6 ^{CDEFG}	23.2 ^{CDE}	294 ^{CDE}	126 ^{BCDEF}	173 ^{BCD}	4.8 ^{BCDE}	131 ^{CDEF}	162 ^B	35 ^{CDEFG}
LU-18	8.5 ^{FGH}	12.6 ^{DEFG}	1.5 ^{AB}	18.3 ^{CDEF}	24.7 ^{DEFGH}	21.2 ^{DEF}	291 ^{CDE}	134 ^{ABCD}	172 ^{BCD}	4.7 ^{CDEF}	133 ^{ABC}	159 ^B	29 ^{FG}
LU-19	10.0 ^{EF}	12.3 ^{DEFG}	1.9 ^{BC}	17.4 ^{DEF}	25.7 ^{DEFG}	21.0 ^{DEF}	293 ^{CDE}	132 ^{ABCD}	162 ^{DEFG}	3.9 ^{FG}	134 ^{ABC}	161 ^B	30 ^{EF}
LU-20	24.1 ^A	31.8 ^A	3.5 ^{CD}	27.9 ^A	36.9 ^A	30.6 ^A	318 ^{BC}	125 ^{BCDEF}	158 ^{DEFGH}	5.6 ^A	124 ^F	158 ^B	38 ^{BCDE}
LW-22	6.3 ^{HI}	5.0 ^{HI}	1.2 ^{AB}	18.2 ^{CDEF}	29.8 ^{ABCD}	25.0 ^{BCD}	366 ^A	122 ^{CDEFG}	144 ^{GH}	4.7 ^{DEF}	138 ^{AB}	170 ^C	36 ^{CDEF}
LU-23	10.0 ^{FGH}	16.3 ^{BCDE}	1.7 ^B	15.5 ^{FG}	21.4 ^{GH}	17.5 ^{FGH}	299 ^{CD}	134 ^{ABCD}	146 ^{FGH}	4.8 ^{BCDE}	129 ^{CDEF}	160 ^B	35 ^{CDEFG}

¹ DMY indicates dry-matter yield; LSL, longest stem length; NDF, neutral detergent fiber; CP, crude protein; CT, condensed tannins.

² Emergence and flower dates are measured from 1 January.

³ Values followed by the same letter within the same column are not significantly different at $P = 0.05$.

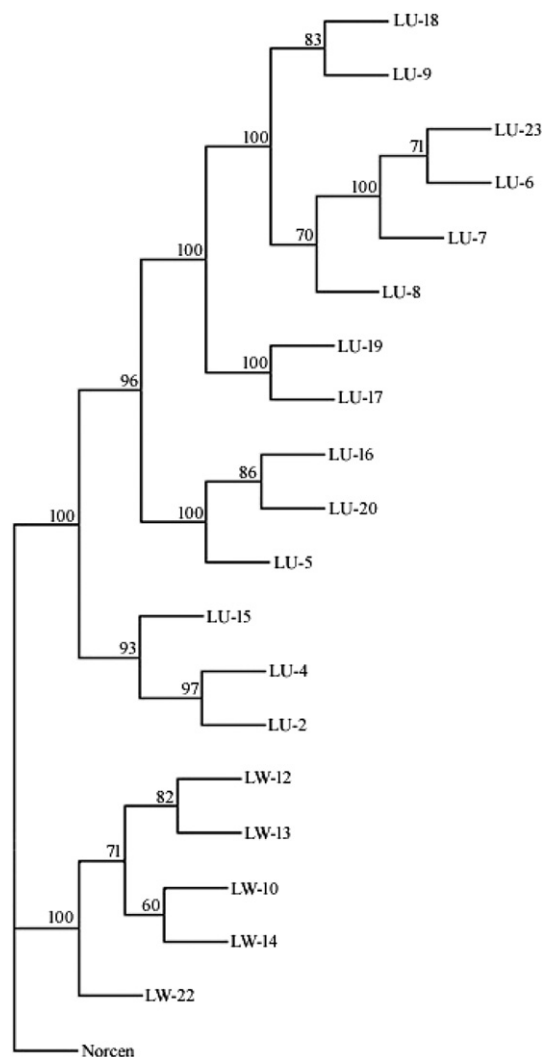


Figure 2. A neighbor-joining (NJ) dendrogram constructed using a pairwise ϕ_{ST} distance matrix of 19 *Lotus* collections with associated bootstrap values (%).

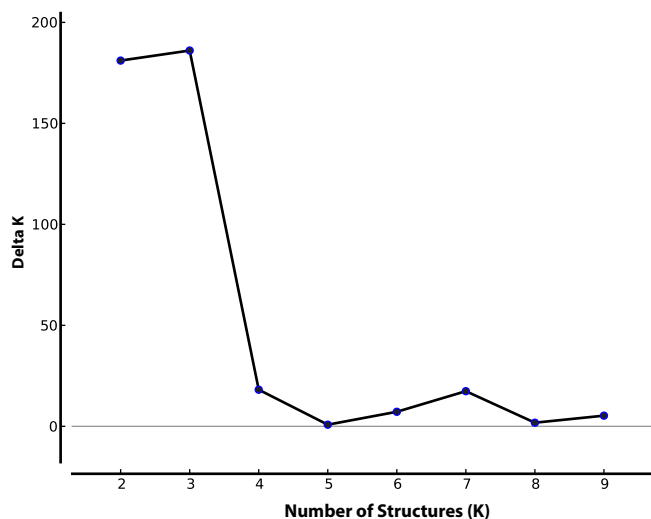


Figure 3. Delta K versus K plot from Bayesian clustering of 14 *Lotus* collections tested from 1 to 9 groups

and LU-4 grouping and the LU-5, LU-16, and LU-20 grouping (Fig. 5). (See Fig. 4.)

Discriminate analysis of common garden traits was used to discriminate the three groups of Utah lotus populations revealed by PCoA and structure analyses. However, because the PCoA and structure analyses split LU-15 individuals between two groups, it was necessary to test two models, with LU-15 in each of two possible groups. The first model, which grouped LU-15 with LU-2/LU-4, had a significant Wilk's Lambda value of $F_{2,11} = 21.11$ ($P = 0.0002$). However, the number of stems was the only trait able to discriminate among the three groups, discriminating LU-2/LU-4/LU-15 with 66.7% accuracy and LU-6/LU-7/LU-8/LU-9/LU-17/LU-18/LU-19/LU-23 with 87.5% accuracy. The second model, which grouped LU-15 with LU-6/LU-16/LU-20, had a Wilk's Lambda value of $F_{6,18} = 10.93$ ($P < 0.0001$). It identified DMY, pod mass, and flowering date as discriminating traits for LU-2/LU-4 and LU-5/LU-15/LU-16/LU-20 with 100% accuracy and the remaining larger group (LU-6/LU-7/LU-8/LU-9/LU-17/LU-18/LU-19/LU-23) with 87.5% accuracy (with the phenotypically small collection LU-8 misclassified with LU-2/LU-4).

Mantel's correlation statistic (Z) showed significant correlations between the genetic and geographic distance matrices ($r = 0.888$, $P = 0.001$) and between the phenotypic and geographic distance matrices ($r = 0.248$, $P = 0.044$). No significant correlations were found between the genetic and environmental, genetic and phenotypic, and phenotypic and environmental distance matrices (Table 3). In addition, no significant Pearson's correlations were found between any PCoA factors and any collection site geographical and environmental characteristics. Similarly, canonical variables were not correlated to geographic or environmental characteristics, indicating that the canonical variables were not representative of any geographical and climatic variables associated with seed source environments. These results suggested that there was little evidence of local adaptation for the measured phenotypic traits and collection-site characteristics.

Discussion

Common Garden Studies

Forage quality and DMY are important plant characteristics for livestock and wildlife grazing (Bhattarai et al., 2010). In 2014, mean NDF and CP for Norcen were 352 and 119 g kg⁻¹, respectively. The forage quality of collections LU-5 and LU-20 were similar to that of Norcen with NDF and CP being 357 and 120 g kg⁻¹, and 318 and 125 g kg⁻¹, respectively. Although these forage quality values in our study were from late-season harvests with mature seed pods, NDF was comparable or better than that reported for summer regrowth of alfalfa (*Medicago sativa* L.) (375–410 g kg⁻¹) and birdsfoot trefoil (323–396 g kg⁻¹) harvested at 10% bloom (Cassida et al., 2000). Similarly, Cassida et al. (2000) reported that CP at early bloom for alfalfa was 200–244 g kg⁻¹ and for birdsfoot trefoil was 192–236 g kg⁻¹, which were considerably greater than our CP values for Utah lotus. Because pod production characteristics were important for us to consider, DMY in our study was harvested at the mature pod stage, which generally is past peak quality for most pasture legumes. It is possible that harvesting *Lotus* plants at the early bloom stage may result in higher forage quality than observed in our study, but this may not be of prime concern for rangeland use.

Low concentrations of CT are important for forage quality because CTs reduce the risk of bloat, improve nutrient absorption, reduce protein degradation in the rumen (Smith and Kelman, 1997), and control intestinal parasites (Novobilisky et al., 2011). Big trefoil (*Lotus uliginosus* Schkuhr) reportedly has high CT concentrations up to 107 g kg⁻¹ (Kelman and Tanner, 1990). Big trefoil with high CT (106 g kg⁻¹) reduced digestibility and absorption of nutrients in sheep studies when compared with big trefoil with low CT levels (46 g kg⁻¹) (Barry and Manley, 1984). It was recommended that genotypes of big trefoil with

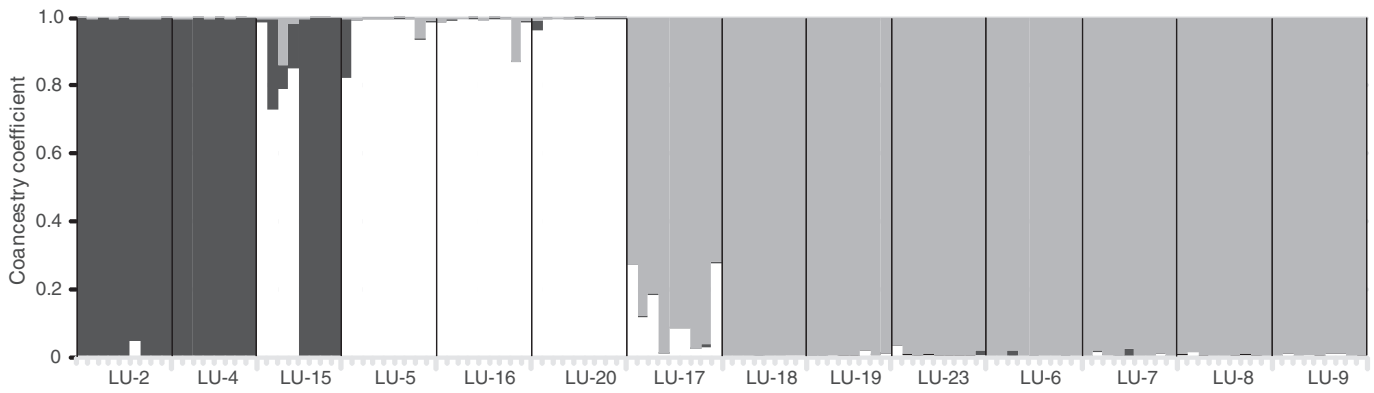


Figure 4. Bar plot of collections grouped at $K = 3$ indicates the three groups within the Utah lotus collections based on Bayesian clustering of 14 Utah lotus collections.

low CT should be selectively bred to have no more than 40 g kg^{-1} CT (Smith and Kelman, 1997). In our study, CT values for Utah lotus ranged from 146 to 199 g kg^{-1} dry matter. The CT values in Utah lotus were on average nearly 10 times greater than that found in Norcen in our study (17.3 g kg^{-1} dry matter) and the study of Grabber et al. (2015). However, collections LU-5 and LU-20 were among the lowest CT concentrations, with 152 and 158 g kg^{-1} dry matter, respectively, which were not different than that for LU-23, which had the lowest CT of 146 g kg^{-1} dry matter. Although Utah lotus has relatively high levels of CT, plant regrowth was heavily grazed by jack rabbits (*Lepus californicus* [Gray]) and/or mule deer (*Odocoileus hemionus* [Rafinesque]) at the Blue Creek site during autumn in both the 2014 and 2015 growing seasons, which suggests that wildlife may tolerate high CT levels.

Genetic Relationships

The least diverse (highest within-collection similarity) accession was Norcen ($S\text{-value} = 0.73$), while all the Utah lotus collections were more diverse, with values between 60% and 71% similarity among plants within collections. The most diverse (lowest within-collection similarity) Utah lotus collection was LU-15 ($S\text{-value} = 0.60$). AMOVA revealed that most genetic variation was apportioned within each collection. Consistent with the similarity values, the AMOVA indicated that the Utah lotus collections had 73% variation within collections and 27% among collections. These data indicated that Utah lotus is a highly outcrossed species that should have ample variation for adapting to environmental changes (Hoffmann and Sgro, 2011).

The most significant grouping with Bayesian clustering was at the species level for $K = 3$ structures. The PCoA analysis, discriminant analysis, and NJ dendrogram also showed support for these same groupings. Indeed, there was bootstrap support between the same three subgroups

mentioned earlier in the dendrogram. The only difference in clustering of collections was that LU-15 in the PCoA analysis did not cluster with any collection but was an intermediate distance between LU-2/LU-4 and LU-5/LU-16/LU-20. These three Utah lotus subgroups were statistically discriminated by phenotypic trait differences with only the LU-15 admixed populations as potentially misclassified, where LU-15 was included with the LU-5/LU-16/LU-20 group. Thus, although LU-15 was genetically closer to LU-2/LU-4, it was phenotypically more similar to LU-5/LU-16/LU-20.

The geographic and genetic distances for Utah lotus were highly correlated, and phenotypic and geographic distances were moderately correlated (see Table 3). This indicated that the further apart the collections were, the more genetically distant they were. The same trend held true for phenotypic distance, albeit with a much smaller coefficient. The two western-most collections from Nevada were grouped apart from the rest of the Utah lotus seed collections in the PCoA analysis (see Fig. 5), which was also supported by the discriminate analysis of measured phenotypic traits. Isolation by distance is a common phenomenon for naturally occurring populations of outbreeding perennials (Warren et al., 1998).

Despite the isolation-by-distance correlation, there is lack of evidence for strong local adaptation for the traits we measured. This lack of evidence was supported by the nonsignificant Mantel's Z tests between the environmental distance matrices and phenotypic distance

Principal Coordinates (PCoA)

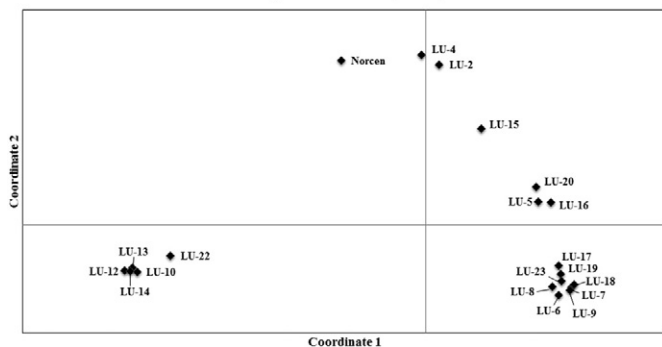


Figure 5. Principal coordinate analysis (PCoA) separations among Utah lotus, and Norcen.

Table 3

Mantel's test statistic (Z) with associated r and P values for various distance matrices for Utah lotus with and without the two collections from Nevada.

	Utah lotus all collections	Utah lotus without Nevada collections
Genetic by geographic		
Z	3 553.9	1 700.0
r	0.888	0.870
P value	0.001	1.000
Genetic by environmental		
Z	2 341 928.0	1 452 039
r	0.108	0.098
P value	0.859	0.836
Genetic by phenotypic		
Z	117.2	71.6
r	0.2352	0.565
P value	0.074	0.998
Phenotypic by geographic		
Z	57 770.3	35 008.5
r	0.2481	0.606
P value	0.044	0.998
Phenotypic by environmental		
Z	40 299 298	29 310 006
r	−0.0854	−0.0489
P value	0.812	0.384

Table 4

Pearson correlation coefficients (r) for dry-matter yield (DMY), number of stems, pod mass, canopy height, canopy width, longest stem length (LSL), neutral detergent fiber (NDF), crude protein (CP), condensed tannins (CT), survival, emergence date, flowering date, and vegetative period of 14 Utah lotus collections.

	DMY	No. of stems	Pod mass	Canopy height	Canopy width	LSL	NDF	CP	CT	Survival	Emergence date	Flowering date
No. of stems	0.71*** ¹	—	—	—	—	—	—	—	—	—	—	—
Pod mass	0.78***	0.47***	—	—	—	—	—	—	—	—	—	—
Canopy height	0.81***	0.73***	0.69***	—	—	—	—	—	—	—	—	—
Canopy width	0.79***	0.74***	0.59***	0.81***	—	—	—	—	—	—	—	—
LSL	0.84***	0.73***	0.69***	0.96***	0.91***	—	—	—	—	—	—	—
NDF	0.56***	0.49***	0.76***	0.70***	0.55***	0.68***	—	—	—	—	—	—
CP	−0.29***	−0.12 ns	−0.59***	−0.45***	−0.22*	−0.42***	−0.67***	—	—	—	—	—
CT	−0.24*	−0.05 ns	−0.17*	−0.22*	−0.17 ns	−0.25*	−0.33**	0.22*	—	—	—	—
Survival	0.36***	0.04 ns	0.27*	0.22*	0.23*	0.26*	0.15 ns	−0.29**	−0.30**	—	—	—
Emergence date	−0.19 ns	−0.68***	−0.19 ns	−0.40***	−0.45***	−0.39***	−0.35**	0.03 ns	−0.21 ns	0.33**	—	—
Flowering date	0.15 ns	−0.35***	0.11 ns	−0.11 ns	−0.14 ns	−0.1 ns	−0.06 ns	0.07 ns	−0.34**	0.25*	0.74***	—
Vegetative period	0.32**	0.58***	0.23*	0.41***	0.31**	0.35**	0.19 ns	0.13 ns	0.11 ns	−0.23*	−0.73***	−0.28*

¹ns indicates not significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Table 5

Factor loadings for three orthogonal factors derived from three composite and two directly (D) measured variables for 14 Utah lotus collections.

Variable	Factor1 _{LU}	Factor2 _{LU}	Factor3 _{LU}
Eigenvalue	3.33	1.05	0.41
Cumulative	0.67	0.88	0.96
Composite1 _{LU}	−0.48	0.79	0.28
Composite2 _{LU}	−0.14	0.89	0.35
Condensed tannins (D)	−0.11	0.34	0.93
Survival (D)	0.97	−0.10	−0.08
Composite3 _{LU}	0.84	−0.49	−0.13

matrices. Additionally, the nonsignificant Pearson correlations and canonical correlations between phenotypic and environmental characteristics indicated that the modeled collection-site environments did not appreciably correspond to measured phenotypic differences. Thus, as decisions are made concerning which particular collections to use in the development of germplasm for Utah lotus, local adaptation need not be of predominant concern.

Many isolation mechanisms have been reported. They may cause genetic differentiation between populations and species, which include physiographic (Transeau, 1941), edaphic (Rajakaruna, 2004), and biotic mechanisms such as pollinators (Ramsey et al., 2003; Kramer et al., 2011) or seed dispersal limitations (Howe and Miriti, 2004) that reduce gene flow between populations. The largest Utah lotus subgroup of

eight collections spanned the Wasatch Mountain Range in central Utah, with collections on both the eastern and western sides and several from the mountain valleys within the mountain range. Although this mountain range could have been a large physiographic barrier that reduced pollinator movements between collection sites of Utah lotus, this was not the case with these eight collections. There was apparently sufficient genetic exchange among these eight collections that genetic differentiation has not occurred.

Implications

A wide range of phenotypic variation was observed in our common garden studies for Utah lotus; however, no strong evidence supporting local adaptation was detected. On the basis of the collections evaluated in our study, results suggested that collections of Utah lotus should be broadly adapted to most environments throughout its range, which may improve seeding success. Collections LU-5 and LU-20 were consistently in the top tier of collections for important plant traits in the common garden evaluations, including DMY, number of stems, pod mass, canopy height, canopy width, longest stem length, and persistence. On the basis of our results, one pooled germplasm source of Utah lotus comprising collections LU-5 (Kane County, Utah) and LU-20 (Washington County, Utah) could be developed for rangeland revegetation in the southern Great Basin and Colorado Plateau. High seed production, seedling recruitment, biomass, and plant height are plant traits previously identified as being good indicators of plant performance in restoration seedings (Pywell et al., 2003; Fischer et al., 2013). As a result, these and other important plant traits could be evaluated more fully in this germplasm in the future to explore the possibility of improving the performance of this germplasm for rangeland revegetation.

References

- Barry, T.N., Manley, T.R., 1984. The role of condensed tannins in the nutritional value of *Lotus pedunculatus* for sheep. *British Journal of Nutrition* 51, 493–504.
- Belesky, D.P., 1999. *Lotus* species used in reclamation, renovation, and revegetation. In: Beuselinck, P.R. (Ed.), *Trefoil: the science and technology of Lotus*. CSSA Special Publication No. 28. American Society of Agronomy, Madison, WI, USA, pp. 133–144.
- Benham, J.J., 2001. Genographer—AFLP/Microsatellite Software. v. 1.6.0. Available at: <http://www.genographer.com/>. Accessed 2 August 2016.
- Bhattarai, K., Bushman, B.S., Johnson, D.A., Carman, J.G., 2010. Phenotypic and genetic characterization of western prairie clover collections from the western United States. *Rangeland Ecology & Management* 63, 696–706.
- Bhattarai, K., Bushman, B.S., Johnson, D.A., Carman, J.G., 2011. Searls prairie clover (*Dalea searlsiae*) for rangeland revegetation: phenotypic and genetic evaluations. *Crop Science* 51, 716–727.

Table 6

Factor scores for the three orthogonal factors derived from three composite variables and two directly measured variables for 14 Utah lotus collections.

Entry	Factor1 _{LU}	Factor2 _{LU}	Factor3 _{LU}
LU-2	−0.2204	1.64614	0.4113
LU-4	−0.7995	0.91617	−1.4441
LU-5	0.47453	−1.8024	−0.6026
LU-6	1.51393	0.49331	0.21993
LU-7	0.65237	0.8156	0.72061
LU-8	−0.3435	0.07905	1.83859
LU-9	−0.7228	0.13993	1.42495
LU-15	0.19902	−1.622	0.44833
LU-16	1.4426	0.76751	−0.4083
LU-17	−0.4409	−0.5194	0.25664
LU-18	−0.7121	0.11321	−0.0169
LU-19	−2.1064	−0.5731	−0.4829
LU-20	1.20419	−1.0437	−0.4976
LU-23	−0.141	0.58961	−1.8679

- Broadhurst, L., Lowe, A., Coates, D., Cunningham, S., McDonald, M., Veski, P., Yates, C., 2008. Seed supply for broadscale restoration: maximizing evolutionary potential. *Evolutionary Applications* 1, 587–597.
- Brooks, M.L., 1999. Alien annual grasses and fire in the Mojave Desert. *Madroño* 46, 13–19.
- Cassida, K.A., Griffin, T.S., Rodriguez, J., Patching, S.C., Hesterman, O.B., Rust, S.R., 2000. Protein degradability and forage quality in maturing alfalfa, red clover, and birdsfoot trefoil. *Crop Science* 40, 209–215.
- Chambers, J.C., Roundy, B.A., Blank, R.R., Meyer, S.E., Whittaker, A., 2007. What makes Great Basin sagebrush ecosystems invulnerable by *Bromus tectorum*? *Ecological Monographs* 77, 117–145.
- Escaray, F.J., Menendez, A.B., Garriz, A., Pieckenstein, F.L., Estrella, M.J., Castagno, L.N., Carrasco, P., Sanjuán, J., Ruiz, O.A., 2012. Ecological and agronomic importance of the plant genus *Lotus*. Its application in grassland sustainability and the amelioration of constrained and contaminated soils. *Plant Science* 182, 121–133.
- Falush, D., Stevens, M., Pritchard, J.K., 2007. Inference of population structure using multilocus genotype data: dominant markers and null alleles. *Molecular Ecology Notes* 7, 574–578.
- Felsenstein, J., 2009. PHYLIP (Phylogeny Inference Package). Version 3.69. University of Washington, Department of Genome Sciences (distributed by author), Seattle, WA, USA.
- Fischer, L., von der Lippe, M., Kowarik, I., 2013. Urban grassland restoration: which plant traits make desired species successful colonizers? *Applied Vegetation Science* 16, 272–285.
- Fleischner, T.L., 1994. Ecological costs of livestock grazing in western North America. *Conservation Biology* 8, 629–644.
- Grabber, J.H., Ridday, H., Cassida, K.A., Griggs, T.C., Min, D.H., MacAdam, J.W., 2015. Yield, morphological, and chemical composition of European- and Mediterranean-derived birdsfoot trefoil cultivars grown in the colder continental United States. *Crop Science* 54, 1893–1901.
- Grabber, J.H., Zeller, W.E., Mueller-Harvey, I., 2013. Acetone enhances the direct analysis of procyanidin- and prodelphinidin-based condensed tannins in *Lotus* species by the butanol-HCl-iron assay. *Journal of Agricultural and Food Chemistry* 61, 2669–2678.
- Grant, W.F., Small, E., 1996. The origin of the *Lotus corniculatus* (Fabaceae) complex: a synthesis of diverse evidence. *Canadian Journal of Botany* 74, 975–989.
- Greene, S.L., 1999. *Lotus* genetic resources: maintaining diversity through conservation. In: Beuselinck, P.R. (Ed.), *Trefoil: the science and technology of Lotus*. CSSA Special Publication No. 28. American Society of Agronomy, Madison, WI, USA, pp. 61–80.
- Hair, J.F., Black, W.C., Babin, B.J., Anderson, R.E., Tatham, T.L., 2006. *Multivariate data analysis*. 6th ed. Pearson Prentice Hall, Upper Saddle River, NJ, USA 699 pp.
- Harrison, R.D., Chatterton, N.J., McArthur, E.D., Ogle, D., Asay, K.H., Waldron, B.L., 2003. Range plant development in Utah: a historical view. *Rangelands Archives* 25–4, 13–19.
- Hijmans, R.J., Cameron, S.E., Parra, J.L., Jones, P.G., Jarvis, A., 2005. Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology* 25, 1965–1978.
- Hoffmann, A.A., Sgro, C.M., 2011. Climate change and evolutionary adaptation. *Nature* 470, 479–485.
- Howe, H.F., Miriti, M.N., 2004. When seed dispersal matters. *BioScience* 54, 651–660.
- Ito, M., Miyamoto, J., Mori, Y., Fujimoto, S., Uchiumi, T., Abe, M., Suzuki, A., Tabata, S., Fukui, K., 2000. Genome and chromosome dimensions of *Lotus japonicus*. *Journal of Plant Research* 113, 435–442.
- Jensen, J.L., Bohonak, A.J., Kelley, S.T., 2005. Isolation by distance, web service. *BMC Genetics* 6, 13.
- Johnson, D.A., Bushman, B.S., Bhattarai, K., Connors, K.J., 2011. Notice of release of Majestic Germplasm and Spectrum Germplasm western prairie clover. *Native Plants Journal* 12, 249–256.
- Johnson, D.A., Bushman, B.S., Connors, K.J., Bhattarai, K., Jones, T.A., Jensen, K.B., Parr, S.D., Eldredge, E.P., 2015. Notice of release of Fanny Germplasm, Carmel Germplasm, and Bonneville Germplasm Searls' prairie clover: selected class of natural germplasm. *Native Plants Journal* 16, 265–275.
- Johnson, D.A., Jones, T.A., Connors, K.J., Bhattarai, K., Bushman, B.S., Jensen, K.B., 2008. Notice of release of NBR-1 Germplasm basalt milkvetch. *Native Plants Journal* 9, 127–132.
- Johnson, R., Stritch, L., Olwell, P., Lambert, S., Homing, M.E., Cronn, R., 2010. What are the best seed sources for ecosystems restoration on BLM and USFS lands? *Native Plants Journal* 11, 117–131.
- Kelman, W.M., Tanner, G.J., 1990. Foliar condensed tannin levels in *Lotus* species growing on limed and unlimed soils in South-Eastern Australia. *Proceedings of the New Zealand Grassland Association* 52, 51–54.
- Kirkbride Jr., J.H., 1999. *Lotus* systematics and distribution. In: Beuselinck, P.R. (Ed.), *Trefoil: the science and technology of Lotus*. CSSA Special Publication No. 28. American Society of Agronomy, Madison, WI, USA, pp. 1–20.
- Kramer, A.T., Fant, J.B., Ashley, M.V., 2011. Influences of landscape and pollinators on population genetic structure: examples from three *Penstemon* (Plantaginaceae) species in the Great Basin. *American Journal of Botany* 98, 109–121.
- Leonard, A.C., Franson, S.E., Hertzberg, V.S., Smith, M.K., Toth, G.P., 1999. Hypothesis testing with the similarity index. *Molecular Ecology* 8, 2105–2114.
- Mantel, N., 1967. The detection of disease clustering and generalized regression approach. *Cancer Research* 27, 1219–1227.
- Meudt, H.M., Clarke, A.C., 2007. Almost forgotten or latest practice? AFLP applications, analyses and advances. *Trends in Plant Science* 12, 106–117.
- Miller, D.A., Beuselinck, P.R., Carlson, I.T., Elling, L.J., 1983. Norcen birdsfoot trefoil. *Crop Science* 23, 1010–1011.
- Novobilsky, A., Mueller-Harvey, I., Thamsborg, S.M., 2011. Condensed tannins act against cattle nematodes. *Veterinary Parasitology* 182, 213–220.
- Parsons, M.C., Jones, T.A., Larson, S.R., Mott, I.W., Monaco, T.A., 2011. Ecotypic variation in *Elymus elymoides* subsp. *brevifolius* in the northern Intermountain West. *Rangeland Ecology & Management* 64, 649–658.
- Peakall, R., Smouse, P.E., 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes* 6, 288–295.
- Peakall, R., Smouse, P.E., 2012. GenALEX 6.5: genetic analysis in Excel. Population genetic software for teaching and research—an update. *Bioinformatics* 28, 2537–2539.
- Pywell, R., Bullock, J., Roy, D., Warman, L., Walker, K., Rothery, P., 2003. Plant traits as predictors of performance in ecological restoration. *Journal of Applied Ecology* 40, 65–77.
- Rajakaruna, N., 2004. The edaphic factor in the origin of plant species. *International Geology Review* 46, 471–478.
- Ramsey, J., Bradshaw, H.D., Schemske, D.W., 2003. Components of reproductive isolation between the monkeyflowers *Mimulus lewisii* and *M. cardinalis* (PHRYMACEA). *Evolution* 57, 1520–1534.
- Rumbaugh, M.D., 1990. Special purpose forage legumes. In: Janick, J., Simon, J.E. (Eds.), *Advances in new crops*. Timberline Press, Portland, OR, USA, pp. 183–190.
- SAS, Statistical Analysis Systems, 2011. SAS/STAT (release 9.3) User's guide. SAS Institute, Inc., Cary, NC, USA.
- SEINet, Southwest Environmental Information Network—Arizona Chapter, 2016. Available at: <http://swbiodiversity.org/seinet/index.php> Accessed 2 August 2016.
- Shaw, N. L., Lambert, S. M., DeBolt, A. M., and Pellant, M. 2005. Increasing native forb seed supplies for the Great Basin. In: Dumroese, R. K., Riley, L. E., and Landis, T. D. [tech. coords.]. *National Proceedings: Forest and Conservation Nursery Associations—2004*. Fort Collins, CO, USA: USDA-FS Rocky Mountain Research Station. p. 94–102.
- Smith, K.F., Kelman, W.M., 1997. Predicting condensed tannin concentrations in *Lotus uliginosus* Schkuhr using near-infrared reflectance spectroscopy. *Journal of the Science of Food and Agriculture* 75, 263–267.
- Steiner, J.J., 1999. Birdsfoot trefoil origins and germplasm diversity. In: Beuselinck, P.R. (Ed.), *Trefoil: the science and technology of Lotus*. CSSA Special Publication No. 28. American Society of Agronomy, Madison, WI, USA, pp. 81–96.
- Stevens, R., MacArthur, E.D., Young, S.A., Massay, G., Cuany, R.S., Johnson, D.A., 1994. Notice of naming and release of “Timp” Utah sweetvetch (*Hedysarum boreale* Nutt.) for soil improvement and early spring forage for both wildlife and livestock. Utah Crop Improvement Assoc., Logan, UT Available at: <http://www.ars.usda.gov/SP2UserFiles/Place/20801000/pdfs/germ14.pdf>. Accessed 2 August 2016.
- St-Pierre, N.R., 2001. Integrating quantitative findings from multiple studies using mixed model methodology. *Journal of Dairy Science* 84, 741–755.
- Transeau, E.N., 1941. Prehistoric factors in the development of the vegetation of Ohio. *The Ohio Journal of Science* 41, 207–211.
- Undersander, D., Greub, L., Leep, R., Beuselinck, P., Wedberg, J., Smith, D., Kelling, K., Doll, J., Cosgrove, D., Grau, C., Peterson, S., Wipfli, M., English, J., 1993. Birdsfoot trefoil for grazing and harvested forage. University Extension, Iowa State University, Ames, IA, USA.
- Vos, P., Hogers, R., Bleeker, M., Reijnders, M., Van de Lee, T., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kruiper, M., 1995. AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Research* 23, 4407–4414.
- Warren, J.M., Raybould, A.F., Ball, T., Gray, A.J., Hayward, M.D., 1998. Genetic structure in the perennial grasses *Lolium perenne* and *Agrostis curtisii*. *Heredity* 81, 556–562.
- Whisenand, S.G., 1990. Changing fire frequencies on Idaho's Snake River Plain: ecological and management implications. The Station. Nov. 1990 (276). General Technical Report INT-US Department of Agriculture, Forest Service, Intermountain Research Station, Ogden, UT, USA.
- Young-Matthews, A., Darris, D., 2011. Plant guide for big deervetch (*Lotus crassifolius*). USDA—Natural Resources Conservation Service, Plant Materials Center, Corvallis, OR, USA.