RESEARCH ARTICLE

Genes in space: what Mojave desert tortoise genetics can tell us about landscape connectivity

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Abstract

Habitat loss and fragmentation in the Mojave desert have been increasing, which can create barriers to movement and gene flow in populations of native species. Disturbance and degradation of Mojave desert tortoise habitat includes linear features (e.g. highways, railways, a network of dirt roads), urbanized areas, mining activities, and most recently, utility-scale solar facilities. To evaluate the spatial genetic structure of tortoises in an area experiencing rapid habitat loss, we genotyped 299 tortoises at 20 microsatellite loci from the Ivanpah valley region along the California/Nevada border. We used a Bayesian clustering analysis to quantify population genetic structure across valley and mountain pass habitats. A spatial principal components analysis was used to further investigate patterns with isolation-by-distance. To explicitly consider landscape features (e.g. habitat and anthropogenic linear barriers), we used maximum likelihood population effects analyses. We quantified recent gene flow through relatedness using a maximum likelihood pedigree approach. We detected three genetic clusters that generally corresponded to valleys separated by mountains, with one genetically distinguishable population in a mountain pass. Pedigree analyses showed second order relationships up to 60 km apart suggesting a greater range of interactions and inter-relatedness than previously suspected. Our results support historical gene flow with isolation-by-resistance and reveal reduced genetic connectivity across two parallel linear features bisecting our study area (a railway and a highway). Our work demonstrates the potential for tortoises to use a range of habitats, spanning valleys to mountain passes, but also indicates habitat fragmentation limits connectivity with relatively rapid genetic consequences.

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Introduction

An important question in conservation ecology is how anthropogenic landscape change impacts movement and population connectivity. Habitat loss and fragmentation can significantly increase the risk of population decline and extinction for native populations by altering natural movement patterns and landscape use (Ewers and Didham 2006; Haddad et al. 2015; Hand et al. 2014). Integrating genetics with landscape ecology provides a framework to examine the role of heterogeneous habitats in shaping genetic diversity and population structure (Holderegger and Wagner 2008; Manel et al. 2003; Storfer et al. 2007). Fortunately, the hard-to-observe process of movement of individuals through a landscape can be inferred by examining genetic structure and relatedness (Dileo and Wagner 2016; Lowe and Allendorf 2010; Slatkin 1985). However, the genetic effects of recent landscape changes that result in habitat loss or linear

barriers are often observable only after a substantial lag time that can range from 1 to 200 generations (Landguth et al. 2010); therefore, the influence of these changes on genetic diversity may not be observed for several decades in species with delayed breeding and long generation times, such as the Mojave desert tortoise (*Gopherus agassizii*).

Historically, the Mojave desert of southern California, southern Nevada, northwestern Arizona, and southern Utah is thought to have exhibited relatively high levels of ecological connectivity (Dickson et al. 2016). For native species like the desert tortoise, which occurs throughout most of this region (Germano et al. 1994; Murphy et al. 2007), highly connected habitat combined with limited individual movement and dispersal have produced a genetic pattern of isolation-by-distance (IBD) with additional differentiation from topographical features (Hagerty and Tracy 2010; Murphy et al. 2007; Hagerty et al. 2011; Shaffer et al. 2015). Isolation-by-distance is characterized by continuous populations where interbreeding is limited by dispersal distance and distant populations are more genetically differentiated (Wright 1943). The lack of major geographical barriers to movement has resulted in low to moderate levels of genetic differentiation range-wide (pairwise F_{ST} 0.011–0.132; Hagerty and Tracy 2010), indicative of gene flow occurring in a stepping-stone like pattern (Murphy et al. 2007; Hagerty and Tracy 2010; Hagerty et al. 2011; Sanchez-Ramirez et al. 2018). This is further supported by radio-telemetry studies of movement and home ranges. For example, a review of Mojave desert tortoise home range size indicates a range from 1 to 53 ha (median 9.2 ha) with animals capable of traveling 470–823 m/day, while males are known to move over 1 km/day (Berish and Medica 2014). However, most daily movements are under 200 m (O'Connor et al. 1994), suggesting that long-distance dispersal primarily occurs sporadically and over multiple generations for this species (USFWS 1994).

The historical landscape, characterized by broad interconnected valleys and mountain passes that influenced the population genetic structure and gene flow we measure in desert tortoises today, has changed. Human presence in the North American deserts has increased since the last century, expressed by rapid urban expansion (Hughson 2009), and a proliferation of vehicular routes from trails to major highways (Leu et al. 2008), which have caused loss and fragmentation of desert habitat. Rapid urban development, such as within Las Vegas valley—once a connective region linking tortoise populations across their range (Britten et al. 1997; Hagerty and Tracy 2010), has resulted in substantial loss of habitat connectivity and reduced movement of animals and gene flow relative to historical conditions. Desert valleys along the state line between Las Vegas, Nevada and the desert cities of southern California have recently undergone substantial habitat alteration. Significant disturbance was initially related to mining throughout the area and has continued to grow since the mid-1800s. The Southern Pacific Railroad was built in the mid-1880s to support mining and transport people and goods, and still bisects the desert today (Tuma and Sanford 2014). The urbanization of desert lands increased throughout the 1900s and Las Vegas is now a major metropolitan area. An interstate highway route (I-15) through the Nevada and California Mojave desert can be traced to the early 1900s, with the interstate we know today largely defined by the mid-twentieth century. The ever increasing highway speeds and traffic, installation of concrete barriers between north and south bound lanes, and desert tortoise exclosure fencing starting in the late 1990s along portions of the highway effectively creates a nearly complete barrier to tortoise movement (Peaden et al. 2017). However, the presence of culverts under I-15 may allow for occasional passage as tortoises are known to use storm drain culverts under other highways (Boarman et al. 1997). The large network of dirt roads across the Mojave, although not an observed barrier to tortoise movement, has numerous negative effects on the quality of desert tortoise habitat (as summarized by Heaton et al. 2008). Most recently, habitat loss has further intensified throughout tortoise habitat with the development of utility-scale solar facilities, which have increased markedly since 2010 (BLM 2010; BrightSource Energy 2014).

Disturbance in arid ecosystems has long lasting impacts (Webb and Wilshire 1980) that may preclude habitat restoration or recovery (USFWS 2011). This poses a serious risk to the long-term persistence of the desert tortoise, which was federally listed as a threatened species under the Endangered Species Act in 1990 largely due to reductions in range and population density (USFWS 1994). Population trends indicate rapid declines associated with human landscape disturbance; specifically, habitat loss and degradation due to urbanization (Averill-Murray et al. 2012; Corn 1994; Doak et al. 1994; Tracy et al. 2004; USFWS 2011). Range-wide, populations have continued to decline since their 1990 listing, reportedly by roughly one-third in the last decade (Allison and McLuckie 2018).

Habitat loss and fragmentation are expected to increase due to ongoing development, which could eventually threaten connectivity for the tortoise (Averill-Murray et al. 2013). For example, of the 16,282 km² of tortoise habitat that lies outside conservation areas, 700 km² has been projected to be lost to utility-scale solar development (Averill-Murray et al. 2013). The timeframe for development is indefinite as projects are being proposed, modified, and constructed continually with no perceivable endpoint. Utility-scale solar placement is also variable and subject to change. As human population growth, urbanization, and utility-scale solar energy construction on public lands continue to significantly reduce habitat for the Mojave desert tortoise (Berry

and Aresco 2014) the likelihood that the species will become reliant on sustained conservation actions increases (Averill-Murray et al. 2012). Given that persistent urban expansion has amplified isolation for tortoises (Averill-Murray et al. 2013), and that development will likely continue, the need to maintain connectivity from California through Nevada and into Utah and Arizona is now more vital than ever. Therefore, understanding existing tortoise population genetic structure is key to assessing the impacts of continued habitat loss and fragmentation.

The area within and surrounding the Ivanpah valley provides a study region replete with historical and more recent potential anthropogenic barriers to tortoise movement and gene flow, as well as natural features that may either facilitate gene flow (large areas of open habitat), or restrict gene flow (mountain passes and expansive dry lakes). Because tortoises are commonly associated with desert valleys, but have been recorded in rugged terrain (O'Connor et al. 1994) and are known to occupy and move through heterogeneous habitat (Morafka and Berry 2002), we hypothesized that tortoises have historically used mountain passes as connective habitat between the Ivanpah valley and adjacent valleys. However, habitat disturbance may alter connectivity. Anthropogenic barriers within the Ivanpah valley include I-15 (50–80 years) and the Southern Pacific Railroad (140 years), while more recent impacts include a golf course (> 20 years) and three utility-scale solar developments (< 10 years). The solar installations were sited in previously undeveloped Mojave desert tortoise habitat, where density was estimated between 1.2 and 10.4 tortoises/km^2 (Ironwood Consulting 2012). Developments in valley habitat, including solar energy facilities on public lands, have not been well studied to evaluate impacts to the species (Lovich and Ennen 2011; USFWS 2011), and understanding population genetic structure and gene flow in these areas is vital if genetic connectivity is to be maintained into the future.

Given the relatively long generation time of the desert tortoise (20–25 years; USFWS 1994) traditional measures of population differentiation (e.g. F_{ST}) may not reflect current landscape conditions. Additional analytical methods such as examining the spatial distribution of first and second order relatives can help to understand more recent movement and dispersal patterns (Vandergast et al. 2019). Using clustering approaches and explicitly testing for effects of individual landscape features may help to better characterize the relative impacts of natural and anthropogenic features on genetic structure.

In this study, we applied a fine-scale sampling scheme and combined pedigree reconstruction and genetic clustering analyses with spatially explicit methods to evaluate recent gene flow and historical genetic structure in relation to anthropogenic and historical landscape features within and surrounding the Ivanpah valley. The specific goals of this research were threefold: (1) to identify the role of historical landscape features with suitable desert tortoise habitat in facilitating genetic connectivity among adjacent valleys, (2) to assess genetic structure and relatedness across a heterogeneous landscape that has undergone recent and rapid habitat disturbances, and (3) to quantify individual and population level patterns of genetic variation to provide a reference for genetic connectivity for future comparisons. A reference of historical genetic connectivity in this system could be important in understanding the role of intact habitat for tortoise persistence relative to ongoing disturbance.

Methods

Study area and sampling

This study was conducted in the central portion of the Mojave desert tortoise range (Nussear et al. 2009), focusing on the Ivanpah valley and surrounding Mesquite valley and Piute valley connected by mountain passes (Fig. 1). Field surveys were conducted in 2015, 2016, and 2017 at ten, 1 km^2 study plots (six in Ivanpah valley, one in Mesquite valley CA, one in Eldorado valley NV, and two in mountain passes between valleys), in a diverse array of suitable habitat. Genetic samples collected from an additional location (Piute valley, near Searchlight NV) prior to 2015 were also included, for a total of eleven locations and 299 genetic samples (Fig. 1). The number of individuals varied by study site (Table 1), which may be indicative of natural tortoise densities across the Mojave Desert. Allison and McLuckie (2018) report adult densities of < 1–22.5 tortoises per km^2. Construction at the Ivanpah Solar Electric Generating System (ISEGS; 14 km^2) on the west side of Ivanpah valley, and Silver State Solar (14 km^2) on the east began in 2010. Tortoises from within the ISEGS footprint were translocated to the north of the facility and those from within Silver State Solar were translocated to the east. At our ISEGS North plot 21 of 53 samples were from translocated animals, and at our Silver State plot 11 of 21 were from translocated animals.

Molecular methods

Genetic (blood) samples were collected using subcarapacial venipuncture (Hernandez-Divers et al. 2002). Samples collected in the field were stored by placing one drop on a Fast Technology for Analysis (FTA) card (Whatman GE Healthcare Life Sciences); each card was air dried and stored individually in a paper coin envelope. All extractions were performed with the DNeasy Blood and Tissue Kit (Qiagen) using the manufacturer's instructions, with two minor changes: samples were incubated at 70 °C for 10 min after the addition of Buffer AL, and the elution step was
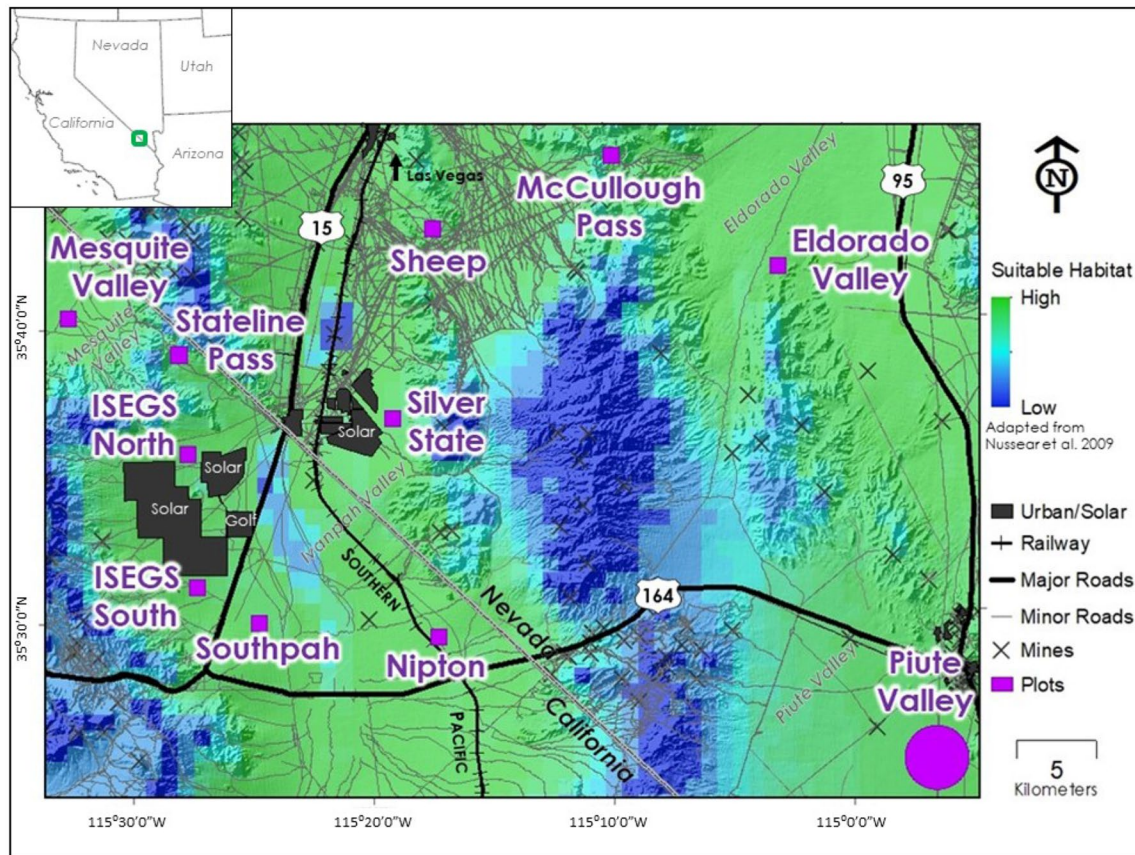
Fig. 1 Map of survey locations centering on the Ivanpah valley, along the California/Nevada border. The 1 km² plots are indicated by purple squares; the additional sample location is indicated by a purple circle that represents the area from which samples were collected. Developments on the landscape include urban/solar (areas where habitat has been lost and/or fenced to exclude tortoises), major roads/railway (linear barriers to connectivity), minor/dirt roads and mines (representing habitat degradation)

Table 1 Genetic diversity statistics for *Gopherus agassizii* by survey location: sample size (N), allelic richness (Ar), mean observed heterozygosity (H_O), mean expected heterozygosity (H_E), inbreeding coefficient (F), and relatedness coefficient (r_{QG}) with 95% confidence interval

Location	N	Ar	H_O	H_E	F	r_{QG}
Mesquite valley	12	5.6	0.796	0.757	0.120	0.061 (0.031–0.091)
Stateline pass	25	6.0	0.816	0.790	0.105	0.019 (0.003–0.035)
ISEGS North	53	6.3	0.805	0.796	0.115	0.013 (0.007–0.020)
ISEGS South	10	5.4	0.838	0.740	0.074	0.073 (0.030–0.121)
Southpah	39	6.2	0.790	0.787	0.118	0.031 (0.022–0.040)
Nipton	32	6.1	0.811	0.783	0.105	0.033 (0.023–0.043)
Silver State	21	6.1	0.786	0.779	0.118	0.021 (0.005–0.034)
Sheep	21	6.0	0.788	0.780	0.118	0.037 (0.022–0.052)
McCullough pass	47	5.8	0.772	0.771	0.128	0.060 (0.052–0.069)
Eldorado valley	15	5.7	0.797	0.770	0.126	0.051 (0.027–0.076)
Piute valley	24	6.2	0.781	0.788	0.124	0.036 (0.022–0.049)
Overall	299	5.9	0.795	0.811	0.115	− 0.003

performed twice with an elution volume of 100 µl for a total final volume of 200 µl. We amplified 20 variable microsatellite loci previously developed for tortoises (Edwards et al. 2003; Hagerty et al. 2008; Schwartz et al. 2003). Amplification of microsatellite loci was performed in 10 µl reactions

with 4 µl Multiplex PCR Plus cocktail (Qiagen), 0.8 µl primer mix, 3.2 µl water, and 2 µl DNA diluted to ≤ 4 ng/µl. Thermocycler conditions were set at 95 °C for 5 min, then 30 cycles were performed with 30 s denaturing at 95 °C, 3 min annealing at 56 °C, and 45 s elongation at 72 °C, finishing

with a 30 min final elongation at 68 °C. PCR product (1 µl) was aliquoted into 10.5 µl HiDi formamide (Thermofisher) with 0.5 µl LIZ500 (Thermofisher) and submitted to Eton Bioscience (San Diego, CA) for genotyping. Each round of genotyping included negative controls to check for contamination. Approximately 10% of the samples were amplified and genotyped twice to assess mistyping and dropout rates. We scored raw data in GeneMarker v.1.90 (SoftGenetics), binned alleles using R 3.5.3 (R Core Team 2019) *MsatAllele* v.1.04 (Alberto 2009), and checked for null alleles with the R package *PopGenReport* v.3.0.0 (Adamack and Gruber 2014). Exact tests for Hardy–Weinberg equilibrium and linkage disequilibrium among microsatellite loci were implemented in GenePop v.4.5 (Rousset 2008) with a Bonferroni correction. Microsatellite loci with inconsistent amplification were not included in the dataset.

Genetic diversity

We assessed standard measures of genetic diversity for the entire dataset and by survey location. We calculated the number of alleles per locus, observed (H_o) and expected heterozygosity (H_e), coefficient of inbreeding (F) using *adegenet* v.2.1.1 (Jombart 2008) in R, allelic richness (*Ar*, Adamack and Gruber 2014), and mean relatedness coefficients (r_{QG}, Queller and Goodnight 1989) with 95% confidence intervals in GenAlEx 6.5 (Peakall and Smouse 2012). Deviations of H_o from theoretical expectations were evaluated using a Bartlett test for equal variance across microsatellite loci to assess homoscedasticity and a paired t-test to compare the observed and expected population means.

Population structure

Genetic structure was evaluated with multiple analytical methods, as well as hierarchically, starting with the entire dataset, then using subsets of the samples based on the genetic clusters detected. We used a Bayesian clustering analysis to infer population structure (Structure v.2.3.4, Pritchard et al. 2000). We ran the admixture model, which assumes each individual draws some fraction of its genome from each of *K* population clusters, with correlated allele frequencies, because allele frequencies are expected to be similar for our survey locations. We estimated the probability of *K* = 1–10 using ten replicate runs of 1,000,000 Markov Chain Monte-Carlo iterations following a burn in of 500,000. We implemented Structure for the entire dataset with sampling location as a prior, which can improve model output when genetic structure is weak (Hubisz et al. 2009). We calculated the mean log probability of the data (*Pr(X|K)* in Pritchard et al. 2000), and second order rate of change (ΔK in Evanno et al. 2005). Structure results were visualized using *PopHelper* in R (Francis 2017).

We also employed a spatial principal components analysis (sPCA) to further investigate cryptic genetic patterns that can result from IBD (Jombart et al. 2008). This multivariate method differs from the previously described Structure analysis by maximizing the variance in individual allele frequencies while accounting for spatial autocorrelation and assuming no population model (Jombart et al. 2008; Prunier et al. 2014). The genetic patterns found using sPCA were compared to 999 randomized Monte-Carlo permutations to test whether observed structure differs from the distribution of random expectations. Eigenvalues are generated through Monte-Carlo simulations and represent both genetic diversity (variance) and spatial structure (spatial autocorrelation as measured by global Moran's *I*). We performed sPCA analyses with the hierarchical approach described above. A spatially explicit connection network of relative neighbors with a genetic distance matrix was created. The product of the variance and the spatial autocorrelation was separated into positive, null, and negative scores representing the magnitude of global (positive) and local (negative) autocorrelation. Global patterns indicate spatial groups or clines, while local patterns detect stronger genetic differences among neighbors than expected among random pairs (Jombart et al. 2008).

We evaluated population genetic differentiation (F_{ST}) between survey locations and between inferred genetic clusters. Linearized F_{ST} ($F_{ST}/(1 − F_{ST})$; Rousset 1997) was calculated between survey locations using Analysis of Molecular Variance (AMOVA, Peakall and Smouse 2012), with an allelic distance matrix and using 999 permutations. We calculated *p*-values for pairwise comparisons of F_{ST} (Jombart 2008) between detected genetic clusters using Weir and Cockerham's '*θ*' (1984). We also tested for historical patterns of IBD using Mantel tests (Mantel 1967), with 999 Monte-Carlo permutations, using genetic and geographic Euclidean distance matrices (Jombart 2008) at multiple levels: (1) among all individuals using each tortoise location; (2) grouping individuals by survey location using the mean locations for each plot; (3) among any genetic clusters detected using mean locations for each cluster; (4) within each distinct genetic cluster independently using tortoise location; and 5) within each individual survey plot using tortoise location.

Model comparisons for population structure

To examine whether recent anthropogenic influences are associated with measurable effects on connectivity among our survey sites, we examined the correlation between pairwise genetic distances using linearized F_{ST} and cost distances reflecting the influence of natural and anthropogenic features on resistance to movement. Comparisons by plot location were analyzed using maximum likelihood

population effects (MLPE) models in the package *ResistanceGA* v.4.0-14 (Peterman 2018) in R. Analyses were limited to individuals genotyped in the ten 1 km² survey sites within and immediately adjacent to the Ivanpah valley (using genetic data from 275 tortoises), excluding the Piute valley location due to the disproportionately large distance from other study sites. Cost surfaces hypothesized to have influenced genetic connectivity over longer time periods (e.g. spanning generations) were created representing: (1) Euclidian distance, where a raster was populated with a single value of no resistance to represent IBD; (2) the inverse of modeled desert tortoise habitat (1—modeled habitat suitability values from Nussear et al. 2009, as in Hagerty et al. 2011) to represent isolation-by-resistance (IBR); (3) the log distance from the interstate rescaled from 0 to 1; and (4) the log distance from the railway rescaled from 0 to 1 (Fig. 2). The log distance surfaces were rescaled from 0 to 1, such that the cost associated with the linear features would not be weighted higher than areas of non-habitat, and the log was used to reflect the more localized effects of these linear features (Fig. 2). While other roads exist in the study area, these are primarily unpaved roads that have accrued more recently. The additional roads exist at a scale that bisects our smaller scale study plots, and while movement may be reduced by these roads (Sadoti et al. 2017), our study animals cross these road networks far more frequently than the larger barriers presented by the interstate highway (no documented crossings) or the railway (two tortoises from the Nipton plot have passed through culverts under the railway once since 2015), which limit the dispersal ability of tortoises (Edwards et al. 2004; Rautsaw et al. 2018).

The relationship of the linearized F_{ST} matrix and cost surfaces was analyzed using genetic algorithms to optimize MLPE analyses. We conducted analyses for all single surfaces individually (using the SS_optim function), as well as all combinations of two, and three surfaces, as well as the full model including all four resistance layers (where multiple surface models were analyzed using the MS_optim function, Peterman pers comm.). The models
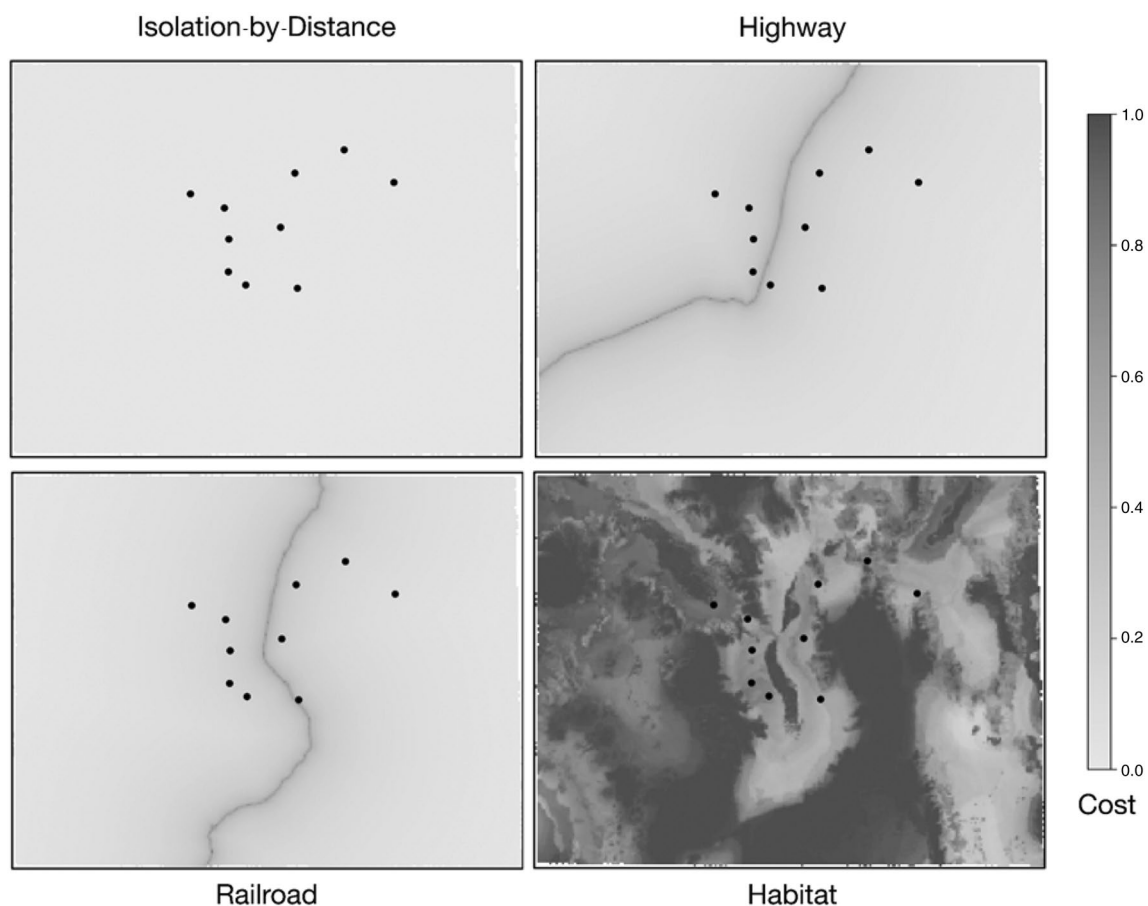
Fig. 2 Cost surfaces used in maximum likelihood population effects (MLPE) models. (Top left) Single value of 0.001 to represent a cost free (Euclidean distance) surface governed only by isolation-by-distance. (Top right) Log of distance to I-15 highway scaled from 0 to 1. (Bottom left) Log of distance to the Southern Pacific Railroad scaled from 0 to 1. (Bottom right) Habitat resistance taken as the inverse of modeled desert tortoise habitat from Nussear et al. 2009 (1—modeled habitat suitability values)

were combined and ranked by Akaike's information criterion (AIC) to identify the best performing model (Table 4).

Relatedness

To detect the spatial scale of movement over the past 1–2 generations, we used the maximum likelihood pedigree reconstruction implemented in COLONY (Jones and Wang 2010) to estimate first and second order relationships among all individuals, and then mapped the geographic locations of first and second order relative pairs. For COLONY runs, we assumed inbreeding with male and female polygamy, and coded individuals as follows: all adult females ($n = 85$) as candidates for maternity, all adult males ($n = 131$) as candidates for paternity, and all individuals as potential offspring. Simulations were run using full-likelihood, medium precision, medium run length, no sibship scaling, and no priors. To avoid excluding pairs based on a single allele, we set the false allele rate to 0.0001. We conducted three independent COLONY analyses varying the random number seed for each. Additionally, the number of runs within each analysis was set to three, increasing the odds of finding the best configurations with maximum likelihood in each COLONY run. We assigned the expected probability of detecting a mother or father to 25% based on the ratios of newly found tortoises on previously surveyed plots using mark-recapture techniques. Log likelihood plots for each run were examined for convergence. Data from all three runs were consolidated and only pairs found in common in all three runs were considered. Of these, only dyads with ≥ 80% probability of relatedness in each of the three runs were kept (Warner et al. 2016).

Results

Data quality

We genotyped 299 samples at 20 microsatellite loci. There was indication of null alleles at two loci (GOA4 and GP61). As the evidence for null alleles at these loci was not consistent across multiple sampling locations these loci were retained. All loci conformed to Hardy–Weinberg equilibrium following Bonferroni correction (corrected p-value = 0.003 for $\alpha = 0.05$), with none consistently in linkage disequilibrium among locations. The error rate, determined by repeat genotyping, in the 20 microsatellite loci retained was 0.77% and all errors were caused by allelic dropout. Primers and locus information can be found in Table S1 (Supporting Information), and raw data are available as a USGS Data Release (https://doi.org/10.5066/P90LIQRI).

Genetic diversity

We summarized estimates of genetic diversity and characteristics for the survey locations and overall based on the microsatellite genotype information (Table 1). The number of alleles per locus ranged from 4 to 43 ($\bar{x} = 15.9$) across loci, and Ar ranged from 5.4 to 6.3 across survey locations (ISEGS South and ISEGS North; the two plots adjacent to a utility-scale solar facility with previously contiguous habitat). Expected heterozygosity was lowest at ISEGS South ($H_E = 0.740$) and highest at ISEGS North ($H_E = 0.796$). There were significant differences in mean H_E between: Mesquite valley and three survey locations (ISEGS North p-value = 0.030; Stateline pass p-value = 0.033; Piute valley p-value = 0.020); ISEGS South and five survey locations (Southpah p-value = 0.020; Nipton p-value = 0.045; Silver State p-value = 0.048; Sheep p-value = 0.048; Piute valley p-value = 0.005); ISEGS North and McCullough pass (p-value = 0.004). The inbreeding coefficient was lowest at ISEGS South ($F = 0.074$) and highest at McCullough pass. ($F = 0.128$). Relatedness r_{QG} ranged from 0.013 at ISEGS North to 0.073 at nearby ISEGS South.

Population structure

STRUCTURE results suggested different numbers of genetic clusters depending on the method used to determine the optimal K value ($Pr(X|K) = 10$ and $\Delta K = 3$; map of assignment to cluster by sampling location Fig. 3a, barplot Fig. 3b). Because $Pr(X|K)$ may overestimate genetic clusters when there are patterns of IBD, we report ΔK (except where $Pr(X|K) = 1$) which may more accurately detect clusters when spatial autocorrelation is present in continuous populations (Evanno et al. 2005; Schwartz and McKelvey 2009). Individuals from McCullough pass formed a unique cluster, while Eldorado valley and Piute valley individuals clustered together. The largest genetic cluster was formed by eight of the eleven plots, and included all survey locations in Ivanpah valley, Stateline pass, and Mesquite valley. Hierarchical analysis of the three main clusters detected additional structure with no strong geographic clustering (Fig. 3c).

Our sPCA results shed additional light on genetic patterns in the study area, finding Ivanpah valley and Mesquite valley differing from locations to the east (McCullough pass, Eldorado valley, Piute valley) using the full dataset (global $r = 0.011$, p-value = 0.011; Fig. 4a). Hierarchical analysis identified additional structure within Ivanpah valley along the east and west sides of the valley, roughly corresponding with linear barriers, that include a 140 year old railway, and 60 + year old highway corridor, which parallel one another across much of our study area (global $r = 0.009$, p-value = 0.031; Fig. 4b). Positive spatial autocorrelation was detected among individuals within the Eldorado/Piute valley cluster
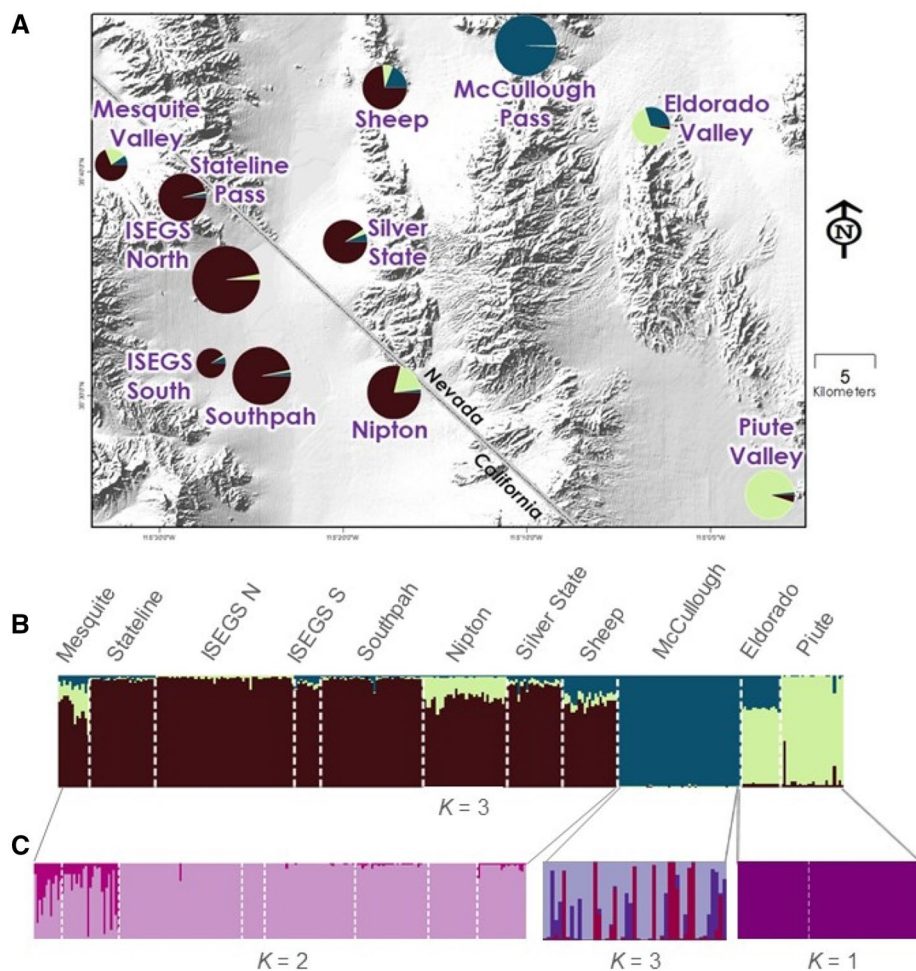
Fig. 3 STRUCTURE results with sample location used as a prior. Number of clusters reported using the Evanno method (ΔK), except where the Pritchard method found one cluster ($Pr(X|K)$). **a** Pie charts representing the proportion of each site's genetic background coming from three main genetic clusters identified prior to hierarchical analysis ($\Delta K = 3$) for the entire study area with sample size ranges from 10 for the smallest pie to 53 for the largest, colors correspond to those found in B. **b** STRUCTURE barplot with each vertical bar representing an individual, color scheme matching that found in A and represent-ing the proportion of each individual's genetic background coming from the three main clusters, with barplots organized generally from the farthest northwest location (Mesquite valley) through a mountain pass (Stateline pass) into Ivanpah valley plots, through a mountain pass (McCullough pass) to farthest southeast location (Piute valley). **c** Results of hierarchical analysis revealing additional structure (Ivanpah/Mesquite valley $\Delta K = 2$, McCullough pass $\Delta K = 3$, Eldorado/Piute valley $\Delta K = 2$, $Pr(X|K) = 1$)

($r = 0.036$, p-value $= 0.033$) and within McCullough pass ($r = 0.049$, p-value $= 0.001$). Evaluation of individuals within Mesquite valley and Stateline pass found no spatial autocorrelation (global $r = 0.036$, p-value $= 0.310$; local $r = 0.361$, p-value $= 0.206$).

Linearized F_{ST} values among sampling locations ranged from 0.003 to 0.040 (ISEGS North to Silver State and Mesquite valley to Eldorado valley; Table 2A). Based on standard permutation across the full dataset genetic differentiation was significant (linearized $F_{ST} = 0.022$, p-value $= 0.001$). All comparisons with McCullough pass and other survey locations were significant, including adjacent plot locations. Significant genetic structure was also found between Mesquite valley and all other locations, with the exception of

neighboring Stateline pass. Eldorado and Piute valleys differed significantly from all other plots, except each other, and Piute valley from one plot in the Ivanpah valley cluster (Silver State). When locations were combined to correspond to the three main inferred genetic clusters, pairwise F_{ST} values ranged from 0.018 to 0.028, with each comparison statistically significant (Table 2B).

We detected IBD across the study area at all levels tested: among all individuals using each tortoise location, grouping individuals by survey location and using the mean geographic location of all individuals in each survey area, among the three genetic clusters (Ivanpah/Mesquite valley, McCullough pass, and Eldorado/Piute valley) using the mean of individuals associated with each cluster as the

Fig. 4 sPCA results of the summary of genetic variability and spatial structure, where white and black squares represent the product of the variance and spatial autocorrelation as scores that are positioned by spatial coordinates. Square size indicates the magnitude of the variance. Negative scores represent local patterns of spatial autocorrelation and positive scores represent global patterns. **a** Map of the entire study area showing a pattern of genetic variability between Ivanpah valley and Mesquite valley and locations to the east, with potential integration at the Sheep and Nipton plots; and **b** Map of Ivanpah valley revealing an east–west genetic pattern
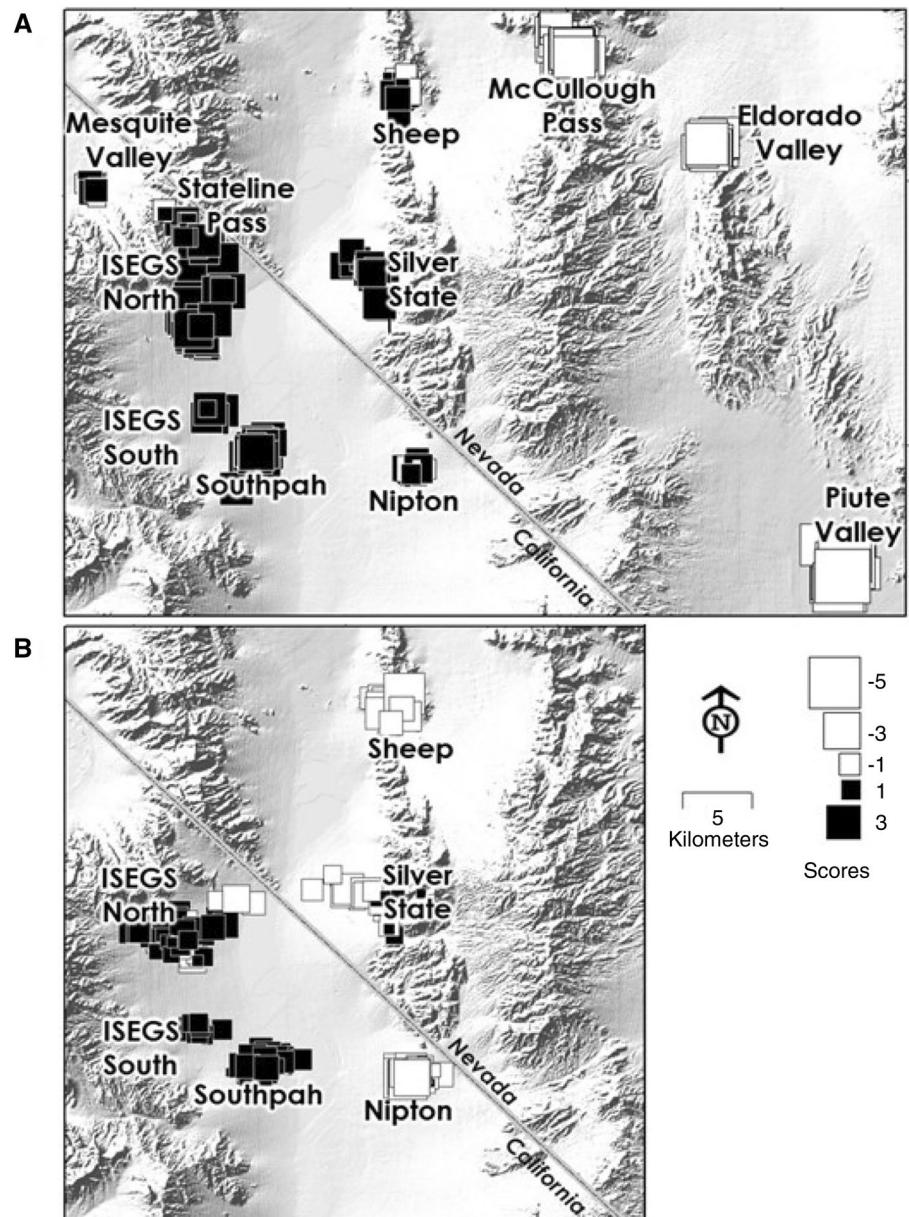
geographic location, and within each distinct genetic cluster. Additionally, a correlation between genetic and geographic distances was found within four individual survey plots (Southpah, McCullough pass, Eldorado valley, and Piute valley; with McCullough pass also constituting a genetic cluster; Table 3). Of the six survey plots where no IBD was detected, three were recipients of translocated tortoises (ISEGS North and Silver State).

Model comparisons for population structure

Model comparisons suggested that the distribution of suitable habitat, the Southern Pacific Railroad, and I-15 were associated with genetic differentiation. The two highest

ranking models both included habitat (as the inverse of the habitat suitability model), which effectively indicates IBR, and is consistent with the findings of Hagerty et al. (2011). Both models also included the Southern Pacific Railroad. These two models differed by their inclusion of I-15, which connects Las Vegas, Nevada and Los Angeles, California. The interstate parallels the railway for most of our study area, and thus likely represents an additive barrier that would result in similar genetic patterns. The highest ranking model (with the lowest AIC) included habitat distance, the interstate, and the railway (Fig. 2). This model had a weight of 53%, and an R^2 value of 0.90 (Table 4). A second model including only the habitat and the railway had similar performance, with a ΔAIC of only 0.2, a weight of 47%, and an

Table 2 Pairwise F_{ST} values (lower) with corresponding p-values (upper)

A	MV	SL	IN	IS	SP	NI	SS	SH	MC	EV	PV
MV	–	0.092	0.001*	0.002*	0.001*	0.001*	0.001*	0.005*	0.002*	0.001*	0.001*
SL	0.013	–	0.400	0.003*	0.006*	0.063	0.397	0.014*	0.013*	0.003*	0.012*
IN	0.028	0.011	–	0.087	0.569	0.581	0.051	0.029*	0.001*	0.010*	0.017*
IS	0.032	0.028	0.018	–	0.020*	0.307	0.193	0.005*	0.009*	0.001*	0.001*
SP	0.035	0.021	0.007	0.024	–	0.495	0.237	0.002*	0.003*	0.001*	0.001*
NI	0.036	0.016	0.012	0.017	0.013	–	0.227	0.009*	0.009*	0.001*	0.015*
SS	0.028	0.012	0.003	0.018	0.014	0.015	–	0.123	0.026*	0.035*	0.101
SH	0.025	0.022	0.019	0.028	0.029	0.025	0.017	–	0.004*	0.001*	0.004*
MC	0.033	0.024	0.025	0.027	0.027	0.025	0.023	0.028	–	0.003*	0.001*
EV	0.040	0.030	0.027	0.041	0.033	0.034	0.022	0.035	0.033	–	0.517
PV	0.037	0.025	0.027	0.039	0.033	0.027	0.021	0.033	0.035	0.019	–

B		IVMV		MC		EVPV
IVMV		–		0.001*		0.001*
MC		0.018		–		0.001*
EVPV		0.018		0.028		–

(A) Comparisons between survey locations: Mesquite valley (MV), Stateline pass (SL), ISEGS North (IN), ISEGS South (IS), Southpah (SP), Nipton (NI), Silver State (SS), Sheep (SH), McCullough pass (MC), Eldorado valley (EV), and Piute valley (PV). (B) Comparisons between the three main genetic clusters: Ivanpah/Mesquite valley (IVMV), McCullough pass (MC), and Eldorado/Piute valley (EVPV). Significant p-values after Bonferroni correction are denoted with an asterisk

Table 3 Isolation-by-distance correlation coefficient values (Pearson's r) using individual geographic locations; by survey location using mean tortoise locations of each survey area; by inferred genetic cluster using mean tortoise locations of each cluster; within each of the three genetic clusters individually: Ivanpah/Mesquite valley (IVMV), McCullough pass (MC), and Eldorado/Piute valley (EVPV); and within each discrete survey location: Mesquite valley (MV), Stateline pass (SL), ISEGS North (IN), ISEGS South (IS), Southpah (SP), Nipton (NI), Silver State (SS), Sheep (SH), Eldorado valley (EV), and Piute valley (PV)

	r	DF	p-value
By individual	0.158	298	0.001
By survey location	0.450	10	0.027
By genetic cluster	0.791	3	0.039
Within IV	0.116	212	0.001
Within MC	0.230	46	0.001
Within EVPV	0.124	38	0.002
Within MV	0.281	11	0.127
Within SL	0.084	24	0.187
Within IN	− 0.002	52	0.512
Within IS	− 0.028	9	0.572
Within SP	0.190	38	0.010
Within NI	0.007	31	0.469
Within SS	0.036	20	0.381
Within SH	0.072	20	0.286
Within EV	0.204	14	0.040
Within PV	0.134	23	0.040

R^2 value of 0.90. All other models had lower performance. The full model included all cost surfaces and ranked 3rd, with a ΔAIC > 14.0, and a model weight of approximately 0. Models including habitat performed better than Euclidean distance alone. All models considered ranked well above the null model, where only the intercept was calculated (Table 4).

Relatedness

Evaluating relationship category through pedigree analysis indicated evidence of first order relationships within eight (Stateline pass, ISEGS North, ISEGS South, Southpah, Nipton, McCullough pass, Eldorado valley, and Piute valley) of the eleven survey locations. The total number of first order relationships discovered within survey locations was 63. All but one pair of relatives were found within the same 1 km² plots. There was one first order relationship between a translocated tortoise from ISEGS North and a resident tortoise from ISEGS South. Because they may have been geographically closer prior to facility construction in 2010, this relationship may not necessarily be due to a natural dispersal event. There were 110 unique second order relationships in the survey area, with 59 of these occurring within the same plot and 51 between plots (Table 5), suggesting tortoise movement on multi-generational time scales has occurred throughout the study area. The Euclidean distances between second order relatives found at different survey locations ranged from 5 to 60 km ($\bar{x}= 22$ km).

Table 4 Maximum likelihood population effects (MLPE) model summaries for each model considered (Model); Log likelihood (Log Lik); the number of parameters (k); resulting Akaike's information criterion score (AIC); change in AIC from the highest ranking model (ΔAIC); model weights (W) as calculated per Anderson and Burnham (2004); and R^2

Model	Log Lik	k	AIC	ΔAIC	W	R^2
Railway + interstate + habitat	159.79	10	− 311.58	0.00	0.53	0.90
Railway + habitat	159.68	7	− 311.37	0.21	0.47	0.90
Railway + interstate + Euclidian distance	152.60	10	− 297.21	14.37	0.00	0.87
Railway + Euclidian distance	152.30	7	− 296.59	14.99	0.00	0.86
Railway	152.16	4	− 296.33	15.25	0.00	0.86
Interstate + Euclidian distance	148.95	7	− 289.89	21.69	0.00	0.84
Interstate + habitat	148.13	7	− 288.26	23.32	0.00	0.80
Interstate	146.57	4	− 285.13	26.45	0.00	0.77
Euclidian distance	146.48	2	− 284.96	26.62	0.00	0.76
Habitat	146.48	4	− 284.96	26.62	0.00	0.76
Null	114.68	1	− 223.35	88.23	0.00	0.00

Table 5 Total number of second order relationships detected using the program COLONY among *Gopherus agassizii* in the Ivanpah valley area: Mesquite valley (MV), Stateline pass (SL), ISEGS North (IN), ISEGS South (IS), Southpah (SP), Nipton (NI), Silver State (SS), Sheep (SH), McCullough pass (MC), Eldorado valley (EV), and Piute valley (PV)

	MV	SL	IN	IS	SP	NI	SS	SH	MC	EV	PV
MV	1										
SL	2	4									
IN	0	1	12								
IS	0	0	3	0							
SP	1	0	4	0	9						
NI	0	3	5	1	1	10					
SS	1	1	0	0	1	1	0				
SH	1	0	0	0	1	0	4	3			
MC	0	0	0	0	3	1	1	2	10		
EV	0	0	0	0	0	0	0	0	3	5	
PV	1	0	2	0	0	4	1	0	0	2	5

Discussion

Mountain passes provide connectivity among valleys

The benefits of connectivity include increased exchange of individuals between habitats, with positive impacts on community interactions (Tewksbury et al. 2002) and improved population size and persistence (Henein and Merriam 1990). The Mojave desert tortoise has long been associated with valley bottoms and bajadas, or coalescing alluvial fans (Germano et al. 1994), and while tortoises are indeed prevalent in these habitats, we also found strong support that mountain passes are occupied by tortoises and provide connectivity between valleys. Connectivity relies on individual or genetic exchange between substantive habitat areas that serve as avenues for dispersal, travel, reproduction, recolonization, and genetic interchange (Beier and Loe 1992). Mountain passes fit this description by allowing gene flow and serving as bridges between neighboring valleys (in comparison with more rugged mountainous areas), and by providing variable levels of connectivity. McCullough pass shows evidence of admixture with Ivanpah valley, Eldorado valley, and Piute valley, and all plots share alleles and/or second order relatives, indicating recent gene flow; albeit at lower levels than Stateline pass. This is possibly due to differences in terrain, available habitat, local population sizes, variation in movement patterns, and/or behavioral differences. Long-term radio-telemetry studies currently being conducted in these areas indicate tortoises are resident in mountain passes, making it likely that these passes also serve as stepping-stone habitats. Mountain pass populations likely contribute to sustained connectivity over generations rather than only intermittent contributions from long distance movements of individuals through passes. Although anthropogenic disturbance is present in the mountain pass locations, it is largely comprised of unpaved roads, which have not presented an absolute barrier to our study animals. However, recent developments on valley floors have the potential to fragment populations. The results of this research provide valuable insights into the significance of mountain passes for supporting landscape connectivity and highlight their importance for conservation planning.

Weak but detectable population structure with gene flow

Bayesian cluster analyses identified three main historical genetic populations: (1) Ivanpah/Mesquite valley through Stateline pass, (2) McCullough pass, and (3) Eldorado/Piute valley. Genetic patterns, revealed by sPCA, differed east to west within the Ivanpah valley. Genetic structure and relatedness within and between valleys indicate that tortoises occupying this central portion of the Mojave Desert do not represent a single panmictic population. Low levels of genetic differentiation within study plots point to recent exchanges of individuals, which is further supported by the presence of second order relationships among study plots, implying that tortoises may require multi-generational dispersal events to maintain connectivity. Our findings reveal weak but detectable genetic structure consistent with the hypothesis that historical habitat was largely continuous and characterized by interconnected valleys, with relatively few restrictive points (e.g., narrow passes between adjacent valleys).

With large areas of connected habitat and fairly contiguous populations, evidence supports historically high levels of gene flow and low levels of genetic differentiation throughout the range of the species (Murphy et al. 2007; Hagerty and Tracy 2010). Our results demonstrate that tortoises in and around the Ivanpah valley were likely not genetically or geographically isolated in the recent past, and that this could be a regionally important zone for connectivity. This is supported by the work of Hagerty et al. (2011) where a high probability of genetic connectivity was predicted in and around Ivanpah valley. These findings make historically connected valleys, like Ivanpah, especially significant for Mojave desert tortoise connectivity given that much of the habitat in the central portion of the range has been lost to the city of Las Vegas, a large urban area where little connective habitat for tortoises remains.

Genetic connectivity in light of habitat loss

Although it appears that the greater Las Vegas valley once served as connective habitat in the central portion of the species range (Britten et al. 1997; Hagerty and Tracy 2010; Nussear et al. 2009), it has been replaced by a large metropolitan area with incumbent infrastructure that represents a barrier to movement and gene flow for desert tortoises. The urbanization of Las Vegas valley elevates the need to preserve desert tortoise population connectivity in adjacent valleys, including Ivanpah, which are now more vital than ever as connections for tortoises from California through Nevada and Utah. However, development pressures extend well beyond the city of Las Vegas to a myriad of human land use practices that result in permanent habitat loss and

fragmentation whereby small actions and influences aggregate into larger actions and effects on the landscape that are not explicitly acknowledged; the "tragedy of fragmentation" (Goble 2009).

Although our results support historical as well as relatively recent gene flow for the Mojave desert tortoise, MLPE analysis revealed a reduction in genetic connectivity within the Ivanpah valley to the east and west of two linear barriers: the Southern Pacific Railroad and I-15. The signal caused by the railway is likely stronger than that of I-15 because this feature has been on the landscape longer, for approximately 140 years, or around seven tortoise generations. Interstate-15 runs nearly parallel to the railway and has presented a potential barrier to tortoises for 50–80 years, or around four tortoise generations. This could foreshadow increased impacts to Mojave desert tortoises from more recently established barriers and large scale developments in the next several tortoise generations, as the lag time to detect genetic changes is measured in generations. For example, most utility-scale solar developments have been on the landscape for less than one tortoise generation, but their potential for isolating tortoises can be seen in the amount of habitat that has been lost in areas that once supported contiguous populations.

While the broader long-term impacts of development will likely reduce connectivity for desert tortoises, translocation of animals from development areas may result in subtle genetic effects that appear to increase genetic diversity and admixture initially. However, this signature is expected to be transient. In this research, the ISEGS North and ISEGS South plots were located on either end of a utility-scale solar facility (ISEGS), and 40% of our samples from ISEGS North were from individuals that were translocated from within the footprint of that facility. This likely contributed to substantial differences between these two plots including: sample size (ISEGS South $n = 10$, ISEGS North $n = 53$), the range of genetic diversity (ISEGS South $Ar = 5.4$, ISEGS North $Ar = 6.3$), relatedness (ISEGS South $r_{QG} = 0.073$, ISEGS North $r_{QG} = 0.013$), and the fact that these plots contained the only first order relationship found among plots.

Management implications

Mojave desert tortoise recovery actions include protecting existing populations and habitat by conserving intact landscapes and connecting functional habitat (USFWS 2011). However, tortoise habitat continues to be lost with connections between populations at risk from development. Within the Ivanpah valley recent habitat loss has been caused by a highly traveled interstate, a railway, a network of dirt roads and off-highway vehicle tracks, towns, a golf course, mining operations, and several large solar facilities. Unfortunately, we do not have a baseline for the effects of these features on desert tortoise connectivity

and gene flow, because genetic studies were not conducted prior to construction (USFWS 2011). Taken together these recent developments have the potential to continue fragmenting habitat and reduce connectivity within current populations. Additionally, our top two MLPE models indicated a significant influence of the Southern Pacific Railroad and/or I-15 on genetic distances, indicating genetic connectivity across these barriers could continue to decrease through time. If connections are sufficiently restricted, we may see increased isolation of the populations residing in Ivanpah valley extending to Eldorado valley, Mesquite valley, Pahrump, and beyond. Though not the focus of this study, other valleys in the Mojave Desert, including Eldorado and Moapa valleys, are experiencing similar habitat loss and fragmentation to that noted in Ivanpah due to roads and solar developments in tortoise habitat, potentially further exacerbating threats to connectivity.

This work provides a basis for determining management actions that could conserve connected tracts of functional habitat between existing blocks of protected land and can be used as a foundation for continued research efforts moving forward. Prioritizing connectivity corridors for the Mojave desert tortoise across southern California and southern Nevada through northwestern Arizona, and southeastern Utah for protection could prevent further isolation of populations that are currently connected via suitable undisturbed areas (Dickson et al. 2016). Alternatively, if habitat quality continues to decline and suitable connectivity is lost, population persistence and recovery may necessitate the creation of corridors through barriers (i.e. such as culverts found under some portions of major highways and railways, *but see* Rautsaw et al. 2018). For tortoises to persist on the landscape a functional ecosystem is required, which has added benefits for other species in the region (Brooks 2000; Averill-Murray et al. 2012). More generally, populations in large and well connected networks are less threatened by extinction (Hanski 1998), therefore, future conservation management plans could benefit by exploring development scenarios that minimize loss of desert tortoise habitat.

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