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Genetically distinct populations of the pygmy rabbit (*Brachylagus idahoensis*) in the Mono Basin of California

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We examined patterns of genetic variation and diversity of extant pygmy rabbit (*Brachylagus idahoensis*) populations across the species' current range in Nevada and California. Our aims were to determine population genetic structure and levels of diversity across the southern portion of the species' range. We genotyped 13 microsatellite loci from 194 fecal samples collected across 14 localities. Our Bayesian cluster analyses found 2 genetically distinct groups: 1 in the Mono Basin of California and the other encompassing all remaining Nevada Great Basin populations. Considering only the Nevada Great Basin group, we found 4 minimally divergent groups that overlap spatially with many individuals maintaining composite genomes with representation from multiple genetic groups. On average, the Mono Basin populations maintain lower levels of genetic diversity than the Nevada Great Basin populations as measured by both heterozygosity and numbers of alleles. The Mono Basin contains the only populations of pygmy rabbits remaining in California. Given their geographic isolation from other known populations and their genetic distinction, these remnant, peripheral populations warrant special attention to ensure maintenance of genetic variation and continued viability.

Key words: Great Basin, lagomorph, Lahontan Trough, Mono Basin, population genetics

The Great Basin Desert of the western United States spans approximately 500,000 km^2 between the Sierra Nevada and the Rocky Mountains and is characterized by low-lying valleys dominated by sagebrush semidesert vegetation (West 1983). Over the past century, sagebrush habitat in the Great Basin has been reduced and degraded by wildfires, invasion of nonnative grasses, improper livestock grazing, pinyon–juniper expansion, urban encroachment, and under-informed management practices (Wisdom et al. 2005). Prior to European settlement, local, low-intensity fires occurred every 30–70 years, but today, fires occur with greater frequency and intensity (Whisenant 1990) and often burn large tracts of land that typically become monocultures of nonnative cheatgrass (*Bromus tectorum*) (Weiss and Verts 1984). Additionally, pinyon–juniper woodlands have been expanding due to altered disturbance regimes and are encroaching into sagebrush communities (Miller and Rose 1999; Miller et al. 2008). These cumulative impacts have reduced and fragmented the already patchy sagebrush landscape and are negatively affecting species that rely on this unique habitat (Davies et al. 2011).

Pygmy rabbits (*Brachylagus idahoensis*) are North America's smallest leporids, weighing only about 450 g and measuring approximately 30 cm in length (Wilde 1978). They are dietary and habitat specialists that require dense big sagebrush (*Artemisia tridentata*) growing on deep, friable soils (Weiss and Verts 1984). Big sagebrush accounts for the majority of their diet year-round and can compose up to 99% of their diet in winter (Green and Flinders 1980). Due to their small size, pygmy rabbits cannot run as fast as other rabbits and rely on sagebrush canopy cover and burrows for protection against a large suite of predators (Heady et al. 2001; Crawford et al. 2010; Price et al. 2010; Camp et al. 2012). While the geographic distribution of the pygmy rabbit includes most of the Great Basin (Fig. 1), its specialized habitat requirements only allow it to occupy a small subset of sites within this range (Janson 1946; Gabler 1997; Heady et. al. 2001).

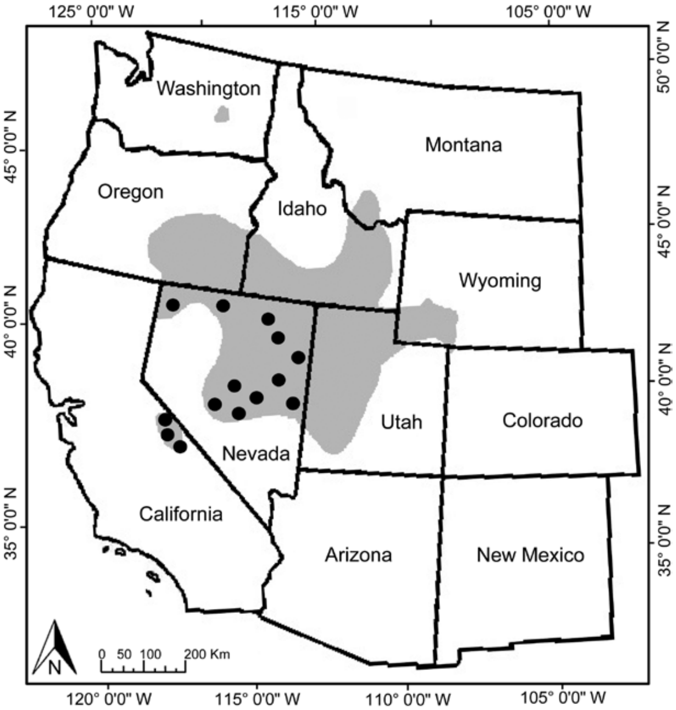

Fig. 1.—Map of the western United States showing the distribution of the pygmy rabbit (*Brachylagus idahoensis*, gray area). Points indicate locations where samples were collected for this study.

Individual colonies are susceptible to rapid declines (Green and Flinders 1980; Weiss and Verts 1984) and the probability of survival of a population of pygmy rabbits is directly related to the amount of contiguous big sagebrush (Dobler and Dixon 1990). Pygmy rabbits rely on cover as protection from predation and as sagebrush cover is eliminated, safe dispersal is reduced (Weiss and Verts 1984; Dobler and Dixon 1990). Recolonization of extirpated areas depends on adequate connectivity to other occupied habitat (Green and Flinders 1980; Crawford et al. 2010). Increased fragmentation and greater isolation of populations can lead to reduced gene flow and a subsequent decline in genetic diversity within the remaining populations (Frankham et al. 2002; Garner et al. 2005). Genetic diversity allows populations more flexibility to respond to changes in the environment and has been shown to be correlated with population fitness (Reed and Frankham 2003; Elias et al. 2013; DeMay et al. 2016). Smaller and more isolated populations are more likely to lose genetic variation through the process of genetic drift.

The fragmentation and loss of mature sagebrush is suspected to have contributed to declines in pygmy rabbit populations throughout their range (Weiss and Verts 1984; Thines et al. 2004; Larrucea and Brussard 2008a). Due to these pressures, the pygmy rabbit is currently categorized as a species of special concern in California and Nevada. The entire species has been considered for federal endangered status, but a range-wide listing was deemed not warranted (Federal Register 2007, 2010). A single distinct population segment as defined by the U.S. Fish and Wildlife Service was identified for pygmy rabbits in Washington State's Columbia Basin and was listed as endangered under the U.S. Endangered Species Act (Federal Register 2003). The Columbia Basin population has since gone extinct in the wild (Becker et al. 2011).

With the continued loss and fragmentation of sagebrush habitat (Knick and Rotenberry 1997), it is important to assess connectivity among pygmy rabbit populations for future conservation planning (Rachlow and Svancara 2006; Estes-Zumpf et al. 2010). Genetic data can provide information on gene flow, genetic diversity, and levels of isolation as well as evolutionary history. Initial genetic studies on pygmy rabbits have been conducted in Idaho (Estes-Zumpf et al. 2010), Wyoming (Thimmayya and Buskirk 2012), Oregon (Warheit 2001), and on the endangered population from Washington's Columbia Basin (Warheit 2001). No data have been available on the genetic differentiation of pygmy rabbit populations at the southern extent of their range. Here, we examine the patterns of genetic variation and diversity found in current pygmy rabbit populations across the species' range in Nevada and California. Our aims were to determine current population genetic structure and the spatial distribution of genetic diversity across the southern portion of the range.

Materials and Methods

Study area.—The Great Basin of North America is defined as a cold-temperate semidesert and is characterized by multiple north-south oriented mountain ranges separated by low-lying valleys. Mountain peaks can be as high as 3,000 m while the valley bottoms are often around 1,200 m in elevation. Our study area encompassed the current range of pygmy rabbit in California and Nevada, United States (Fig. 1). This included portions of Mono County in California and portions of Washoe, Humboldt, Pershing, Lander, Eureka, Elko, Churchill, White Pine, Nye, and Lincoln counties in Nevada, incorporating an area of about 165,000 km^2. Collections were made mostly on public lands managed by the Bureau of Land Management and United States Forest Service (Fig. 1).

Samples.—Sampling was conducted broadly throughout the species' current range in Nevada and California and was concentrated on areas recently surveyed and known to have pygmy rabbits (Larrucea and Brussard 2008b; Fig. 1). Fecal pellets were collected primarily during the winter months when ambient temperatures and the restricted winter diet of pygmy rabbits increase the likelihood of extracting DNA from fecal material (Kocher et al. 1989; Adams et al. 2011). In addition, snow cover made tracks and fecal pellets highly visible, which aided in locating samples. Winter collection also reduces potential confusion between pygmy rabbit fecal pellets and the pellets of young-of-the-year cottontail rabbits (*Sylvilagus* spp.) and black-tailed jackrabbits (*Lepus californicus*).

Each sample consisted of 6 or more pellets from an individual burrow entrance or single deposition. Single depositions were classified as a distinct conglomerate of similarly aged pellets. Fecal samples were collected using sterile forceps and were placed into individual paper envelopes. The location of samples was recorded using a handheld GPS unit and the location, date, and a general description were written on the sample envelope. Envelopes were kept on ice and then in a −80°C freezer until DNA extraction.

Laboratory methods.—We extracted whole, genomic DNA from frozen fecal pellets using QIAamp stool kits (Qiagen, Valencia, California) and the standard protocol for isolation of DNA from stool for human DNA. An initial test comparing the genotype from a single pellet to others in the same deposition pile revealed identical results, allowing us to combine multiple pellets from the same deposition into 1 extraction. This enhanced overall amplification success. We used 4 fecal pellets per individual, as this has been indicated to yield the optimal amount of DNA necessary for amplification of *B. idahoensis* DNA and exclusion of interspecific contamination in downstream analyses (Torstrom et al. 2013). To minimize the possibility of contamination between individual samples, we flame-sterilized all instruments twice between individuals. We made 2 modifications to the extraction protocol in order to maximize the amount of rabbit DNA eluted and minimize the amount of nontarget (i.e., plant) DNA in our final sample. First, we washed the fecal pellets in stool lysis buffer, gently inverting each sample 20 times before incubating at room temperature for 120 min. Following incubation, we maintained the standard protocol until the final elution step. In this step, we substituted molecular grade water for elution buffer, and incubated the samples on the extraction membrane at 55°C for 10 min prior to centrifugation to release DNA from the membrane.

We resolved genotypes at 16 microsatellite loci. We used a combination of primers proven polymorphic in *B. idahoensis*: A2, A10, A121, A124, A133, D103, D118, D126 (Estes-Zumpf et al. 2008), Sat 5, Sat 7, Sat 8, Sat 12, Sat 16, Sol 44, Sol 08, and Sol 30 (Estes-Zumpf et al. 2010). We carried out amplification in 10 µl multiplex reactions using 1 µM of each primer (forward primer labeled with one of four fluorescent tags: NED, VIC, PET, or 6FAM), 4 µl of Qiagen HotStarTaq PCR Master Mix (Qiagen), and 3 µl of H_2O, with thermocycler settings of: initial denaturation at 94°C for 15 min; 35 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 1 min 30 s, extension at 72°C for 1 min; and a final extension at 72°C for 5 min. We combined amplified products with a LIZ size standard and HiDye, and resolved genotypes on an ABI 3730 DNA Analyzer (Applied Biosystems Inc., Foster City, California) at the Nevada Genomics Center. Allele sizes were identified using GeneMarker software v1.85 (SoftGenetics, State College, Pennsylvania) and verified by eye.

Analysis.—We estimated the average number of alleles per locus, private alleles, and unbiased heterozygosity using GenAlEx (Peakall and Smouse 2006, 2012). In addition to reporting the average number of alleles per locus within each population (N_A), we used a rarefaction approach as implemented in the ADZE software package (version 1.0—Szpiech et al. 2008) to allow comparisons of allelic diversity (richness; A_X) between localities with different sample sizes.

We quantified population genetic subdivision by estimating the standardized pairwise F_{ST} among populations using the approach of Meirmans (2006) as implemented in GenAlEx. To determine the number of distinct genetic clusters across the sampled region, we conducted Bayesian clustering analysis in the program STRUCTURE 2.3.4 (Pritchard et al. 2000; Falush et al. 2007). We applied the admixture model of ancestry and the independent allele frequency model. We ran 10 iterations each of $K = 1–10$ with a burn-in of 1,000,000 steps followed by 1,000,000 MCMC replicates. We used the delta K approach of Evanno et al. (2005) to determine the optimal number of distinct genetic clusters within the sample (K). We also explored the correlated allele frequency model in STRUCTURE but found similar results so only report those of the independent allele frequency model. To further visualize population differentiation, we conducted a principal coordinates analysis in GenAlEx.

RESULTS

DNA was obtained from 194 fresh fecal pellet samples collected from 14 localities in Nevada and California (Fig. 1). Due to allelic dropout and monomorphic loci, we excluded 3 loci (Sat 08, Sat 12, and Sol 30) and used 13 microsatellite loci for our analyses.

Bayesian STRUCTURE analysis supported $K = 2$ with a southwestern group in the Mono Basin region of California (Bodie, Mono Lake, Crowley Lake) genetically differentiated from the remaining Nevada Great Basin populations. The distinction between these 2 groups is also evident in principal coordinates space (Fig. 2). The Mono Basin and Nevada Great Basin groups differed by an average pairwise F_{ST} of 0.22 (min. = 0.14, max. = 0.31) while within-group average pairwise F_{ST} was 0.08 in the Mono Basin and 0.08 in the Nevada Great Basin group (Table 1).

We next removed the highly differentiated Mono Basin group from the analysis and only considered the Nevada Great Basin populations. With only the Nevada Great Basin populations, the STRUCTURE analysis supported $K = 4$, suggesting 4 genetic

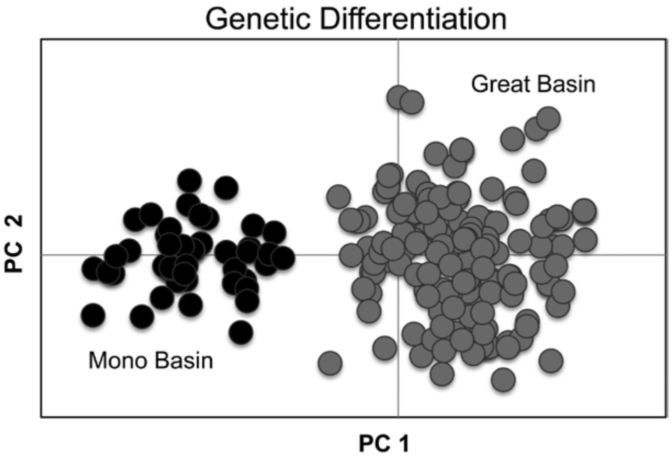

Fig. 2.—Principal coordinates analysis showing genetic differentiation of Mono Basin populations of pygmy rabbits (*Brachylagus idahoensis*) from Nevada Great Basin populations based on 13 microsatellite loci. The 2 groups of populations primarily segregate from each other on principal coordinates axis 1.

clusters across this region. These groups are much more subtly differentiated than the magnitude of difference between the Mono Basin and Nevada Great Basin genetic clusters. Nearly half of the sampled individuals (48%) have $q > 0.80$ for 1 of these 4 clusters, yet clusters are spatially overlapping and many individuals are admixed (Fig. 3).

On average, the Mono Basin populations maintain 3.48 alleles per locus and average heterozygosity of 0.55 in comparison to Nevada Great Basin populations that maintain an average 5.18 alleles per locus and average heterozygosity of 0.71 (Table 2). When we resample the data set and standardize the sample sizes to $n = 9$ or $n = 13$, the Mono Basin populations

Table 1.—Pairwise F_{ST} among sampled pygmy rabbit (*Brachylagus idahoensis*) populations are shown. Divergences between Mono Basin region and Nevada Great Basin populations are highlighted in gray. The average pairwise F_{ST} between Mono Basin and Nevada Great Basin groups is 0.22. Average within-group F_{ST} is 0.08 in the Mono Basin group and 0.08 in the Nevada Great Basin group.

Population	Bodie	Mono Lake	Crowley Lake	Belmont	Reese River Valley	Illipah	Egan-Jakes Valley	Steptoe Valley/ Cave Lake	Eastern Antelope Valley	Elko	North Elko	Grass Valley	Santa Rosas	Vya
Bodie	0.00													
Mono Lake	0.08	0.00												
Crowley Lake	0.10	0.06	0.00											
Belmont	0.20	0.15	0.17	0.00										
Reese River Valley	0.26	0.19	0.22	0.06	0.00									
Illipah	0.23	0.18	0.24	0.07	0.10	0.00								
Egan-Jakes Valley	0.31	0.23	0.29	0.06	0.13	0.08	0.00							
Steptoe Valley/Cave Lake	0.24	0.20	0.21	0.07	0.11	0.05	0.10	0.00						
Eastern Antelope Valley	0.26	0.20	0.24	0.07	0.12	0.06	0.08	0.12	0.00					
Elko	0.23	0.17	0.21	0.04	0.09	0.06	0.06	0.05	0.06	0.00				
North Elko	0.27	0.22	0.24	0.05	0.08	0.07	0.07	0.08	0.08	0.02	0.00			
Grass Valley	0.25	0.21	0.21	0.07	0.12	0.12	0.12	0.13	0.10	0.13	0.11	0.00		
Santa Rosas	0.18	0.14	0.16	0.05	0.05	0.06	0.07	0.05	0.07	0.04	0.04	0.05	0.00	
Vya	0.20	0.14	0.18	0.06	0.11	0.03	0.08	0.07	0.08	0.05	0.07	0.10	0.06	0.00

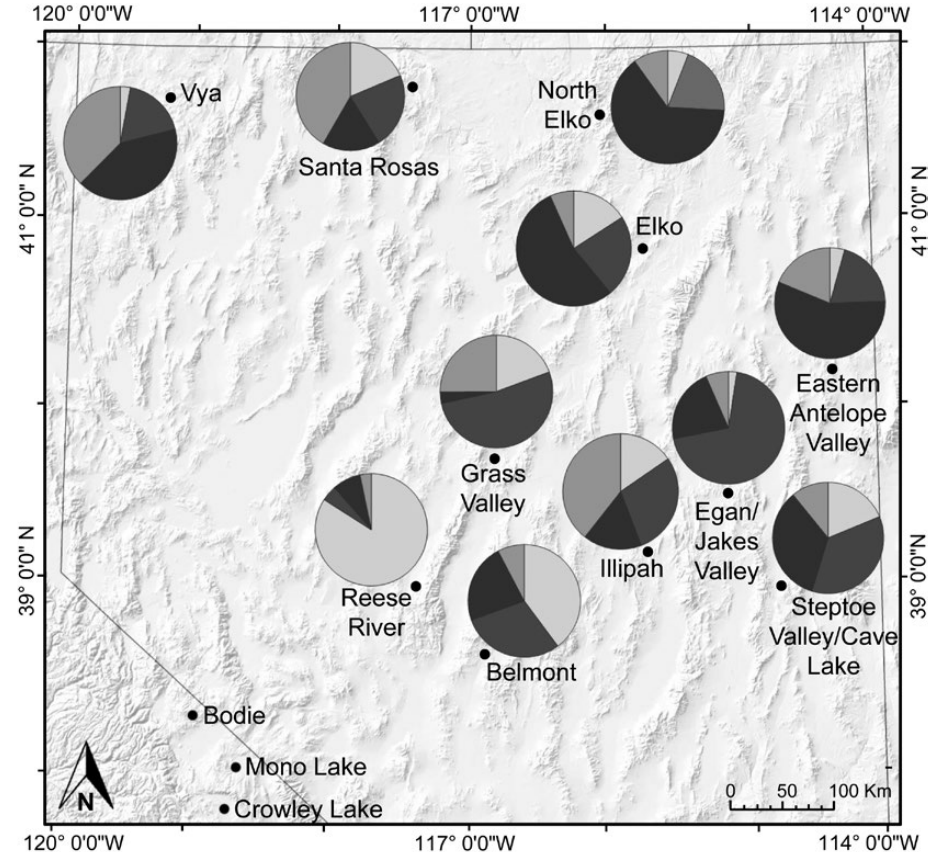

Fig. 3.—Spatial distribution of 4 minimally divergent genetic clusters across pygmy rabbit (*Brachylagus idahoensis*) populations of Nevada's Great Basin Desert. Pie charts show the frequency of each of the 4 genetic groups at that locality. The level of spatial overlap of these genetic groups suggests historic isolation and differentiation followed by genetic connectivity and mixing across the range.

Table 2.—Genetic diversity of pygmy rabbit (*Brachylagus idahoensis*) populations in the Mono Basin and Nevada Great Basin. Sample size (*n*), mean number of alleles per locus (N_A) with *SE*, allelic richness corrected for sample size at $n = 10$ (A_9) and $n = 15$ (A_{13}), and mean unbiased heterozygosity (uHE) with *SE* are shown.

	n	N_A	A_9	A_{13}	uHE
Mono Basin					
Bodie	17	3.38 (0.31)	2.75	3.01	0.52 (0.05)
Mono Lake	13	3.38 (0.18)	2.75	2.97	0.58 (0.02)
Crowley Lake	16	3.69 (0.29)	2.93	3.20	0.56 (0.06)
Nevada Great Basin					
Belmont	20	6.08 (0.59)	3.99	4.57	0.72 (0.03)
Reese River Valley	18	5.31 (0.41)	3.64	4.12	0.67 (0.04)
Illipah	10	4.23 (0.34)	3.65		0.69 (0.04)
Egan-Jakes Valley	8	3.62 (0.21)			0.66 (0.03)
Steptoe Valley/Cave Lake	7	4.38 (0.35)			0.73 (0.04)
Eastern Antelope Valley	10	4.69 (0.33)	3.29		0.74 (0.02)
Elko	9	5.23 (0.40)	4.23		0.73 (0.03)
North Elko	10	5.77 (0.46)	4.39		0.76 (0.03)
Grass Valley	6	3.85 (0.32)			0.65 (0.05)
Santa Rosas	27	7.00 (0.59)	4.03	4.69	0.71 (0.02)
Vya	23	6.77 (0.58)	4.25	4.94	0.74 (0.03)

have lower allelic richness (ave. $A_9 = 2.81$, ave. $A_{13} = 3.06$) than Nevada Great Basin populations (ave. $A_9 = 3.90$, ave. $A_{13} = 4.58$; Table 2). When we resample the regional pool of individuals from the Mono Basin ($n = 46$) and Nevada Great Basin ($n = 148$) and standardize the sample size to $n = 46$, we find that the Mono Basin has significantly lower mean allelic richness ($A_{46} = 4.09$) than the Nevada Great Basin ($A_{46} = 7.54$; Wilcoxon signed-rank test, $z = 3.16$, $P = 0.0016$). The Mono Basin does not maintain any unique alleles relative to the Nevada Great Basin, while the latter maintains 64 alleles that we did not recover in the Mono Basin region.

DISCUSSION

Mono Basin populations.—The only known remaining populations of pygmy rabbits in California are substantially genetically differentiated from populations in Nevada. Populations in and around the Mono Basin are about 160 km from the nearest neighboring populations and our data suggest that this spatial isolation has had genetic consequences. It is likely that isolation of these populations was initiated long ago with warming climates during the Holocene and continued with other more recent changes to the landscape (Grayson 2000, 2006). Pygmy rabbits likely populated the Mono Basin prior to the end of the Pleistocene when pollen data from the region show that the vegetation of a lower-elevation region called the Lahontan Trough was a sagebrush-dominated community (Grayson 1993). This low-elevation region extends from the Mojave Desert in the south up to the Columbia Plateau in the north (Grayson 1993). As the climate warmed, the habitat of the Lahontan Trough shifted and today these lower elevational areas are dominated by greasewood (*Sarcobatus*) and saltbush (*Atriplex*) communities, which are unsuitable for pygmy rabbits. Hence, pygmy rabbit populations in the Mono Basin have likely been isolated from other populations since the end of the Pleistocene or the Middle Holocene about 10,000 to 5,500 years ago (Grayson 1987) and join other described population isolates in the Mono Basin including sage-grouse (*Centrocercus urophasianus*—Benedict et al. 2003; Oyler-McCance et al. 2005) and the dark kangaroo mouse (*Microdipodops megacephalus polionotus*—Hafner et al. 2006).

Despite their isolation, pygmy rabbits in the Mono Basin show a slightly lower level of differentiation than was seen in the geographically isolated population in Washington. Pairwise F_{ST} values between the extirpated Washington population and Oregon populations ranged from 0.29 to 0.32 (Warheit 2001; Elias et al. 2013), whereas Mono Basin and Nevada Great Basin groups differed by an average pairwise F_{ST} of 0.22. The geographic distance between the Columbia Basin in Washington and the nearest extant populations in central Oregon (500 km) is greater than the distance between the Mono Basin and Nevada's Great Basin populations (160 km). Even prior to isolation, it is likely that greater genetic differences existed between the Washington and Oregon populations. Additionally, at the time of analysis, the Washington rabbits had already been reduced to very low numbers (< 30—Hays 2001), whereas the Mono Basin appears to support a good number of pygmy rabbits (E. S. Larrucea, pers. obs.) and therefore maintains relatively higher levels of genetic variation.

Nevada Great Basin populations.—When we removed the highly differentiated Mono Basin group from the analyses, we found more subtle genetic differentiation across populations in Nevada. Great Basin populations of pygmy rabbits are characterized by 4 minimally divergent groups that overlap spatially. The differentiation among these groups suggests historic isolation and differentiation, but the spatial overlap of these groups suggests subsequent historic connectivity and mixing across the sampled region. The fossil record of the region supports such a pattern as well. Prior to the end of the Pleistocene (~10,000 years ago), pygmy rabbits were distributed as far south as New Mexico (Grayson 1987). A wetter climate at that time meant that sagebrush vegetation existed throughout more southerly and lower-elevation areas (Betancourt et al. 1990; Thompson et al. 1993). However, at the end of the Pleistocene and then again during the Middle Holocene (8000–5000 years ago), evidence suggests that temperatures increased and precipitation decreased leading to a decline in sagebrush (Thompson et al. 1993). The fossil record shows dramatic range retractions as well as reduced abundances of pygmy rabbits during these periods (Lyman 1991; Grayson 2000, 2006).

During one or more periods of range retractions, pygmy rabbit populations may have diverged due to increasing isolation. The numerous north-south oriented mountain ranges of the Great Basin, as well as the low-lying playas between them, naturally bisect the sagebrush community, which can lead to isolation and spatial structuring of populations. As the range of the pygmy rabbit once again expanded, some of these divergent groups came back into contact and gene flow once again was able to occur between formerly more isolated populations. The lack of substructure we observed among Nevada's contemporary pygmy rabbit populations indicates that gene flow is or has recently occurred among populations of pygmy rabbits. Pygmy rabbit colonies are currently found in most of Nevada's intermountain valleys within the species range, increasing the likelihood of connectivity of populations throughout the Nevada Great Basin region (Larrucea and Brussard 2008b).

Genetic diversity.—While the Mono Basin populations are not dramatically low in genetic diversity (3.5 alleles per locus, average heterozygosity of 0.55), they are statistically significantly less diverse than the Great Basin populations. They are similar in average heterozygosity to other peripheral populations such as those found in Wyoming ($H_e = 0.58$—Thimmayya and Buskirk 2012). Before increasing through interbreeding with individuals from other regions, the average heterozygosity of the endangered Columbia Basin population in Washington was 0.4 (Warheit 2001; Elias et al. 2013). Isolated populations have an increased tendency to lose genetic variation, which increases the risk of extinction due to a reduced ability to adapt to environmental change (DeMay et al. 2017). Despite the level of differentiation of Mono Basin populations from the Great Basin populations sampled here, the Mono Basin does not appear to maintain any unique microsatellite alleles relative to the Nevada Great Basin populations. This could be the result of loss of genetic diversity in the Mono Basin region through genetic drift, or that this peripheral population never had a genetic composition that was fully representative of the Nevada Great Basin.

The average diversity we observed in the Nevada Great Basin populations ($H_e = 0.71$) is similar to the average heterozygosity of 0.75 found in the Lemhi Valley of Idaho (Estes-Zumpf et al. 2010). These 2 regions are more central to the overall distribution of pygmy rabbit. Central populations are generally less isolated and therefore experience a higher rate of gene flow, preserving variability (Lewontin 1974).

Future projections.—Indications are that the pygmy rabbit may once again be facing a period of range retraction (Thines et al. 2004; Larrucea and Brussard 2008a). Today, pygmy rabbits throughout Nevada and California face increasing habitat loss as changes in climate and land use have altered the structure and dynamics of the Great Basin's native flora and fauna (Chambers and Wisdom 2009). However, contemporary loss of sagebrush habitat is likely occurring at a greater rate than during historic range retractions, and global climate change is expected to intensify these changes (Rowe and Terry 2014). The Great Basin has already warmed 0.3–0.6°C over the 20th century and is predicted to warm an additional 2.5–4°C by 2100 (Hayhoe et al. 2004; Grayson 2011). Changes brought about by warming at the end of the Pleistocene–Middle Holocene may provide an indication of future impacts of global warming (Murphy and Weiss 1992; Rowe and Terry 2014). A warmer, drier climate may cause sagebrush communities to retract from more southern and low-elevation areas. Additionally, and more immediately, warmer temperatures have meant a region-wide decline in snowpack and earlier spring melt (Mote et al. 2005). Pygmy rabbits rely on winter snowpack, in which they create extensive subnivian tunnels. These tunnels protect them from predators and extreme temperatures and provide access to upper levels of sagebrush vegetation (Katzner and Parker 1997). A reduction in snowpack may therefore reduce winter survival rates and could increase the chances of colony extirpation.

The escalating loss of sagebrush habitat will exacerbate the already fragmented nature of habitat that pygmy rabbits rely on. Smaller populations have a greater risk of reduced genetic variation, and isolation of populations can cause gradual differentiation and an overall loss of diversity. Since genetic variation is relevant to the health and viability of populations, it should continue to be monitored in future management plans. Peripheral populations can act as sources of novel genetic variation due to rare or unique alleles and can be important in the maintenance of genetic diversity in a species as a whole, but they also have a greater probability of extirpation (Mayr 1970; Channell and Lomolino 2000). Populations of pygmy rabbits in the Mono Basin are currently spatially and genetically isolated from the remainder of the occupied range and already show significant genetic differentiation. These populations are at the low end of genetic variation among populations sampled here and warrant special attention to ensure maintenance of variation given their level of isolation.

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