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RESEARCH ARTICLE

Holocene-era landscape conditions affect genetic connectivity in a sagebrush obligate species, the pygmy rabbit (*Brachylagus idahoensis*)

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Abstract

Context Environmental changes produce discontinuities in suitable habitat. However, drawing inference into the effects of these changes on contemporary genetic patterns is often difficult. Recent approaches for evaluating landscape resistance facilitate increased understanding of landscape effects on gene flow.

Objectives We investigated the effects of pluvial lakes and sagebrush cover on genetic connectivity for the pygmy rabbit (*Brachylagus idahoensis*), a sagebrush obligate species. We predicted that sagebrush-based surfaces would be more explanatory than pluvial lake surfaces. Furthermore, we predicted that habitat characteristics during the mid-Holocene would explain genetic differentiation better than those during the late-Pleistocene.

Methods We leveraged a genetic dataset to evaluate the explanatory power of landscape resistance surfaces. We generated resistance surfaces that represent varying degrees of resistance associated with pluvial lakes and sagebrush cover, then projected sagebrush distribution back to the mid-Holocene and late-Pleistocene.

Results Representations based on sagebrush distribution were more explanatory than those based on pluvial lakes. Projections of sagebrush distribution back in time indicate concordance between genetic connectivity and mid-Holocene sagebrush distribution. Limited numbers of dispersal pathways were apparent among study regions, suggesting spatially restricted corridors of connectivity.

Conclusions We demonstrate that shifts in vegetative cover can shape contemporary patterns of genetic connectivity. By coupling testing of resistance surfaces with estimates of past vegetative change, we provide insights into the time scales over which genetic differentiation may occur. Given projections of future declines in sagebrush, maintaining sagebrush cover will be critical to population persistence of pygmy rabbits.

Keywords Biogeography · Connectivity · Genetics · Great Basin · Resistance surfaces · Hindcasting

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Introduction

Landscape structure and configuration can act either to facilitate or impede the movement of animals, resulting in complex patterns of genetic differentiation among populations (Anderson et al. 2010; Storfer et al. 2010). Over time, these landscape influences shape the spatial distribution of genetic variation (Spear et al. 2010; Zeller et al. 2012; Graves et al. 2014). The relationship between genetic differentiation and the landscape can be characterized using a variety of models, ranging from simple representations where genetic differentiation linearly increases with distance between populations (isolation-by-distance; Wright 1943; Slatkin 1993) to more spatially-complex models. For example, landscape features that entirely prevent gene flow between populations can be represented by isolation-by-barrier models, where pairs of populations are considered connected or not using a binary classification scheme (Merriam et al. 1989; Cushman et al. 2006). More sophisticated models, however, can incorporate complex influences of landscape features on gene flow, often referred to as isolation-by-resistance models (Cushman et al. 2006; McRae 2006; Miller et al. 2018).

The emphasis on spatially-explicit representation of genetic connectivity has led to a suite of methodological advances that focus on characterizing and representing landscape resistance to identify drivers of fundamental aspects of organismal movement and fitness such as: (a) how willing an organism is to cross through a particular area, (b) the energetic costs of moving through an area, (c) the effects on survival incurred by an organism while moving through an area, and (d) reproductive success once an organism reaches the target area (Zeller et al. 2012). The composite spatial representation of these resistance values—often called a resistance surface (McRae 2006; Spear et al. 2010)—can then be used to identify movement corridors, assess the effects of landscape change on genetic connectivity, and investigate how environmental factors affect connectivity (Zeller et al. 2012). While these approaches have substantially improved our collective understanding of the effects of landscape features on genetic differentiation, the time scales at which genetic effects of landscape alterations will become evident is dictated by evolutionary and life history traits (e.g. generation time, survival rates, and population size through time) as well as species-specific habitat requirements (Nybom and Bartish 2000; Riginos et al. 2011; Richardson 2012). Thus, studies interested in assessing the effects of landscape features on gene flow should carefully consider how evolutionary history and life history traits might affect expected patterns of genetic differentiation, particularly in dynamic landscapes (Anderson et al. 2010).

The Great Basin of North America has long served as a model for understanding island biogeography. Much of this historical work (Brown 1971; Billings 1978; Wilcox et al. 1986; Floyd et al. 2005) has focused on numerous and isolated mountain ranges that support woodland, forested, and alpine environments separated by large expanses of cold desert shrubs and unvegetated playas that occupy valley floors. During the wetter and cooler periods of the Pleistocene large pluvial lakes extended across many of the low-lying valleys (Reheis et al. 2014). For terrestrial animals, pluvial lakes have served as barriers leading to genetic discontinuities that are still apparent in taxa whose distributions span these ancient barriers. As the climate warmed during the Holocene these pluvial lakes gave way to shrublands, particularly those dominated by sagebrush (*Artemisia* spp.; Rhode and Madsen 1995). Pollen analysis in the Bonneville Basin and Owens Valley suggests that as the warming continued, sagebrush communities gave way to a drier community of shrubs collectively known as the salt desert scrub that supports a different suite of species, including *Atriplex* spp. and *Sarcobatus* spp., with a key transition date occurring around 10,000 YBP (Mensing 2001; Louderback and Rhode 2009).

Although these biogeographic transitions have shaped distributions for most species inhabiting the Great Basin to a certain extent, the effects of biogeographic change may be most apparent for species with specialized habitat requirements, particularly if those are intrinsically tied to vegetative conditions and transitions (Barnagaud et al. 2011; Auffret et al. 2017; Dondina et al. 2017). As a sagebrush obligate species, the pygmy rabbit (*Brachylagus idahoensis*) has likely experienced distributional shifts associated with biogeographic transitions of the sagebrush community (Green and Flinders 1980; White et al. 1982; Shipley et al. 2006). Pygmy rabbits depend extensively on sagebrush habitat for foraging, with sagebrush representing as much as 99% of their diet during winter months (Green and Flinders 1980; Crowell et al. 2018). Furthermore, as the smallest North American leporid, dispersal distances are relatively small (1–3 km; Estes-Zumpf et al. 2010) and highly dependent upon the availability of sagebrush for protection from predators (Heady et al. 2001; Crawford et al. 2010; Price et al. 2010; Larrucea et al. 2018).

As previously continuous tracts of sagebrush become fragmented due to invasive species, increased wildfire, anthropogenic activities, and climate change, this species has experienced dramatic declines range wide (Weiss and Verts 1984; Larrucea and Brussard 2008a; Crawford et al. 2010, Byer et al. 2021). As a result, while fossil evidence suggests that this species was previously widespread throughout the Great Basin (Grayson 1987, 2006; Commendador and Finney 2016), its current range is limited to relatively isolated colonies (Heady et al. 2001). A number of studies have assessed the genetic consequences of this species' fragmented range (Estes-Zumpf et al. 2010; Thimmayya and Buskirk 2012; Larrucea et al. 2018), with these studies suggesting relatively low genetic substructure in Idaho and Wyoming (Estes-Zumpf et al. 2010; Thimmayya and Buskirk 2012). However, Larrucea et al. (2018) found that the Mono Basin of California appeared to be genetically distinct from other southern Great Basin populations in Nevada based on genetic clustering analyses. Although Larrucea et al. (2018) suggest that this genetic discontinuity likely reflects Holocene-era shifts in sagebrush distribution, Lake Lahontan and other pluvial lakes covered much of this area during the last glacial maximum (roughly 22,000 YBP; Reheis et al. 2014), and have shaped the distributions of many mammals (Riddle et al. 2014), including Mohave ground squirrels (*Xerospermophilus mohavensis*; Bell et al. 2010) and American pikas (*Ochotona princeps*; Galbreath et al. 2010), as well as invertebrates (Graham et al. 2013).

Contextualizing the relative contributions of these two factors—more ancient shifts in the distribution of terrestrial habitat and more recent vegetative change—to genetic differentiation for the pygmy rabbit may provide insights into the effective time scales over which genetic discontinuities may emerge for this and other Great Basin taxa. Herein, we investigate the relative roles of these hypothesized drivers of genetic differentiation in pygmy rabbits. We used a dataset of individuals genotyped at 13 microsatellite loci to evaluate the relative power of several representations of landscape resistance to explain genetic patterns. We posed the following hypotheses. Given the importance of sagebrush for maintaining viable populations of this species, the vegetative community may play a predominant role in connectivity between extant pygmy rabbit populations. Accordingly, we predicted that resistance layers derived based on the distribution of sagebrush should explain more variation than layers derived based on the historical distribution of pluvial lakes. However, concordance between landscape resistance and genetic connectivity should also reflect the time scales at which habitat features influenced connectivity; thus, surfaces created based on habitat characteristics expected during the mid-Holocene would likely explain genetic differentiation better than models created based on late-Pleistocene habitat features, particularly given that microsatellite loci often capture more contemporary patterns of genetic differentiation relative to other markers (Hauser et al. 2011; DeFaveri et al. 2013; Sethi et al. 2016). Our results illustrate the importance of calibrating genetic investigations of landscape resistance based on the timescales of drivers of interest in order to clarify links between past, present, and future habitat change and patterns of genetic differentiation.

Methods

Study area

Our study area consists of the Nevada and eastern California portions of the Great Basin, which represent the southwestern portion of the range of pygmy rabbits. This area is a cold desert/semidesert, characterized by extreme temperatures and north–south trending mountain ranges that are frequently covered in sparse pinyon-juniper woodlands. The lowest-elevation portion of the study area includes the Lahontan Trough, which is characterized at present by sparse salt desert scrub communities consisting primarily of *Atriplex* and *Sarcobatus* species. Some, but not all, of these areas were also inundated by pluvial lakes during the Pleistocene (Fig. 1). Sagebrush communities (*A. tridentata* spp., *A. nova*, *A. arbuscula*) are the most common vegetation

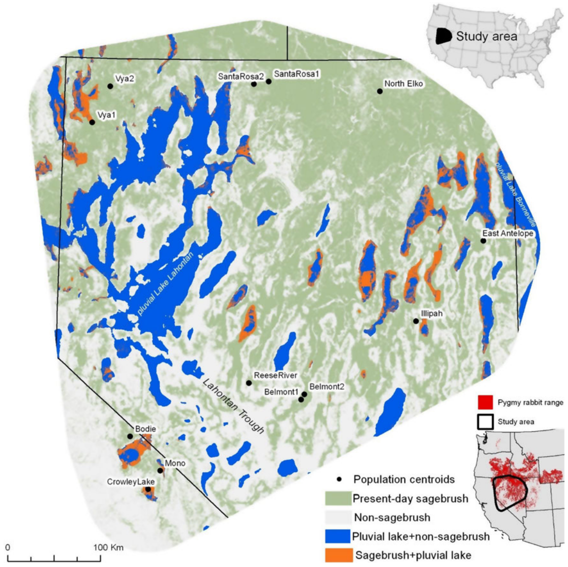

Fig. 1 Map showing the distribution of pluvial lakes (blue and orange) and sagebrush (green and orange), with population centroids indicated as points. Note that the distribution of the pygmy rabbit in California and Nevada (inset) coincides with the distribution of sagebrush cover in the displayed region. For the non-sagebrush hypothesis, high resistance to movement was assigned to the non-sagebrush gray cells. For the pluvial lake hypothesis, high resistance was assigned to the blue and orange cells. Both hypotheses show high resistance for the blue cells

communities in the study area and make up 47% of the total vegetation within the study area; other vegetation communities include salt desert scrub and pinyon-juniper woodlands (Larrucea and Brussard 2008b). While most sampling localities were within Nevada, three (Bodie, Crowley Lake, and Mono, Fig. 1) were in California; as in previous studies (Larrucea et al. 2018), we refer to these three localities collectively as the Mono Basin.

Sample collection and laboratory methods

Although previously described in Larrucea et al. (2018), we briefly describe sample and genetic data collection methodology here. Samples were collected from currently-known active pygmy rabbit colonies throughout the Nevada and California portions of the Great Basin during the winter months of 2013–2016. Tracks in snow cover and visual observation of pygmy rabbits were used to find fresh fecal pellets. We collected six or more fecal pellets from burrow entrances or single depositions, and recorded locations of samples using handheld GPS units. We stored fecal samples in paper envelopes, and kept samples in a − 80 °C freezer to await further processing. We then used QIAamp stool kits (Qiagen, Valencia, California) to extract genomic DNA from four fecal pellets per individual, and extracted 10% of the samples twice to check sample quality (hereafter referred to as QAQC samples).

Sampling localities were then delineated for population-based analyses in two ways. First, during field collection efforts, sampling was ceased when sign (i.e., fecal pellets and burrows) began to disappear. This means that most samples were collected in aggregated localities, reflecting the fragmented nature of this species' distribution. Second, to remove cases where only several samples were collected from sampling localities, we then aggregated our 194 individual samples into thirteen localities requiring a minimum of five individuals per site spaced no more than 5 km from one another to approximate dispersal distances for this species; these sampling localities generally coincided with those delineated in Larrucea et al. (2018), and contained an average of 10 individuals with some localities having as many as 16 individuals. Ultimately, we then used the centroids of the samples within a site to represent site locations (Fig. 1). Given that these thirteen sampling localities are distanced from each other an average of 80 km (minimum = 15 km) and that pygmy rabbits are thought to disperse an average of about 1–3 km (Estes-Zumpf et al. 2010), we feel secure in labeling these as separate localities. While 13 sampling localities is a relatively small number of localities for population genetic studies, alternative configurations led to sampling localities with too few individuals per locality (five or less). Thus, while these 13 localities do not precisely correspond to any previously-published accounts of the number of genetic clusters for this species in the southern Great Basin (Larrucea et al. 2018, Byer et al. 2021), these localities represented our efforts to reach an optimal compromise between number of localities, sample size per locality, and a priori expectations of genetic structure.

Genotyping

As described in Larrucea et al. (2018), following extraction, we carried out three independent PCR reactions for each sample at all 13 microsatellite loci

(Estes-Zumpf et al. 2008, 2010; Larrucea et al. 2018). This meant that the QAQC samples had 6 datasets and all other samples had three. If the genotype was readily scorable and at least two out of the three genotypes matched, we kept that sample's genotype in the dataset. If they were not easily scorable or if all three did not match, we removed those data. Given that samples were collected non-invasively from burrow entrances, Larrucea et al. (2018) performed an initial test comparing the genotype from single pellets to others in the same deposition pile. This revealed identical genotyping results, confirming that these single deposition piles come from single individuals. Departures from Hardy–Weinberg (HW) equilibrium and global tests of linkage equilibrium (LD) among all pairs of loci and populations were tested using GENEPOP v4.0 (Raymond and Rousset 1995; Rousset 2008). The test for HW equilibrium used the heterozygote deficiency method (Raymond and Rousset 1995), which is a global test that examines either the population(s) or locus but not both simultaneously. The test of LD examined the relationship between genotypes at each pair of loci (i.e., composite LD; Weir 1996). After Bonferroni correction, none of the loci deviated from HW expectations in any population. With lack of deviation from HW equilibrium and a very complete dataset (i.e. no evidence of null homozygotes), we had no reason to expect high frequency null alleles in the dataset. Of the 1092 pairwise tests for linkage disequilibrium across loci and populations, 7 locus pairs appeared to be significantly correlated in up to 2 populations. Given the lack of consistency across multiple populations, all loci were retained for analysis.

We quantified population genetic subdivision by estimating the standardized pairwise F_{ST} among localities using the approach of Meirmans (2006) as implemented in GenAlEx; prior to analysis, all F_{ST} values were converted into linearized $F_{ST}\left(\frac{F_{ST}}{(1-F_{ST})}\right)$ for downstream analysis (Slatkin 1995; see Table S1 for pairwise linearized F_{ST} values used in downstream analyses). Although other distance metrics have been used for landscape genetic analyses of resistance, equivalent results to those presented below were obtained for an alternate distance metric (D_{est}); thus, we present only results based on linearized F_{ST} for simplicity. Furthermore, while individual-based genetic approaches represented a potential avenue for analysis of this dataset, initial tests indicated very poor fit between individual-based measures of genetic differentiation and landscape variables; thus, we proceeded with population-based analyses using the 13 localities described above.

Landscape variables and resistance surface approaches

We represented pluvial lakes using a digital version of the late-Pleistocene pluvial lake high stands developed by Reheis (1999) available online at https://pubs.usgs.gov/mf/1999/mf-2323/. Polygons were converted to raster, resampled to 200-m resolution, and coded to represent areas within pluvial lake high stands versus all other areas. The modern distribution of sagebrush and non-sagebrush vegetation was derived from the Southwest Regional GAP data (Homer et al. 2015). The GAP land cover dataset is a remotely–sensed land cover product derived from Landsat imagery at 30-m resolution. We classified all vegetation types that consisted primarily of sagebrush in the sagebrush category and all other vegetation types and non-vegetative land cover as non-sagebrush. To facilitate comparison with the pluvial lake layer and to ensure that the connectivity analysis could run in a timely manner given computational constraints, we resampled the raster to 200-m resolution using majority resampling in ArcGIS 10.3.1 (ESRI 2015). We then used two approaches to represent and characterize landscape resistance: tests of the goodness of fit for a priori resistance and connectivity hypotheses, and a genetic algorithm approach for optimizing resistance values. Although optimization approaches perform well for selecting optimal combinations of resistance values based on genetic differentiation, they are computationally intensive, and may thus be intractable to use for large numbers of potential surfaces. Furthermore, comparing the output of optimization-based approaches with more conventional a priori tests of resistance values may reveal the relative benefits and costs associated with each approach.

Approach 1: a priori tests of resistance values

We used our a priori approach to selecting resistance values as an initial test of the effects of pluvial lakes, sagebrush, and effective distance metric on gene flow. For each of the two binary raster layers—sagebrush and pluvial lake—we tested ten different relative resistance levels for non-sagebrush and pluvial-lake pixels, respectively: 2, 4, 8, 16, 32, 64, 128, 256, 512, and 1024, representing the relative difficulty of gene flow through the less favorable land cover type; less resistant pixels were given a value of 1. We evaluated these alternate resistance values at two spatial scales: the entire study area (Nevada and Mono Basin), and Nevada alone. In each case, the binary resistance surface was used as the predictor variable and linearized F_{ST} was used as the response.

For each resistance surface, we calculated three different measures of ecological distance. The first two are cost-weighted distance and least-cost path length. Cost-weighted distance and least-cost path length are two related measures derived by identifying the least-cost path across a resistance raster that connects two points. Cost-weighted distance represents the sum of resistance values along the least-cost path, whereas least-cost path refers to the length of the single best route (e.g., in meters) that minimizes the accumulated resistance required to move between the two points on a raster surface (Etherington and Holland 2013). In contrast, unlike least-cost path length, cost-weighted distance accounts for the increased difficulty in traversing highly resistant habitats within the least-cost path (Etherington and Holland 2013). For example, the least-cost path may cover a short distance in Euclidean space, yet the ecological cost of moving across such a surface may be very high, resulting in high mortality or high energetic costs to the organism. To calculate least-cost path length and cost-weighted distance between sampling localities we ran Landscape Genetics Toolbox (Etherington 2011) for each of the ten resistance levels for each of the two hypotheses (pluvial lake and sagebrush). For each model we evaluated the proportion of the genetic variance explained using the effective distance.

In contrast to cost-weighted distance and least-cost path length that both characterize a single optimal route, circuit theory simultaneously considers all possible pathways connecting two points on a resistance surface at once (McRae and Beier 2007). We used Circuitscape software 4.0.5 (Shah and McRae 2008) and calculated cumulative current between all pairs of points representing our localities. As with cost-weighted distance and least-cost path length we evaluated the amount of genetic variance explained by these models. Rather than testing significance of resistance surfaces based on all distance metrics, we instead used multiple regression on distance matrices (MRM) as implemented in package "ecodist" (Goslee and Urban 2007) with matrices of cumulative current derived for each covariate (sagebrush and pluvial lakes) and Euclidian distances as predictors and genetic distance as the response. We fit models that considered each predictor matrix separately, and in each case, we interpreted significance of each predictor at $\alpha = 0.05$ based on 1000 permutations. We repeated this procedure for both the entire study area and for Nevada alone.

We also created and evaluated two null hypotheses for comparison with pluvial lake and sagebrush resistance surfaces: the first represented increasing genetic differences with increasing Euclidean distance (hereafter termed the isolation-by-distance model), whereas the second represented geographically indeterminate separation between Mono Basin and Nevada populations due to the low elevation Lahontan Trough (hereafter termed the isolation-by-barrier model). The isolation-by-barrier hypothesis was a non-geographic representation of connectivity in which pairs of points crossing the barrier were coded as a value of 1 and pairs of localities within regions (Nevada vs. Mono Basin) were coded as 0. Given the complexity involved in selecting resistance values, we used these two alternative hypotheses as mechanism-agnostic null hypotheses of patterns of gene flow; if either of these surfaces explained equal or more variation compared to the pluvial or sagebrush-based resistance surfaces, we then interpreted the pluvial or sagebrush-based surfaces as being no more explanatory than null representations. Given the non-spatial nature of these two hypotheses, we only summarize Euclidian distance for each of these hypotheses.

Approach 2: genetic algorithm-based resistance optimization

In contrast to the first approach testing a priori combinations of resistance values for goodness-of-fit, we also used an optimization-based approach to do the following: (a) produce optimal resistance surface values and assess explanatory power for pluvial and sagebrush surfaces for comparison with our a priori landscape resistance approach, and (b) evaluate the explanatory power of composite layers based on combinations of pluvial and sagebrush resistance surfaces, particularly given the spatial overlap between pluvial lake high stands and sagebrush distribution (Fig. 1). In order to accomplish these goals, we used package "ResistanceGA" in R version 3.6.3, which uses genetic algorithms in tandem with Circuitscape to produce optimal combinations of resistance values for supplied surfaces (Peterman 2014; R Core Team 2020). We used the function "MS_optim" to produce a composite representation of the two surfaces. As for the first approach, we used linearized F_{ST} as the response, and supplied the binary sagebrush and pluvial lake surfaces as input surfaces for categorical optimization. Most default settings for both "GA.prep" and "jl.prep" were used, and we utilized log likelihood as the objective function for optimization. We also used function "SS_optim" to optimize the sagebrush and pluvial lake surfaces separately. Finally, we calculated sample size-corrected Akaike's Information Criterion (AICc; Sugiura 1978) for both the single and multi-surface optimized surfaces, as well as a null surface, and a surface based on distance alone. Following (Burnham and Anderson 2002), any surface within 2 ΔAICc of the best surface was considered biologically important.

Historical sagebrush distribution and landscape connectivity

In addition to testing resistance due to modern vegetation, we also hindcasted sagebrush distribution back in time using climate data from the WorldClim dataset (Fick and Hijmans 2017) and applied the most explanatory resistance value from the a priori modern sagebrush vs. non-sagebrush resistance surface. Three time periods were selected from the downscaled (200 m) paleoclimate data from the WorldClim version 1.4 climate data (Fick and Hijmans 2017). These time periods included the present-day, mid Holocene (6000 YBP), and the late glacial maximum (22,000 YBP). Modern day projections are based on the CMIP5 model (Taylor et al. 2012). We used the following four bioclimatic models to reconstruct sagebrush distribution for all three time periods: average temperature, mean diurnal range of temperature, temperature seasonality, and annual temperature range. Although we originally intended to include precipitation variables in this model, inclusion of these variables produced hindcasts that were substantially less accurate than models without; thus, we proceeded with hindcasts based on thermal variables alone. Note that we did not reconstruct pluvial lake distribution beyond last glacial maximum; uncertainty in the spatial distribution of all pluvial lakes in our study area made considering dynamic lake extents intractable.

We developed distribution models for sagebrush based on the above four variables using a random forest modeling approach. Random forest is an ensemble of classification trees and has been used widely in species distribution modeling because it has many valuable properties such as not relying on distribution assumptions and having good predictive performance (Cutler et al. 2007). To construct the model, we randomly selected 10,000 points from the modern-day binary sagebrush/non-sagebrush map for model training and an additional 10,000 random points for model validation of which 47% were located in sagebrush and 53% were in non-sagebrush. Random forest models were run in the R package "randomForest" (Liaw and Wiener 2002) using the Marine Geospatial Ecology Tools (Roberts et al. 2010) to facilitate transfer of the data between ArcMap and R and to create maps for the three time periods (present-day, mid-Holocene, Pleistocene). We used default settings which included growing 500 trees and using 2 variables for splitting. We used the Youden Index (Youden 1950) to separate sagebrush from non-sagebrush, which resulted in a threshold value of 0.462. Finally, we applied the most predictive resistance level (non-sagebrush resistance = 798.46) to all three time periods (present-day based on climate projections, mid-Holocene, last glacial maximum) and related each resistance surface to our pairwise genetic distance matrix. To calculate cumulative current, we used Circuitscape software 4.0.5 (Shah and McRae 2008) as we did for the modern vegetation using the thirteen populations as source and destination points.

Results

Approach 1: a priori tests of resistance values

Surfaces that included Mono Basin indicated that the sagebrush surfaces had more explanatory power than the pluvial lake surfaces at all resistance values (Fig. 2a). For the sagebrush surfaces, variance explained increased up to a non-sagebrush resistance of 32 ($R^2_{current}$ = 0.72), followed by a very gradual

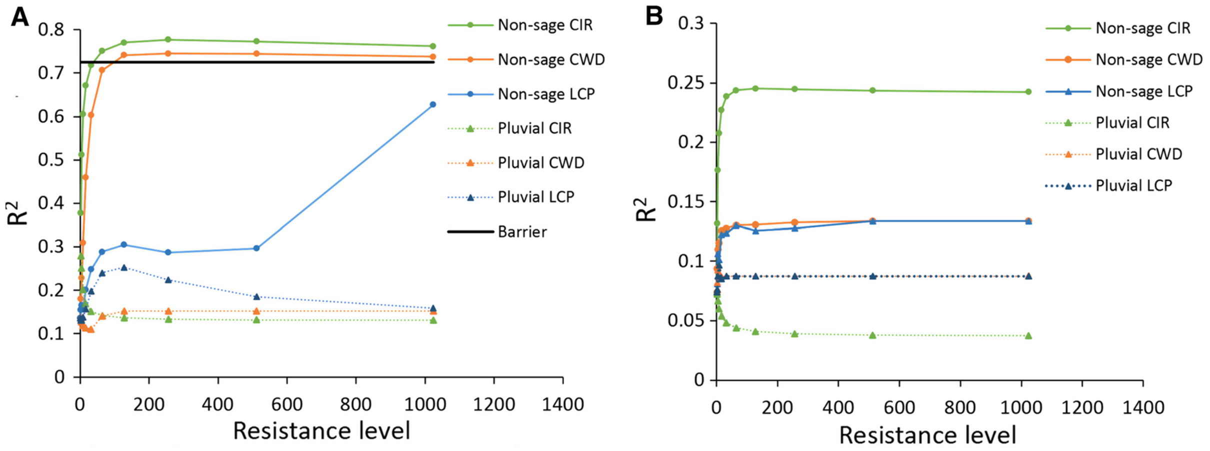

Fig. 2 Genetic distance versus ecological distance for **a** all localities including the Mono Basin localities and **b** only for the Nevada localities. The models tested two different hypotheses (non-sagebrush vegetation as resistance relative to sagebrush and pluvial lake high stands as resistance relative to non-pluvial lakes), ten different resistance levels (2, 4, 8, 16, 32, 64, 128, 256, 512, and 1024 times), and three different methods for measuring ecological distance (*LCP* least-cost path length, *CWD* cost-weighted distance, *CIR* cumulative current from circuit theory). We also compared these models to a non-geographic barrier model (that assessed complete separation between the Mono Basin and the Nevada localities; black line) and an Isolation-by-Distance (IBD) model that set all cells to a resistance value of one (point of convergence among lines near Y-axis)

increase up to a resistance level of 128 ($R^2_{current}$ = 0.77), at which point there is an asymptote for higher resistance values and maximum R^2 at a resistance level of 256 (Fig. 2a). In contrast, the pluvial lake hypothesis explained far less variance, never exceeding $R^2 = 0.30$ (Fig. 2a). The isolation-by-distance surface explained relatively little variance ($R^2 = 0.136$) while the barrier surface explained considerably more ($R^2 = 0.725$), suggesting that non-spatial representation of complete isolation between Mono Basin and Nevada populations explains only slightly less variance ($\Delta R^2 = 0.052$) than the best sagebrush surface. Cumulative current for the most predictive sagebrush surface (non-sagebrush resistance = 256) was a significant predictor of genetic distance ($F = 140.155$, $p = 0.001$), as was current for the most predictive pluvial lakes surface (pluvial-lake resistance = 2; $F = 29.474$, $p = 0.001$) and Euclidian distance ($F = 11.951$, $p = 0.004$).

Surfaces that excluded Mono Basin (and included only Nevada localities) explained less variance overall (maximum $R^2 = 0.25$; Fig. 2b). For the Nevada-only sagebrush surfaces, explanatory power increased up to a non-sagebrush resistance of 128, at which point explained variance reached an asymptote (Fig. 2b). In contrast, increases in resistance assigned to pluvial lake high stands for the pluvial lakes surfaces produced little-to-no effect on variance explained (maximum of 0.10; Fig. 2b). In Nevada, resistance surfaces explained more genetic variance compared to the null model of isolation-by-distance ($\Delta R^2 = 0.171$). Cumulative current for the most predictive Nevada-alone sagebrush surface was a significant predictor of genetic distance ($F = 13.932$, $p = 0.013$), as was Euclidian distance ($F = 3.424$, $p = 0.019$), but not cumulative current for the most predictive Nevada alone pluvial lakes surface ($F = 3.346$, $p = 0.083$).

Cumulative current derived from circuit theory consistently outperformed cost-distance and least-cost path length. For sagebrush surfaces that included the Mono Basin, representing distances based on circuit theory and cost-distance produced similar results (Fig. 2a). In contrast, the variance explained by least-cost path length increased initially, plateaued, and then increased dramatically again going from non-sagebrush resistance values of 512–1024 (Fig. 2a). For the sagebrush surfaces that excluded Mono Basin, distances based on circuit theory outperformed cost-distance and least-cost path distances ($R^2_{current}$ = 0.25, $R^2_{cost\text{-}weighted}$ = 0.13, $R^2_{least\text{-}cost}$ = 0.13; Fig. 2b).

Approach 2: resistance surface optimization

Optimization of resistance surface values using package "ResistanceGA" indicated lowest AICc for the model based on sagebrush distribution alone, with no other models within 2 ΔAICc of this model (Table 1). For this top model, non-sagebrush habitat was assigned a resistance of 481.93; furthermore, this optimal surface had a slightly higher R^2 (0.83) than the most explanatory sagebrush surface derived using our a priori approach ($R^2_{current}$ = 0.78). The composite surface based on a weighted combination of sagebrush and pluvial surfaces (sagebrush = 62.4%, pluvial = 37.6%) was ultimately not competitive with the sagebrush surface (ΔAICc = 5.34) and explained less variance than the sagebrush layer (R^2 = 0.825; Table 1). This composite surface assigned lowest resistance to sagebrush located in pluvial lake high stands, and highest resistance to non-sagebrush habitat located outside of pluvial high stands (Table 1). The pluvial surface ultimately ranked below an isolation-by-distance surface (distance ΔAICc = 95.516, R^2 = 0.268; pluvial ΔAICc = 96.234, R^2 = 0.339; Table 1). Furthermore, unlike the composite layer, the pluvial surface assigned highest resistance to pluvial high stands (Table 1).

Historical sagebrush distribution and landscape connectivity

The sagebrush model only using temperature variables performed well with an overall accuracy of 73%, an area under the ROC curve of 0.816, a root-mean square error of 42.2%, and relatively balanced sensitivity (77%) and specificity (70%). The most important variables were average temperature (37% of variable importance) followed by temperature range (24%), temperature seasonality (23%), and diurnal range (16%). Projections of sagebrush distribution in the present-day bear great resemblance to the maps of sagebrush based on remotely sensed land cover at 80% overlap, although the projected sagebrush distribution models appear more generalized, yet capture the general patterns (Fig. 3). Projections of sagebrush distribution to the Holocene suggest relatively similar patterns of connectivity between the mid-Holocene and present day with 88% overlap. In contrast, during the last glacial maximum, much of the sagebrush

Table 1 (a) AICc values for genetic algorithm-based optimization of landscape resistance; (b) assigned resistance values assigned to each habitat classification for each optimized surface

Surface	k	AICc	ΔAICc	R^2	LL
(a)					
Sagebrush	3	− 272.759	0.000	0.827	141.713
Composite (sagebrush + pluvial)	5	− 267.414	5.345	0.825	141.993
Distance	2	− 177.243	95.516	0.268	93.222
Pluvial	3	− 176.526	96.234	0.339	93.596
Null	1	− 146.263	126.496	0.000	76.313

Surface	Nonsage	Pluvial	Sage, nonpluvial	Sage, pluvial	Nonsage, nonpluvial	Nonsage, pluvial
(b)						
Sagebrush	481.933	1.000	1.000	1.000	481.933	481.933
Composite (sagebrush + pluvial)	2356.000	0.210	2.990	1.000	1180.500	1178.500
Distance	1.000	1.000	1.000	1.000	1.000	1.000
Pluvial	1.000	1.660	1.000	1.660	1.000	1.660
Null	1.000	1.000	1.000	1.000	1.000	1.000

k number of parameters, *AICc* corrected Akaike's Information Criterion, *ΔAICc* difference in AICc relative to top surface, R^2 model fit, and *LL* log likelihood

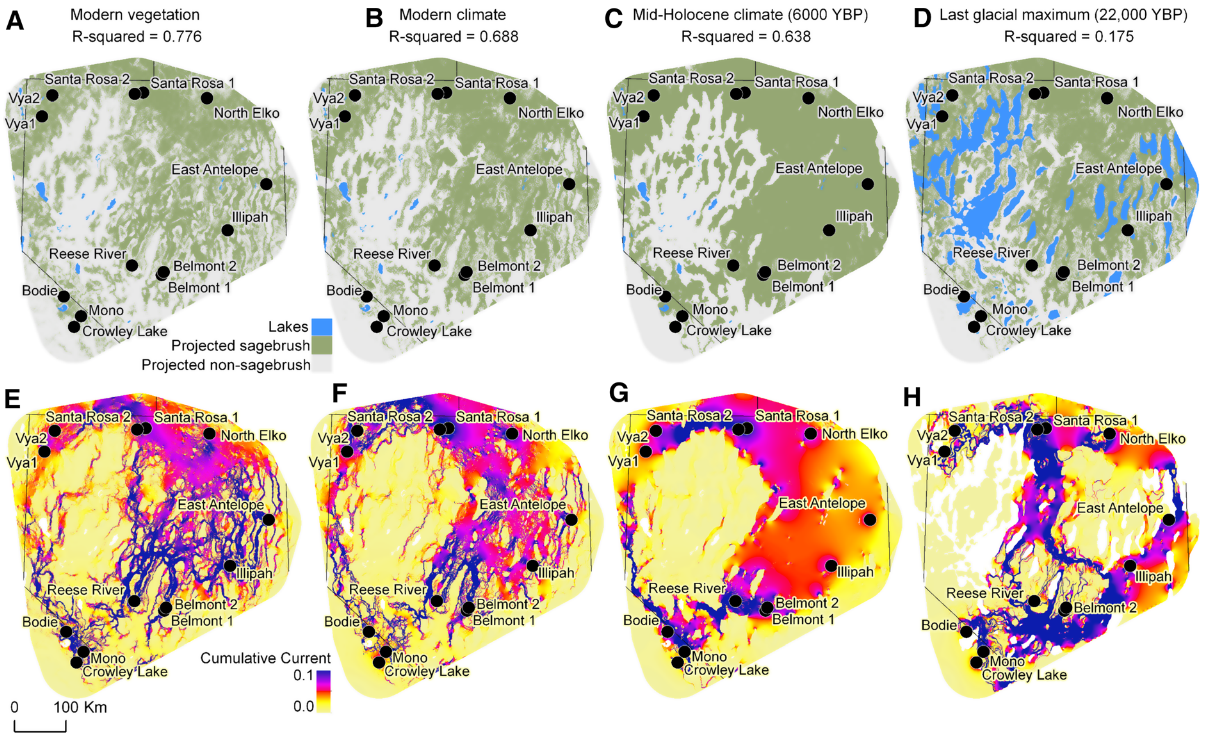

Fig. 3 Sagebrush habitat, non-sagebrush habitat, and water bodies for **A** modern climate based upon modern vegetation maps, **B** sagebrush distribution reconstructed from modern climate, **C** sagebrush distribution reconstructed from mid-Holocene climate (6000 YBP), and **D** sagebrush distribution reconstructed for the late Pleistocene (22,000 YBP). Cumulative current derived from circuit analysis using genetic population centroids as nodes (i.e. identifying potential movement among all of the population centroids to all other population centroids). Cumulative current was calculated for **E** modern climate based upon modern vegetation maps, **F** sagebrush distribution reconstructed from modern climate, **G** sagebrush distribution reconstructed from mid-Holocene climate (YBP), and **H** sagebrush distribution reconstructed for the late Pleistocene (YBP). Large water bodies are shown in white rather than blue for **E**–**H** for clarity. Coefficient of determination (r-squared) was calculated by comparing the amount of genetic variation explained to the ecological distance derived from Circuitscape for each pair of sampling localities. All surfaces used the most predictive non-sagebrush resistance of 798.46

habitat that would have facilitated movement for pygmy rabbit is predicted to have been located at lower elevations than present day, while the very lowest elevations were filled with pluvial lakes. The projected Pleistocene sagebrush distribution overlapped with the modern distribution 65%. Resistance surfaces based on projected sagebrush distribution in the current day, Holocene, and last glacial maximum suggested better fit between resistance surfaces and genetic differentiation for the mid-Holocene (R^2 = 0.688), followed by the current day (R^2 = 0.638) and last glacial maximum (R^2 = 0.175), suggesting concordance between mid-Holocene sagebrush distribution and present day genetic differentiation (Fig. 3).

Discussion

Traditional studies of biogeography use geological information to reconstruct past changes in population connectivity and distribution, often relying on representation of historical events in geological records (Crisci et al. 2006). Although the field is still developing, landscape genetics provides a suite of novel tools that can bolster studies of biogeography, specifically by using observed patterns of genetic differentiation to test hypotheses related to the influences of past geological and ecological change (Rissler 2016). In this study, we leveraged recently-collected genetic data for the pygmy rabbit to explore hypotheses related to genetic divergence across the southern Great Basin (Larrucea et al. 2018). By evaluating the relative fit of landscape resistance surfaces parameterized to represent past biogeographic events—specifically, shifts in the distribution of sagebrush habitat in the mid-Holocene and historical barriers presented by pluvial lakes present during the last glacial maximum—to observed genetic data, our approach allowed for consideration of the appropriate time scales involved in shaping contemporary genetic pattern. Our analyses revealed several key findings: (a) mid-Holocene shifts in sagebrush distribution have played a predominant role in shaping genetic differentiation, and (b) a limited number of possible dispersal pathways may connect the Mono Basin and Nevada, whereas connectivity within Nevada may be driven by multiple possible dispersal pathways.

Sagebrush and pluvial lake resistance

Across all analyses, the clear importance of contemporary and historical patterns of sagebrush distribution was evident in the high explanatory power of sagebrush-based resistance surfaces, with particularly evident disruptions to gene flow between the Mono Basin and Nevada. While sagebrush surfaces were only slightly more explanatory than our isolation-by-barrier surfaces depicting no gene flow between Nevada and the Mono Basin, we view sagebrush surfaces as more realistic depictions of the ecological processes producing genetic differentiation between Nevada and the Mono Basin. Furthermore, recent coalescent simulations indicate that rates of gene flow between Mono Basin and Nevada are low, but non-zero, indicating that some level of historic gene flow was present between these areas, likely along the relatively few areas of the Lahontan Trough that had sufficient sagebrush cover for traversal (Byer et al. 2021). Since the pygmy rabbit is a sagebrush obligate that relies upon sagebrush for diet and cover (White et al. 1982; Shipley et al. 2006; Camp et al. 2012), this suggests a strong, temporally-consistent link between sagebrush cover and both habitat suitability and connectivity. In contrast, although the historical distribution of pluvial lakes likely played a role in the biogeography of a number of Great Basin species (Wells 1983; Reheis 1999; Reheis et al. 2014), effects of pluvial lake distribution on pygmy rabbit connectivity were weak overall ($R^2 < 0.30$), with highest proportions of explained variance when low resistance values were assigned to pluvial lake high stands (Tables S2, S3). Furthermore, the optimization analyses indicated that pluvial surfaces did not explain any more variance than representations based on distance alone (Table 1).

Genetic subdivision in pygmy rabbits appears to be related to mid-Holocene warming and declines in sagebrush cover, given the observed higher explanatory power of resistance surfaces based on mid-Holocene sagebrush distribution. This pattern is consistent with localized fossil records, including those from caves such as Homestead Cave in Utah (Grayson 2006) and Owl Cave in Idaho (Commendador and Finney 2016), which document drastic declines in pygmy rabbits as sagebrush declined. For example, at Homestead Cave virtually all pygmy rabbit records are from prior to 8300 YBP with virtually no recolonization afterward (Grayson 2006). Fossil records collected from southern New Mexico (Harris 1990) and southern Nevada (Haynes 1965) suggest the distribution of pygmy rabbit during the Pleistocene may have extended far south of its current range, indicating dramatic range contraction due to vegetative change in the mid-Holocene.

There are several caveats to our approach, however. First, we note that competing growth (e.g. trees and grasses) that potentially occupied portions of the sagebrush niche may account for some of the lower predictive power of the Pleistocene resistance surfaces; our approach did not consider other vegetative types beyond sagebrush. Second, uncertainty in climate projections may also potentially contribute to the weaker relationship between gene flow and sagebrush distribution, particularly if different species of sagebrush have contrasting bioclimatic niches. Since our efforts only considered distribution of sagebrush overall rather than considering each species separately, we could not consider the contributions of sagebrush species of particular dietary importance, such as big sagebrush (*Artemisia tridentata*), to genetic differentiation and gene flow. Further work is needed to connect changes in the distribution of particular sagebrush species to gene flow in pygmy rabbits. Additionally, given that our use of microsatellite markers likely captured more recent genetic patterns than alternative markers (such as single-nucleotide polymorphisms), our results may simply indicate that contemporary genetic pattern is shaped by contemporary landscape processes. Concurrent work with single-nucleotide polymorphisms indicates that genetic differentiation in Nevada likely occurred in the very recent past (100–300 years), whereas differentiation between the Mono Basin and Nevada was more ancient (> 30,000 years ago); thus, the results presented herein indicate that more ancient divergence between genetic lineages may have been re-enforced by more contemporary shifts in sagebrush distribution (Byer et al. 2021).

Influences of scale and distance metric

Although effects of sagebrush were apparent for all analyses, our results were clearly influenced by the geographical extent of the analysis, with explanatory power decreasing dramatically when surfaces were tested for Nevada alone (excluding the Mono Basin). Although somewhat expected given that Larrucea et al. (2018) found only weak subdivision and geographic overlap among inferred genetic clusters in Nevada, this suggests contrasting processes involved in gene flow limitation depending on spatial scale. Studies in other regions of the pygmy rabbit's range (eastern Idaho, Estes-Zumpf et al. 2010; southern Wyoming, Thimmayya and Buskirk 2012) have suggested little genetic divergence at finer scales than those considered in this study, suggesting widespread local gene flow. It is also possible, however, that lower genetic differentiation throughout Nevada—and reduced explanatory power for sagebrush resistance surfaces—are at least partly reflective of the distribution of available sagebrush habitat throughout this region since the mid-Holocene. Large discontinuities in sagebrush distribution are only present in the western and southern portions of Nevada, and only appear to separate localities in Nevada from those in the Mono Basin (Figs. 1, 3). Thus, sagebrush distribution may be a limiting factor for connectivity between Nevada and Mono Basin localities, and modern declines in sagebrush cover in Nevada may be expected to limit connectivity throughout this region as well.

Choice of scale appeared to interact strongly with metrics of effective distance as well, as effective distances computed using circuit theory were more explanatory than other distance metrics computed for the Nevada-alone surfaces, highlighting the importance of multiple dispersal pathways in this part of the range (McRae and Beier 2007). This is consistent with the geography of the basin and range province in which broad and connected valleys dominated by sagebrush alternate with north–south trending mountain ranges; this topographic complexity likely leads to multiple optimal dispersal pathways between adjacent populations (Murphy et al. 2010; Guarnizo and Cannatella 2013). In contrast, the similarity between cost-distance and circuit theory at the broadest extent suggest that a single pathway or handful of pathways connected Nevada localities with the Mono Basin, evident in the relative sparsity of sagebrush cover in the southwestern part of Nevada (Figs. 1, 3).

Methodological considerations

Although we used two approaches for selecting optimal resistance settings for each layer—an approach based on testing a priori sets of resistance values against genetic differentiation, and a genetic algorithm-based approach for optimizing resistance values that best explain genetic differentiation– both ultimately supported the importance of sagebrush cover over last glacial maximum pluvial lake distribution, but with differences in resistance values and explanatory power. With the a priori approach, variance explained increased rapidly up to a non-sagebrush resistance value of 32; however, at resistance values greater than 128 variance explained only increased slightly up to a resistance value of 798 ($R^2 = 0.79$). In contrast, the single-surface optimization based on sagebrush distribution suggested an optimal resistance setting of 481.93 for non-sagebrush habitat ($R^2 = 0.83$). Thus, quantitative values for non-sagebrush resistance were quite different between approaches, but explanatory power was only slightly improved for the optimization approach.

Although optimization approaches have a number of advantages over a priori approaches to assigning resistance values (Peterman 2014; Peterman et al. 2014), our results suggest that careful choice of a priori resistance values for exploration should theoretically produce results that are somewhat similar to those produced by optimization approaches. In our study, tests of a priori resistance surface values eased the computational burden of testing large numbers of connectivity hypotheses, and allowed expedient consideration of multiple time scales, effective distance metrics, and non-geographic hypotheses of connectivity. Given the substantial computational resources needed for genetic algorithm-based approaches, we primarily used these approaches for evaluating the explanatory power of composite surfaces, which may not be easily accommodated by a priori approaches. Thus, each approach has costs and benefits that must be considered. In particular, tests of a priori resistance surface parameterizations will likely be intractable for continuous surfaces given the large number of parameter combinations necessary to cover parameterization options (Peterman 2014). Since our surfaces each only contained two classes, it was possible to explore a wide variety of high resistance settings for each surface; thus, in our case, categorical surfaces were efficiently parameterized using either a priori or optimization-based approaches.

Conservation implications

Consistent relationships between sagebrush cover and gene flow suggest strong links between dietary specialization, patterns of habitat selection and gene flow. Sagebrush obligates (such as the pygmy rabbit) appear to exhibit particularly strong genetic structure associated with sagebrush distribution, with noticeable genetic subdivisions associated with sagebrush habitat fragmentation. Although immediately apparent in genetic differences between Mono Basin and Nevada populations for the pygmy rabbit (Larrucea et al. 2018; this study), the Greater sage-grouse also shows strong differentiation between populations near the Mono Basin (often referred to as the bi-state population) and populations throughout Nevada (Oyler-McCance et al. 2005). As additional genetic studies are conducted on sagebrush-obligate species in the southern Great Basin, it is possible that the same genetic subdivisions east and west of the Lahontan Trough will be apparent in other sagebrush obligates. Furthermore, our finding that relatively few dispersal pathways connect the Mono Basin and Nevada may indicate that greater attention should be paid to maintaining and restoring sagebrush cover along the border between California and Nevada.

Although currently unexplored for the pygmy rabbit, sagebrush-associated reductions in gene flow between Mono Basin and Nevada populations may lead to adaptive divergence (Kawecki and Ebert 2004; Stiebens et al. 2013; Tigano and Friesen 2016). Although reduced gene flow alone may be insufficient to produce adaptive divergence between spatially-separated populations (Tigano and Friesen 2016), reductions in gene flow paired with divergence in local vegetation and climate conditions have produced observed signatures of adaptive divergence in other sagebrush specialists. For example, previous work on the Gunnison sage-grouse (*Centrocercus minimus*) has documented signatures of adaptive divergence in genes associated with detoxification, which may indicate adaptation to spatial variation in plant secondary compounds (PSC) found in sagebrush (Zimmerman et al. 2019), particularly given observed spatial variation in oil content for big sagebrush (Powell 1970). Although these approaches require large numbers of Single-Nucleotide Polymorphisms (SNPs), a number of emerging genomic tools can be used to identify candidate loci that may be adaptively divergent (Schoville et al. 2011; Savolainen et al. 2013). When paired with associations between historical shifts in sagebrush distribution documented in this present study, genomic insights into local adaptation will provide unprecedented insights into the repercussions of past biogeographic changes. Given the scope of environmental change and anthropogenic alteration of habitat projected through the twenty-first century, considering the effects of historical climate and biogeography on contemporary patterns of genetic diversity will allow for better prediction of how sensitive species will respond to future environmental change.

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Data availability The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Code availability All code used for this study will be made available upon reasonable request.

Declarations

Conflict of interest The authors have no conflicts of interest to disclose.

Ethical approval This research was conducted in compliance with Scientific Collection Permits from the states of Nevada, California, and Oregon, and under oversight of the University of Nevada Reno's Animal Care and Use Committee.

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