Paleoclimate effects and geographic barriers shape regional population genetic structure of blackbrush (*Coleogyne ramosissima*: Rosaceae)

Bryce A. Richardson and Susan E. Meyer

Abstract: *Coleogyne ramosissima* Torr. (blackbrush) is a dominant xerophytic shrub species in the ecotone between the warm and cold deserts of interior western North America. Amplified fragment length polymorphisms (AFLPs) were used to survey genetic diversity and population genetic structure at 14 collection sites across the species range. Analysis revealed significant population differentiation ($F_{ST} = 0.103$, $p < 0.0001$) and reasonably high levels of genetic diversity (expected heterozygosity; $H_E = 0.26$), a surprising result for a putative paleoendemic species. Model-based Bayesian clustering, principal coordinates analysis, and neighbor-joining analysis all produced support for the existence of two metapopulations, the first centered on the Mojave Desert and the second on the Colorado Plateau. These genetic data, coupled with information from Late Pleistocene and Holocene packrat (genus *Neotoma* Say and Ord, 1825) middens, illustrate a demographic history in which eastern and western distributions were disjunct during the Last Glacial Maximum and remained so through the Holocene, forming the present-day metapopulations in the Mojave Desert and Colorado Plateau. This strong regional genetic differentiation has implications for population persistence and migration in response to future climate change, as well as for shrubland restoration following anthropogenic disturbances such as annual grass invasion and wildfire.

Key words: amplified fragment length polymorphisms, biogeography, Colorado Plateau, Mojave, postglacial colonization.

Résumé : *Coleogyne ramosissima* Torr. (blackbrush) constitue une espèce arbustive xérophyte de l'écotone entre les déserts chauds et froids de l'intérieur de l'ouest Nord-Américain. Les auteurs ont utilisé les polymorphismes de la longueur des fragments amplifiés (PFLAs) pour examiner la diversité génétique et la structure génétique des populations sur 14 sites de récolte distribués sur l'ensemble de l'aire de l'espèce. Les analyses révèlent une différenciation significative des populations ($F_{ST} = 0{,}103$, $p < 0{,}0001$) ainsi que des degrés relativement élevés de diversité génétique (hétérozygosie attendue; $H_E = 0{,}26$), un résultat surprenant pour une espèce présumée paléoendémique. Une modélisation basée sur le regroupement bayésien, l'analyse des coordonnées principales et l'analyse des liaisons entre voisins (neighbor-joining analysis), toutes supportent l'existence de deux métapopulations, la première centrée sur le Désert Mojave et la seconde sur le Plateau du Colorado. Ces données génétiques, couplées avec l'information provenant des monticules de détritus de rats du genre *Neotoma* Say and Ord, 1825, illustrent une histoire démographique dans laquelle les distributions orientales et occidentales se sont disjointes au cours du maximum de la dernière glaciation, pour demeurer telles quelles tout au long de l'Holocène, formant les métapopulations du Désert Mojave et du Plateau du Colorado. Cette forte différenciation génétique régionale a des implications pour la persistance des populations et leur migration en réaction au changement climatique future, ainsi que pour la restauration des arbustaies, suite aux perturbations anthropiques comme l'invasion par des graminées annuelles et les incendies.

Mots-clés : polymorphismes des longueurs des fragments amplifiés, biogéographies, Plateau du Colorado, Mojave, colonisation postglaciaire.

[Traduit par la Rédaction]

Introduction

Past climate change represented by the Last Glacial Maximum (LGM, ca. 25 000 to 13 000 year before present (BP)) followed by Holocene warming (ca. <10 000 year BP) has had a profound impact on plant species distributions and population genetic structure. Numerous studies detailing the strata of macrofossils and pollen cores have shown dynamic range shifts of plant species during the Holocene. Genetic analyses in the context of paleoecology have been instrumental in inferring glacial refugia and postglacial colonization (Hewitt 2000; Hu et al. 2009). Population genetic and phylogeographic studies have elucidated intraspecific details of plant demographic responses to the LGM and Holocene climate change. For example, many studies have identified glacial refugia and pathways of colonization during Holocene warming (e.g., Petit et al. 2002; Richardson et al. 2002). Moreover, this research provides insight into ongoing and future responses plant species may undergo with climate change (Petit et al. 2008).

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B.A. Richardson and S.E. Meyer. USDA Forest Service, Rocky Mountain Research Station, Shrub Sciences Laboratory, 735 North 500 East, Provo, UT 84606, USA.

Corresponding author: Bryce A. Richardson (e-mail: brichardson02@fs.fed.us).

Table 1. Geographic locations, site attributes, sample size, and genetic diversity indices of blackbrush (*Coleogyne ramosissima*) site collections used in this study.

Map no.	Collection site	Site abbreviation	Longitude	Latitude	Elevation (m)	Mat (°C)	Gsp (mm)	*N*	H_E	PP
Western Mojave										
1	Lone Pine	LPIN	–118.088	36.640	1134	15.3	43	27	0.217	81.5
2	Haiwee Res	HAIW	–117.975	36.154	1229	14.4	55	20	0.210	46.2
3	Joshua Tree	JTNP	–116.353	34.077	1266	14.6	56	22	0.244	87.7
4	Lost Horse Mine	LHMR	–116.153	33.952	1467	13.6	78	26	0.203	43.1
Eastern Mojave										
5	Wee Thump	WTHP	–115.105	35.515	1393	15.1	111	33	0.218	47.7
6	Potosi Pass	PPRD	–115.432	36.010	1211	15.7	94	30	0.206	46.2
7	Lee Canyon	LEEC	–115.541	36.421	1485	13.1	107	32	0.201	67.7
8	Sawmill Road	SMRD	–115.068	36.698	1559	12.4	127	25	0.217	72.3
Mojave Transition										
9	Toquerville	TOQO	–113.327	37.264	1132	14.7	123	21	0.251	80
Colorado Plateau										
10	Little Rockies	LROK	–110.651	37.761	1684	11.7	99	21	0.273	87.7
11	South Bluff	SOBL	–109.694	37.281	1442	12.2	98	27	0.250	89.2
12	White Mesa	WMES	–109.473	37.436	1607	11.4	119	28	0.266	87.7
13	Needles District	NEED	–109.760	38.168	1494	11.9	114	24	0.280	89.8
14	Gemini Bridge	GBTO	–109.677	38.651	1407	12.2	117	17	0.223	84.6

Note: Map no., numbered locations in Fig. 1; mat, mean annual temperature; gsp, growing season precipitation; *N*, number of samples; H_E, mean expected heterozygosity; PP, polymorphic locus percentage.

The southwestern deserts of North America are rich in Late Pleistocene and Holocene macrofossils due to an abundance of packrat (genus *Neotoma* Say and Ord, 1825) middens and favorable climates for preservation. Studies of these middens have revealed a dynamic movement of plant assemblages during the Holocene, and at some sites fossil records extend well into the Late Pleistocene, ca. 50 000 years BP (Coats et al. 2008). Hundreds of excavated middens in the northern Mojave Desert (MD) and Colorado Plateau (CP) have revealed a transition from more mesic to xeric vegetation starting in the early Holocene (Spaulding 1983; Koehler et al. 2005; Coats et al. 2008). These data are valuable when combined with information on neutral genetic variation because they make it possible to construct the biogeographic and demographic histories of plant species as they were affected by past climate change.

Blackbrush (*Coleogyne ramosissoma* Torr.) is a xerophytic shrub that occupies the ecotones between warm and cold deserts in the MD and CP ecoregions. The species is most abundant on bajadas shallowly underlain by caliche hardpan (MD) and on shallow, coarse-textured residual soils (CP), mainly occupying an elevational envelope between 800 and 1600 m (West 1983). In these areas blackbrush is typically the dominant or co-dominant vegetation. On the CP, blackbrush occupies the lower elevations, whereas in the MD, elevations below 800 m, including valley bottoms, are usually dominated by creosote bush (*Larrea tridentata* (Sessé & Moc. ex DC.) Coville). At higher elevations, blackbrush is replaced by cold desert plant communities generally dominated by species of sagebrush (genus *Artemisia* L.). Blackbrush sites on the CP typically experience colder winter low temperatures and more frequent summer monsoonal precipitation than blackbrush sites in the MD (Table 1). Blackbrush is relatively shallow-rooted for a desert shrub and has been shown to be better at taking up and using water from summer rainfall events than other desert shrubs (Lin et al. 1996). This suggests that it has a competitive advantage in areas with shallow soils and relatively frequent summer monsoons.

Although blackbrush is a member of the Rosaceae, it has several unique characteristics making it an outlier within this family. The genus *Coleogyne* Torr. is monotypic and is phylogenetically differentiated from other rosaceous tribes (Potter et al. 2007). Blackbrush is also unusual for the Rosaceae in having wind pollination, an obligately outcrossing breeding system (Pendleton and Pendleton 1998), opposite leaves, and a chromosome number of eight. Because its taxonomic isolation implies the past extinction of close congeners, blackbrush has been considered to have a relictual distribution and to therefore represent a paleoendemic species (Stebbins and Major 1965). Some have interpreted this to mean that blackbrush itself has a relictual distribution relative to its former distribution, and could be genetically depauperate. However, blackbrush does not generally fit the definition of a paleoendemic very well. It is a regionally dominant species with a wide geographic distribution rather than a rare species with a restricted distribution (Fig. 1). The only study to date on molecular genetic variation in this species (Schuster et al. 1994) reported a relatively high percentage of polymorphism (78.9%) based on 23 allozyme loci in a single collection from northern Arizona.

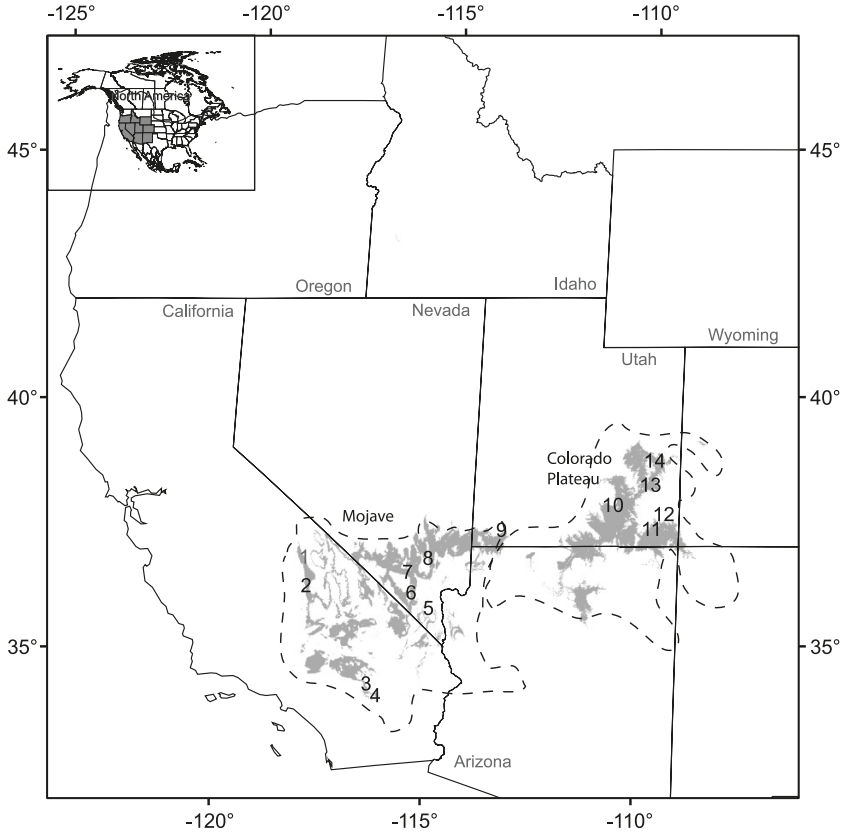
The objectives of this study were as follows: (*i*) assess the genetic diversity and population genetic structure of blackbrush stands at representative sites from across its distribution and (*ii*) examine evidence for relictual versus nonrelictual distribution for this species by combining population genetic data with paleoecological data from previous studies to evaluate putative refugia and postglacial movements.

Materials and methods

Sample collection and DNA extraction

Blackbrush leaf tissue was collected from plants represent-

Fig. 1. Blackbrush (*Coleogyne ramosissima*) site collection locations represented by numbers 1–14 (Table 1) are overlaid with the predicted distribution of the species (in gray), based on the bioclimatic profile. Methods are described in Rehfeldt et al. (2006) and Ledig et al. (2010). The dash lines show the approximate boundaries of the Mojave Desert and Colorado Plateau ecoregions.



ing stands at 14 sites; five sites on the CP of southern Utah and nine sites in the MD of southern Nevada and California, USA (Table 1; Fig. 1). Leaf tissue was collected directly from the collection sites or from greenhouse-grown plants from site-collected seeds. Samples were dried in silica beads using a vacuum chamber for 48 h. Approximately 15 mg of dried material were ground into a powder using a TissueLyser II and DNA extractions followed the DNeasy plant extraction kit protocol (Qiagen, Inc., Valencia, Calif., USA) DNA yield was quantified by a NanoDrop 2000 spectrophotometer (Thermo Scientific, Wilmington, Del., USA).

Amplified fragment length polymorphism analysis

The amplified fragment length polymorphism (AFLP) procedure was modified from Vos et al. (1995). Approximately 350 ng of genomic DNA was digested using 10 U (1 U ≈ 16.67 nkat) of *Eco*RI and *Mse*I at 37 °C for 3 h in a total volume of 20 µL. Adaptors were ligated to DNA fragments at 16 °C for 8 h using 60 U of T4 ligase with 50 µmol/L *Mse*I and 5 µmol/L *Eco*RI adaptors in a final volume of 40 µL. Ligated DNA fragments were then diluted 1:10 with TE buffer (10 mmol/L Tris, 0.1 mmol/L EDTA, pH 7.5) prior to pre-amplification PCR. Pre-amplification PCR was conducted in 30 µL reactions containing 6 µL of DNA template, 200 µmol/L dNTPs, 300 nmol/L *Eco*RI and *Mse*I primers (E+0, M+C extensions), 10× PCR buffer, 3 mmol/L $MgCl_2$, and 1.5 U of Ampli*Taq* DNA polymerase (Applied Biosystems, Carlsbad, Calif., USA). Pre-amplification products were diluted 1:10 with TE buffer followed by selective amplification. Selective amplification was conducted with three combinations of +2 *Eco*RI and +3 *Mse*I primers with the following extensions: AG-CAA, AG-CTC, AC-CAG, respectively. PCR reactions in a 25 µL volume contained 5 µL diluted pre-amplification product, 10× PCR buffer, 2.4 mmol/L MgCl, 300 µmol/L dNTPs, 100 nmol/L *Eco*RI and 300 nmol/L *Mse*I primers, and 1 U of Ampli*Taq* Gold polymerase. The *Eco*RI primers were modified with 6-FAM fluorescent dye (Integrated DNA Technologies, Coralville, Iowa, USA) for band visualization on the sequencer.

Selective amplification products were diluted 1:4 in sterile, distilled water and the fragments were separated on an ABI 3730xl DNA sequencer (Applied Biosystems, Carlsbad, Calif., USA) at the University of Wisconsin Biotechnology Center. An internal size standard, Geneflo-625 (Chimerx, Inc., Milwaukee, Wis., USA), was added to each sample to calculate fragment size. Fragment size calculation and scoring were conducted using GeneMarker version 1.85 (Softgenetics, Inc., State College, Pa., USA). Amplified fragment length polymorphism marker bands within a size range of 75–500 base pairs and peak-height threshold of 150 or greater were scored. All bands with these criteria were visually checked in all samples for presence (1) or absence (0). To insure repeatability of AFLP bands, two to three samples from each of the 96-well reactions were replicated from the initial DNA extraction step. In addition, each pre-amplification and selective amplification reaction contained negative controls.

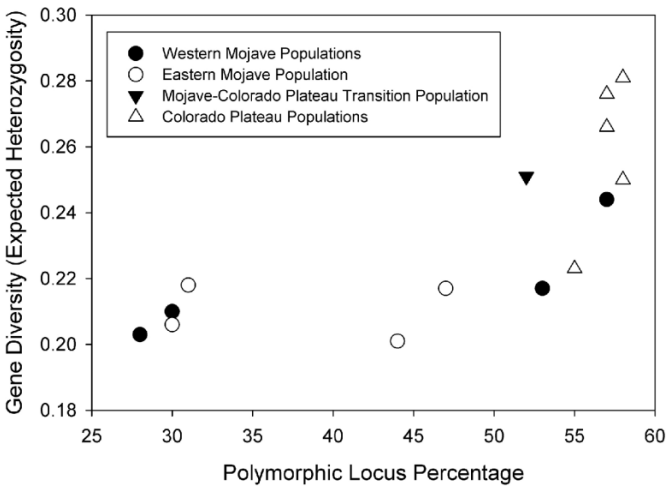
Data analyses

Polymorphic AFLP loci were analyzed only if the band was present in two or more individuals. Using a Bayesian method to estimate the frequency of null alleles (Zhivotovsky 1999) and following the methods of Lynch and Milligan (1994), unbiased expected heterozygosity (H_E) was calculated using AFLP-SURV version 1.0 (Vekemans 2002) for within and among collection sites. An unpaired t test was used to test for significant differences in H_E between sites grouped according the ecoregions MD and CP, omitting the transitional site Toquerville. To test for significant genetic structure (F_{ST}) between collection sites, the data were randomly resampled (1000 iterations) in AFLP-SURV. To construct a bootstrapped, neighbor-joining tree, 100 replicates of pairwise genetic distances matrices (Nei 1978) were calculated in AFLP-SURV. These distance matrices were graphically display using CONSENSE and NEIGHBOR packages of PHYLIP version 3.6 (Felsenstein 2004). We also used Nei's pairwise genetic distance matrix for the isolation by distance analysis using Mantel correlation (Jensen et al. 2005). Principal coordinates analysis was performed by grouping individuals based on their collection site and creating a distance matrix (Cavalli-Sforza and Edwards 1967) in FAMD version 1.2 (Schlüter and Harris 2006). Eigenvector values from each of the collection sites were regressed with latitude and longitude coordinates. Genetic structure was also analyzed without a priori assumptions using STRUCTURE version 2.3, a model-based method for determining the number of populations (K) using probabilistic assignment of individuals (Pritchard et al. 2000). To estimate K and assignment of individuals to populations, five simulations were run for each K (K = 1–10). To account for the dominant nature of AFLPs, each model was run with a method recommended for recessive alleles (Falush et al. 2007). The models were run under the assumption of admixture, and used a burn-in length of 15 000 followed by 50 000 iterations. Previous model runs of different lengths indicated 50 000 iterations were adequate for convergence of parameter estimates. To determine the appropriate model (K), a second-order rate of change test was applied to log probability scores for K = 1–10 (Evanno et al. 2005).

Results

A total of 65 polymorphic AFLP bands were scored in 353 individuals from three selective amplification primer sets. Identical patterns of presence or absence of bands were observed in three replicated samples, demonstrating repeatability. Expected heterozygosity ranged from 0.20 at Lee Canyon to 0.28 at Needles District. The mean expected heterozygosity across all sites was 0.260. Most of this heterozygosity, 0.233 (89.6%), was explained within sites. The remaining genetic variance, 0.027 (10.4%), was found between sites, equating to significant genetic structure (F_{ST} = 0.103, $p < 0.0001$). The percentage of polymorphic loci varied considerably from 43% at Lost Horse Mine to 89% at Needles District and South Bluff (Table 1). CP collections typically had higher levels of expected heterozygosity and more polymorphic loci than MD collections (Fig. 2). An unpaired t test between the MD and CP sites, excluding the Mojave–Colorado Plateau transitional site, Toquerville (n = 13), showed significantly higher heterozygosity in the CP (mean values: MD = 0.2145, CP = 0.2584; t = 4.404; p = 0.001).

Fig. 2. Gene diversity (expected heterozygosity) based on 65 amplified fragment length polymorphism loci plotted against polymorphic loci percentage for 14 blackbrush (*Coleogyne ramosissima*) populations.



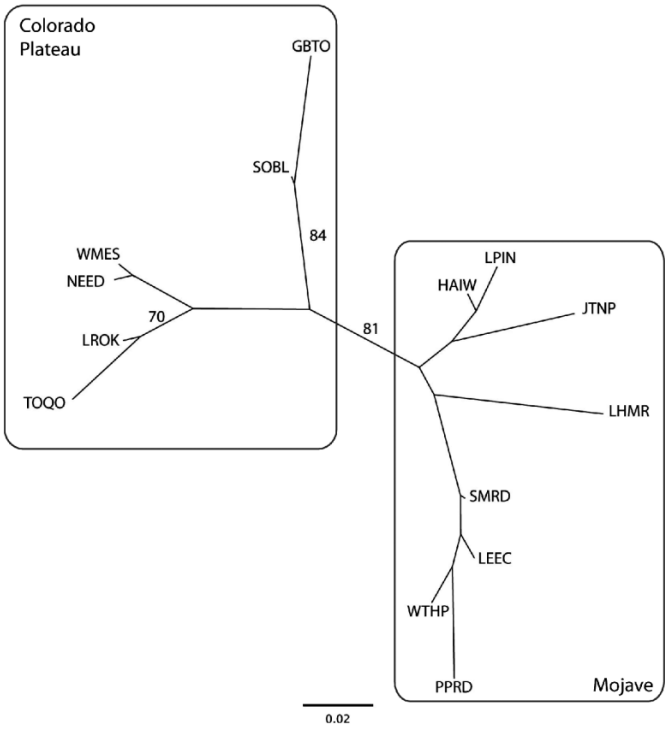
An examination of the relationship between geographic distance and genetic distance at the collection site level demonstrated the existence of a significant isolation by distance effect in this data set (R^2 = 0.358, $p < 0.001$). A neighbor-joining tree supported the existence of two major clades in blackbrush (Fig. 3). This clade division coincided with the ecoregion boundary between the MD and CP. All sites west of the Utah–Nevada border form a MD clade, while sites east of the border form a CP clade. The Toquerville site was placed within the CP clade; however this site is considered to be transitional, but within the eastern margin of the MD ecoregion. Further support for these site relationships was provided by the principal coordinates analysis. The first principal coordinate explained 56.7% of the genetic variation and placed the Toquerville site intermediate to MD sites and CP sites. Eigenvector values from this principal coordinate were regressed on latitude and longitude coordinates of the sites. Both latitude and longitude showed a significant relationship with the eigenvectors, however longitude had a superior fit (Fig. 4; R^2 = 0.837, $p < 0.0001$). This relationship suggests that longitudinal distance is a significant factor in the distribution of blackbrush genetic variation. However, the division into two distinct geographic groups is a primary driver of this relationship with longitude.

A hypothesis of two metapopulations divided between the MD and CP ecoregions was tested with the program STRUCTURE. A series of population models (K = 1–10) were simulated with the data. The second-order rate change was maximized with the model K = 2. A bar plot of assignment probabilities (Fig. 5) of each individual illustrates a consistent pattern with low admixture between MD and CP populations. Slightly more admixture (i.e., lower assignment probabilities) was found at the transitional site, Toquerville.

Discussion

The population genetic data presented in this study do not

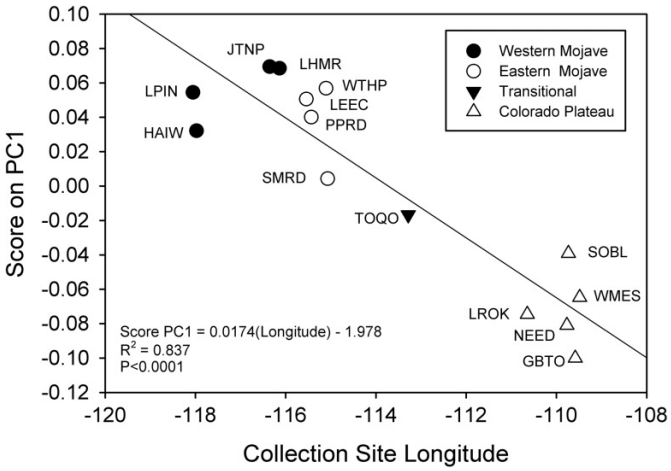
Fig. 3. Neighbor-joining tree based on 65 amplified fragment length polymorphism loci and 14 blackbrush (*Coleogyne ramosissima*) collection sites. Bootstrap support of ≥70 are shown along the branches. Collection sites occupying the Colorado Plateau and Mojave Desert are circumscribed.



support the idea that blackbrush is a relictual paleoendemic that is genetically depauperate. Distinctive character attributes (e.g., wind pollination, opposite leaves, chromosome number = 8) and phylogenetic analyses support the claim that this species has a unique and isolated position within the Rosaceae (Potter et al. 2007). Although this evidence suggests that blackbrush could be an ancestral lineage within the Rosaceae, it does not provide any support for relictual endemism during the past glacial and interglacial cycles. As discussed below, the data from this study and from paleoecological records support an alternative hypothesis, suggesting that blackbrush maintained adequate population sizes and multiple populations during the LGM and early Holocene, and did not experience major genetic bottlenecks.

Blackbrush fossils have been detected in many of the excavated packrat middens in the CP and MD ecoregions. During the Late Glacial (ca. 12 000 year BP), blackbrush was known to occur at midden sites around 275 m along the Arizona–California border (King and Van Devender 1977; Cole 1990). These sites are ca. 60 km north of the Mexico border, south of the modern MD, and outside of the predicted contemporary climate profile for blackbrush (Fig. 1). The northern limits of known fossils during this same time period (ca. 12 000 year BP) were detected in middens along the Grand Canyon in northern Arizona below 1000 m (Cole 1990; Coats et al. 2008). These data suggest multiple blackbrush refugia at low elevation during the LGM. During the Holocene, blackbrush is believed to have expanded northward ca. 500 km and upward ca. 700 m in the CP (Cole 1990). Fossils dating from 9750 year BP at 1400 m in Utah support this northward and upward colonization (Coats et al. 2008).

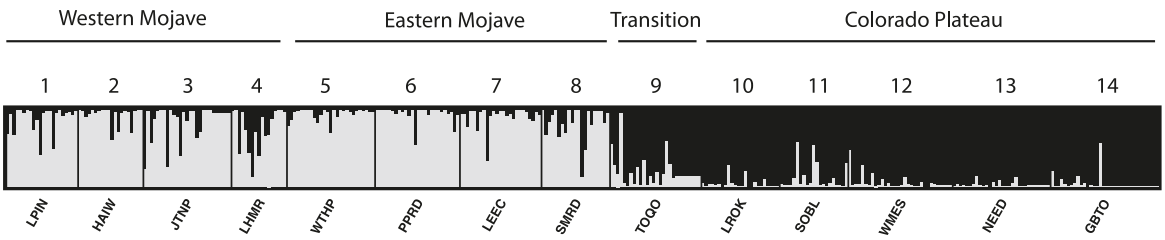
Fig. 4. Eigenvector scores on the first principal coordinate (PC1) from principal coordinates analysis using amplified fragment length polymorphism data regressed on collection site longitude for 14 blackbrush (*Coleogyne ramosissima*) populations included in the study. PC1 accounted for 56.7% of the total variance.



The significantly lower heterozygosity ($p = 0.001$) found in the MD compared to the CP ($H_E = 0.214$ and 0.258, respectively) could be explained by the different climatic, ecological and edaphic properties of these ecoregions. First, fossil evidence places blackbrush presence in the southern MD at 10 210 year BP (Spaulding 1983). Unlike midden data from the CP, middens from the MD show no evidence of blackbrush at upland sites farther north (i.e., above 35°N latitude) until 4000 year BP. Dominance by cold desert plant communities including juniper (*Juniperus osteosperma* (Torr.) Little) persisted in these areas until 10 000 year BP (Koehler et al. 2005), which may have excluded blackbrush recruitment. Second, during the Holocene warming and drying, blackbrush could have moved upward locally, but colonization across desert valleys to suitable upland habitat further north may have been impeded by unfavorable valley climate and soils. Third, studies have shown 183 mm of annual precipitation is needed to sustain blackbrush (Beatley 1974). Episodes of marginal precipitation during the early Holocene may have played a role in slower colonization and (or) local extirpation in the MD. For example, Hunter and McAuliffe (1994) found that the occurrence of blackbrush fossils from middens at a site in the eastern MD coincided with the Little Ice Age (ca. 600 year BP). They hypothesized that this cooler and wetter period was responsible for blackbrush establishment in an area where it was previously absent.

The paleoecological data suggest the northern extent of blackbrush was limited to the lower elevations of the Colorado River drainage during the Late Glacial, and that topographical features above 1200 m were probably barriers to colonization. The topographical features that likely influenced the genetic differentiation between the MD and CP metapopulations were the high plateaus that rim the western and southern edge of the CP (i.e., the Kaibab, Kaiparowits, and Kolob Plateaus). Cold desert plant communities dominated lower elevations in these areas during the Late Glacial (Coats et al. 2008), imposing a substantial geographic barrier to gene flow between the two metapopulations. Even today, these lower elevation areas support cold desert shrub com-

Fig. 5. A barplot of individuals from 14 blackbrush (*Coleogyne ramosissima*) collection sites using the program STRUCTURE. Each bar represents an individual with assignment probabilities to each of two populations. The labels above and below the barplot refer to the site number and abbreviation, respectively. The labels and corresponding site information are described in Table 1. Sites are noted as belonging to eastern, western Mojave Desert, transitional, or Colorado Plateau.



munities. This disjunct distribution is evident in the predicted contemporary climate profile (Fig. 1). The only possible corridor connecting the two metapopulations during the Late Glacial would have been at the lowest elevations in the Grand Canyon and along the Colorado River to the south. This corridor would have become progressively more discontinuous as Holocene warming proceeded because of the incursion of warm desert communities at low elevations. Genetic sampling of blackbrush stands in and around the Grand Canyon would help to clarify the extent to which the Colorado River represented a migration corridor.

Although current and past barriers to gene flow have shaped the genetic structure between MD and CP blackbrush metapopulations, the contrasting climates of blackbrush habitat in the two ecoregions may also have had an impact on adaptive genetic variation (Table 1). Seed germination and establishment studies have shown differential responses between blackbrush seed collected from the MD and CP. A reciprocal seeding experiment between sites at Arches National Park (Moab, Utah, USA) on the CP and Hurricane (near St. George, Utah, USA) in the MD found that local seed established better than imported seed. Superior seedling establishment was attributable to appropriate responses to habitat-specific emergence timing cues (Meyer and Pendleton 2004). Similarly, laboratory germination trials showed that CP seed collections were more cue responsive at colder winter temperatures, whereas MD seed sources were more cue responsive at warmer winter temperatures (Pendleton and Meyer 2004). Ecophysiological measurements showed that blackbrush plants from colder, high-elevation collections grew more efficiently at lower temperature than plants from warmer, low-elevation collections (Summers et al. 2009). These studies suggest that blackbrush exhibits a range of adaptive responses that are associated with different ecoregional environments. Common garden studies of blackbrush are ongoing and should better define the ranges of adaptive variation in this species.

Several other plant species exhibit geographic distributions similar to that of blackbrush, centered in the deserts of the southwest but with a secondary distribution in the lower river drainages of the CP. These include *Psorothamnus fremontii* (Torr. ex A. Gray) Barneby, *Xylorhiza tortifolia* (Torr. & A. Gray) Greene, *Nicotiana obtusifolia* M. Martens & Galeotti, and *Yucca baccata* Torr. These species probably share a similar Pleistocene and post-Pleistocene history with blackbrush, but without a fossil record or population genetic data, this hypothesis is difficult to evaluate. For this reason, blackbrush remains the ideal species to serve as a model for understanding both past and future plant responses to climate in this region.

The population genetic structure in this study, coupled with paleoecological data, supports a divided postglacial demographic history for blackbrush. This division between CP and MD ecoregions appears to be caused by a major barrier to gene flow created by north-trending high plateaus in south-central Utah and northern Arizona. This low gene flow, coupled with differential selection pressures owing to climatic differences between the two regions, has also resulted in metapopulations with contrasting trait syndromes that are adaptive in their respective regions.

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References

Beatley, J.C. 1974. Phenological events and their environmental triggers in the Mojave Desert ecosystems. Ecology, **55**(4): 856–863. doi:10.2307/1934421.

Cavalli-Sforza, L.L., and Edwards, A.W.F. 1967. Phylogenetic analysis: models and estimation procedures. Evolution, **21**(3): 550–570. doi:10.2307/2406616.

Coats, L.L., Cole, K.L., and Mead, J.I. 2008. 50,000 years of vegetation and climate history on the Colorado Plateau, Utah and Arizona, USA. Quat. Res. **70**(2): 322–338. doi:10.1016/j.yqres.2008.04.006.

Cole, K.L. 1990. Reconstruction of past desert vegetation along the Colorado River using packrat middens. Palaeogeogr. Palaeoclimatol. Palaeoecol. **76**(3–4): 349–366. doi:10.1016/0031-0182(90)90120-V.

Evanno, G., Regnaut, S., and Goudet, J. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol. Ecol. **14**(8): 2611–2620. doi:10.1111/j.1365-294X.2005.02553.x. PMID:15969739.

Falush, D., Stephens, M., and Pritchard, J.K. 2007. Inference of population structure using multilocus genotype data: dominant markers and null alleles. Mol. Ecol. Notes, **7**(4): 574–578. doi:10.1111/j.1471-8286.2007.01758.x. PMID:18784791.

Felsenstein, J. 2004. PHYLIP (Phylogeny Inference Package).

Distributed by the author. Department of Genome Sciences, University of Washington, Seattle.

Hewitt, G. 2000. The genetic legacy of the Quaternary ice ages. Nature, **405**(6789): 907–913. doi:10.1038/35016000. PMID: 10879524.

Hu, F.S., Hampe, A., and Petit, R.J. 2009. Paleoecology meets genetics: deciphering past vegetational dynamics. Front. Ecol. Environ, **7**(7): 371–379. doi:10.1890/070160.

Hunter, K.L., and McAuliffe, J.R. 1994. Elevational shifts of *Coleogyne ramossissima* in the Mojave Desert during the Little Ice Age. Quat. Res. **42**(2): 216–221. doi:10.1006/qres.1994.1071.

Jensen, J.L., Bohonak, A.J., and Kelley, S.T. 2005. Isolation by distance, web service. BMC Genetics 6: 13. v.3.16 [computer program]. Available from http://ibdws.sdsu.edu/ [accessed 25 June 2011].

King, J.E., and Van Devender, T.R. 1977. Pollen analysis of fossil packrat middens from the Sonoran Desert. Quat. Res. **8**(2): 191–204. doi:10.1016/0033-5894(77)90045-X.

Koehler, P.A., Anderson, R.S., and Spaulding, W.G. 2005. Development of vegetation in the Central Mojave Desert of California during the late Quaternary. Palaeogeogr. Palaeoclimatol. Palaeoecol. **215**(3–4): 297–311. doi:10.1016/j.palaeo.2004.09.010.

Ledig, F.T., Rehfeldt, G.E., Saenz-Romero, C., and Flores-Lopez, C. 2010. Projections of suitable habitat for rare species under global warming scenarios. Am. J. Bot. **97**(6): 970–987. doi:10.3732/ajb.0900329. PMID:21622467.

Lin, G., Phillips, S.L., and Ehleringer, J.R. 1996. Monosoonal precipitation responses of shrubs in a cold desert community on the Colorado Plateau. Oecologia (Berl.), **106**: 8–17.

Lynch, M., and Milligan, B.G. 1994. Analysis of population genetic structure with RAPD markers. Mol. Ecol. **3**(2): 91–99. doi:10.1111/j.1365-294X.1994.tb00109.x. PMID:8019690.

Meyer, S.E., and Pendleton, B.K. 2004. Factors affecting seed germination and seedling establishment of a long-lived desert shrub (*Coleogyne ramosissima*: Rosaceae). Plant Ecol. **178**(2): 171–187. doi:10.1007/s11258-004-3038-x.

Nei, M. 1978. Estimation of average heterozygosities and genetic distance from small number of individuals. Genetics, **89**(3): 583–590. PMID:17248844.

Pendleton, B.K., and Meyer, S.E. 2004. Habitat-correlated variation in blackbrush (*Coleogyne ramosissima*: Rosaceae) seed germination response. J. Arid Environ. **59**(2): 229–243. doi:10.1016/j.jaridenv.2003.12.009.

Pendleton, B.K., and Pendleton, R.L. 1998. Pollination biology of *Coleogyne ramosissima* (Rosaceae). Southwest. Nat. **43**(3): 376–380.

Petit, R., Brewer, S., Bordács, S., Burg, K., Cheddadi, R., Coart, E., Cottrell, J., Csaikl, U.M., van Dam, B., Deans, J.D., Espinel, S., Fineschi, S., Finkeldey, R., Glaz, I., Goicoechea, P.G., Jensen, J.S., König, A.O., Lowe, A.J., Madsen, S.F., Mátyás, G., Munro, R.C., Popescu, F., Slade, D., Tabbener, H., de Vries, S.G.M., Ziegenhagen, B., de Beaulieu, J.-L., and Kremer, A. 2002. Identification of refugia and post-glacial colonisation routes of European white oaks based on chloroplast DNA and fossil pollen evidence. For. Ecol. Manage. **156**(1–3): 49–74. doi:10.1016/S0378-1127(01)00634-X.

Petit, R.J., Hu, F.S., and Dick, C.W. 2008. Forests of the past: a window to future changes. Science, **320**(5882): 1450–1452. doi:10.1126/science.1155457. PMID:18556547.

Potter, D., Eriksson, T., Evans, R.C., Oh, S., Smedmark, J.E.E., Morgan, D.R., Kerr, M., Robertson, K.R., Arsenault, M., Dickinson, T.A., and Campbell, C.S. 2007. Phylogeny and classification of Rosaceae. Plant Syst. Evol. **266**(1–2): 5–43. doi:10.1007/s00606-007-0539-9.

Pritchard, J.K., Stephens, M., and Donnelly, P. 2000. Inference of population structure using multilocus genotype data. Genetics, **155**(2): 945–959. PMID:10835412.

Rehfeldt, G.E., Crookston, N.L., Warwell, M.V., and Evans, J.S. 2006. Empirical analyses of plant-climate relationships for the western United States. Int. J. Plant Sci. **167**(6): 1123–1150. doi:10.1086/507711.

Richardson, B.A., Brunsfeld, S.J., and Klopfenstein, N.B. 2002. DNA from bird-dispersed seed and wind-disseminated pollen provides insights into postglacial colonization and population genetic structure of whitebark pine (*Pinus albicaulis*). Mol. Ecol. **11**(2): 215–227. doi:10.1046/j.1365-294X.2002.01435.x. PMID: 11856423.

Schlüter, P.M., and Harris, S.A. 2006. Analysis of multilocus fingerprinting data sets containing missing data. Mol. Ecol. Notes, **6**(2): 569–572. doi:10.1111/j.1471-8286.2006.01225.x.

Schuster, W.S.F., Sandquist, D.R., Phillips, S.L., and Ehleringer, J.R. 1994. High levels of genetic variation in populations of four dominant aridland plant species in Arizona. J. Arid Environ. **27**(2): 159–167. doi:10.1006/jare.1994.1055.

Spaulding, W.G. 1983. Late Wisconsin macrofossil records of desert vegetation in the American Southwest. Quat. Res. **19**(2): 256–264. doi:10.1016/0033-5894(83)90009-1.

Stebbins, G.L., and Major, J. 1965. Endemism and speciation in the California flora. Ecol. Monogr. **35**(1): 1–35. doi:10.2307/1942216.

Summers, H.A., Smith, B.N., and Hansen, L.D. 2009. Comparison of respiratory and growth characteristics of two co-occuring shrubs from a cold desert, *Coleogyne ramosissima* (blackbrush) and *Atriplex confertifolia* (shadscale). J. Arid Environ. **73**(1): 1–6. doi:10.1016/j.jaridenv.2008.09.010.

Vekemans, X. 2002. AFLP-SURV version 1.0. Distributed by the author. Laboratoire de Génétique et Ecologie Végétale, Université Libre de Bruxelles, Belgium.

Vos, P., Hogers, R., Bleeker, M., Reijans, M., van de Lee, T., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M., and Zabeau, M. 1995. AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res. **23**(21): 4407–4414. doi:10.1093/nar/23.21.4407. PMID:7501463.

West, N.E. 1983. Colorado Plateau—Mohavian blackbrush semi-desert. *In* Temperate deserts and semi-deserts. *Edited by* N.E. West. Elsevier, Amsterdam, the Netherlands. pp. 399–411.

Zhivotovsky, L.A. 1999. Estimating population structure in diploids with multilocus dominant DNA markers. Mol. Ecol. **8**(6): 907–913. doi:10.1046/j.1365-294x.1999.00620.x. PMID:10434412.