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TECHNICAL REPORT

Phase III Desert Tortoise Genetic Connectivity and Diversity Report

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The speed and intensity of contemporary anthropogenic change calls into question the capacity of extant species to respond and cope with environmental impacts. One possible response may be evolutionary adaptation; however, uncertainties around adaptive divergence and gene flow limit our abilities to predict their importance for the persistence of sensitive species in fragmented landscapes. The Mojave Desert Tortoise (Gopherus agassizii) is native to the Mojave Desert of North America that has been strongly impacted by habitat fragmentation and human development, and is listed as a threatened species under the ESA. Their slow life history is associated with relatively slow adaptive divergence, casting doubt as to their relative capacity to respond adaptively to environmental change. In this study, we used landscape and population genomic toolsets to explore the interplay between adaptive divergence and gene flow in the Mojave Desert Tortoise. Using genetic samples collected across the species' range, we used genotype-environment analyses (GEAs), population genomic analyses, and analyses of population connectivity to characterize neutral and adaptive genetic divergence, identify the predominant environmental gradients driving divergence, identify the spatial scales at which gene flow and dispersal occur, and characterize how adaptive divergence is related to quantitative estimates of gene flow. We produced a dataset of 19,131 single nucleotide polymorphisms (SNPs) from 697 individuals, with an overall missing data percentage of 24.5%. We found an average observed heterozygosity of 0.1826 range-wide, with a global genetic differentiation value of 0.1471. The best supported clustering solution was five clusters, similar to previous studies of genetic structure in this species, that corresponded to the north, east, south, west, and central Mojave Desert.

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Abstract

The speed and intensity of contemporary anthropogenic change calls into question the capacity of extant species to respond and cope with environmental impacts. One possible response may be evolutionary adaptation; however, uncertainties around adaptive divergence and gene flow limit our abilities to predict their importance for the persistence of sensitive species in fragmented landscapes. The Mojave Desert Tortoise (*Gopherus agassizii*) is native to the Mojave Desert of North America that has been strongly impacted by habitat fragmentation and human development, and is listed as a threatened species under the ESA. Their slow life history is associated with relatively slow adaptive divergence, casting doubt as to their relative capacity to respond adaptively to environmental change.In this study, we used landscape and population genomic toolsets to explore the interplay between adaptive divergence and gene flow in the Mojave Desert Tortoise. Using genetic samples collected across the species' range, we used genotype-environment analyses (GEAs), population genomic analyses, and analyses of population connectivity to characterize neutral and adaptive genetic divergence, identify the predominant environmental gradients driving divergence, identify the spatial scales at which gene flow and dispersal occur, and characterize how adaptive divergence is related to quantitative estimates of gene flow. We produced a dataset of 19,131 single nucleotide polymorphisms (SNPs) from 697 individuals, with an overall missing data percentage of 24.5%. We found an average observed heterozygosity of 0.1826 range-wide, with a global genetic differentiation value of 0.1471. The best supported clustering solution was five clusters, similar to previous studies of genetic structure in this species, that corresponded to the north, east, south, west, and central Mojave Desert.

Our GEA analyses produced a dataset of 632 putatively adaptive SNPs and revealed coherent environmental gradients that contrasted hotter, drier regions from cooler, wetter areas, and sandier edaphic conditions from those associated with silt and clay. Our results indicate that temperature, along with edaphic conditions, are likely very important factors for continued persistence of Mojave Desert Tortoises. We found patterns of isolation-by-distance along with unexpectedly high rates of generational migration associated with the central cluster through the east and north clusters. Linear regressions of heterozygosity and inter-individual genetic distance were significant for both the adaptive and neutral datasets, with the adaptive dataset exhibiting a marginally steeper slope for these relationships, indicating that higher levels of gene flow correlate with particularly pronounced adaptive genetic variation. We found evidence for stronger neutral genetic diversity in the central Mojave Desert, where genetic divergence is low and gene flow is high, along with strong adaptive genetic diversity in the northeast Mojave, where genetic divergence and migration rates are high. These results highlight that tortoise habitat in the central Mojave is likely of great importance to genetic connectivity, serving as critical linkage areas across the range, while areas closer to the edge of the range, especially the northeast, west, and to a lesser extent south, may be of key importance to adaptive potential for the species. Our results illustrate that both adaptive divergence and gene flow can be high for tortoises, and that maintaining a connected network, from the core of the species range through

to adaptive edge, is likely critical to preserving the species under current and future environmental conditions.

Introduction

Anthropogenic environmental change, which refers to human-caused alterations to earth's physical and climatic systems, is one of the most severe threats to global biodiversity, and is expected to drastically alter the distribution and abundance of earth's biodiversity (Walther *et al.* 2002; Parmesan & Yohe 2003; Parmesan 2006; Geyer *et al.* 2011; Groves *et al.* 2012; Pacifici *et al.* 2015; Jones *et al.* 2016). The ways in which organisms may respond to and cope with environmental change is receiving increasing attention as projections of impacts due to anthropogenic change become increasingly dire (Walther *et al.* 2002; Parmesan 2006; Sih *et al.* 2011; Travis *et al.* 2013). Although some species may respond to environmental change through range shifts, the magnitude and frequency of dispersal events necessary to facilitate this mode of response may be unlikely to occur in highly fragmented landscapes, potentially requiring alternative, *in situ* responses (Opdam & Wascher 2004; Travis *et al.* 2013; Oliver *et al.* 2015; Rojas *et al.* 2021).

Another important mode of an organisms response to environmental change includes evolutionary adaptation (Hoffmann & Sgro 2011; Hällfors *et al.* 2016; Meester *et al.* 2018; Diniz-Filho & Bini 2019; Walsworth *et al.* 2019), and there is no question that adaptive processes have facilitated the persistence of natural populations in the face of historical environmental change (Millar & Woolfenden 1999; Moritz & Agudo 2013). Unfortunately, the speed and intensity of contemporary environmental change outpaces historical events (Loarie *et al.* 2009; Burrows *et al.* 2011), calling into question the capacity of extant species to respond sufficiently to rapidly environmental change, either through movement or adaptive processes (Thomas *et al.* 2004; Bellard *et al.* 2012; Stanton *et al.* 2015)

As species have responded to past environmental change, both movement (accompanied by gene flow) and response to selection through adaptive divergence have very likely occurred, but the interplay between these mechanisms of response are poorly understood (Kawecki & Ebert 2004; Kawecki 2008; Aitken & Whitlock 2013; Tigano & Friesen 2016). Theoretical models predict that adaptive divergence decreases as gene flow increases, typically due to increased spatial homogenization of gene pools (Sousa *et al.* 2013; Tigano & Friesen 2016). On the other hand, there are numerous cases in which adaptive divergence may actually be promoted by increased gene flow, such as through dispersal of individuals carrying advantageous alleles or demographic benefits of dispersed individuals associated with gene flow (Kawecki & Ebert 2004; Aitken & Whitlock 2013; Micheletti *et al.* 2018).

In an increasingly fragmented world, uncertainties around adaptive divergence and gene flow limit our collective abilities to predict their importance for the persistence of sensitive species in fragmented landscapes (Kawecki & Ebert 2004; Tigano & Friesen 2016). The Mojave

Desert Tortoise (*Gopherus agassizii*), native to the Mojave Desert of North America, has been strongly impacted by habitat fragmentation and human development (Nafus *et al.* 2013; Tuma *et al.* 2016; Sánchez-Ramírez *et al.* 2018). A number of anthropogenic causes of habitat fragmentation – including roads and fences – have had documented negative impacts on gene flow (Latch *et al.* 2011; Dutcher *et al.* 2020) and survival in free-ranging tortoises (Doak *et al.* 1994; Hughson & Darby 2013; Tuma *et al.* 2016). Furthermore, the slow life history of the Mojave Desert Tortoise – typified by delayed sexual maturity, relatively low reproductive output, and sensitivity to reductions in adult survival – is often associated with relatively slow adaptive divergence through time, casting doubt as to the relative capacity of this species to respond adaptively to environmental change (McKinney 1997; McKinney & Lockwood 1999; Williams *et al.* 2008).

In this study, we used landscape and population genomic toolsets to explore the interplay between adaptive divergence and gene flow in the Mojave Desert Tortoise. Using a heretofore unprecedented array of samples collected across the range of the desert tortoise, we used a mix of genotype-environment analyses (GEA), population genomic analyses, and analyses of population connectivity to: 1) characterize neutral and adaptive genetic divergence across the Mojave Desert, 2) identify the predominant environmental gradients driving both neutral and adaptive divergence across multiple scales, 3) identify the spatial scales at which gene flow and dispersal occur, and 4) characterize how adaptive divergence is related to quantitative estimates of gene flow.

Methods

STUDY AREA AND SAMPLE COLLECTION

Our study area encompassed the Mojave Desert, and the Sonoran subdivision of the Colorado Desert, situated in the American southwest (Fig. 1). To leverage samples from the entire range of the Mojave Desert Tortoise, we drew upon previously-collected blood samples (dating back to 2004) and newly-collected blood samples (collected between 2018 and 2022). The largest single source of samples came from a sample set collected between 2004-2006 and 2010-2012 (n = 615), comprised largely of whole blood samples and blood specimen collection cards (FTA, Whatman GE Healthcare Life Sciences; Hagerty *et al.* 2010; Hagerty & Tracy 2010). We sourced remaining previously-collected samples across the Mojave Desert from a number of collaborators from the University of Nevada-Reno (UNR), U.S. Geological Survey (USGS), U.S. Fish and Wildlife Service (USFWS), and San Diego Zoo Wildlife Alliance (SDZWA) (n = 358), again comprising both whole blood samples and collection cards. Finally, we supplemented this sample set with targeted field surveys in identified gaps in the distribution of available samples, often prioritized towards edge habitats that have not been the subject of historic survey efforts (n = 193). Although described elsewhere (Hagerty *et al.* 2010; Sánchez-Ramírez *et al.* 2018; Dutcher *et al.* 2020), blood samples taken during the course of these field surveys were drawn according to standard protocols, with all blood samples collected and preserved on GenSaver 2.0

cards (Ahlstrom GenTegra). These 1,166 samples represent, to our knowledge, the most spatially-comprehensive sample set for this species assembled to date.

DNA EXTRACTION AND LIBRARY PREPARATION

We extracted DNA from all samples using Qiagen Investigator and DNEasy blood and tissue kits (Qiagen), and quantified DNA concentrations using both Quant-iT PicoGreen dsDNA and Qubit dsDNA broad range assays (ThermoFisher Scientific). We then selected a total of 697 samples for sequencing based on a) maximizing the spatial coverage and b) maximizing sample quality and concentration (when possible, > 20 ng/uL) for samples included in the final library, followed by library preparation for double-digest restriction enzyme-associated sequencing (ddRAD; Peterson *et al*. 2012). We digested genomic DNA using EcoRI and MseI then ligated enzyme-specific adapters with individual-specific barcodes to genomic DNA fragments, which facilitates individual identification following sequencing. Following polymerase chain reaction (PCR) amplification of these fragments using Illumina sequencing primers, we used a Pippin Prep to size select PCR products to a size of 200-400 bp. Barcoded individual libraries were then pooled into a single library for sequencing on the Illumina Novaseq sequencing platform at the University of Texas-Austin.

SNP DISCOVERY AND GENOTYPING

Following receipt of multiplexed sequencing data, we used custom Perl scripts to remove potential contaminants and demultiplex this dataset into individual samples. We then used ipyrad v 0.9.8.1 (Eaton & Overcast 2020) to perform a reference assembly to the Mojave Desert Tortoise genome (Tollis *et al.* 2017; Dolby *et al.* 2020), call single nucleotide polymorphisms (SNPs), and genotype individuals. Initial tests of potential parameter combinations for this assembly indicated that the following alterations to default settings represented the best compromise between missing data and read depth per locus: mindepth_majrule = 5, mindepth_stat = 10, min_samples_locus = 5. The post-assembly SNP dataset was then exported as a VCF file for further filtering for minor allele frequency (MAF) and locus-level call rates using a similar procedure to that presented in O'Leary *et al.* (2018) using package “dartR” (Gruber *et al.* 2018).

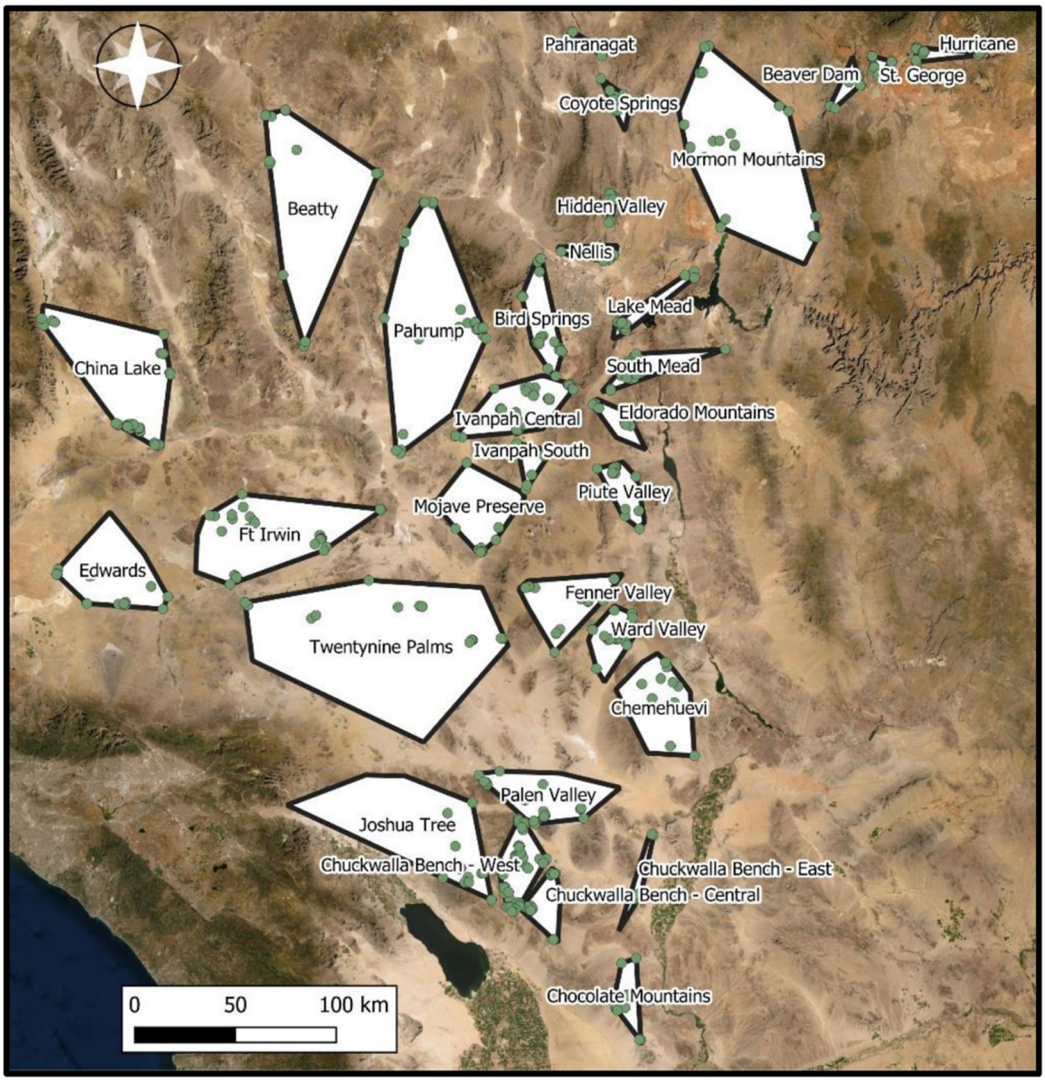

Fig. 1: Distribution of samples selected for sequencing (points; n = 697 points) and delineated sampling location (n = 31 white polygons). Any samples within polygon boundaries were assigned to that sampling location.

GENOME-WIDE GENETIC STRUCTURE AND CLUSTERING

To assess population genetic structure, we first consolidated samples into core sampling localities based on *a priori* knowledge of spatial boundaries between sampling locations (Fig. 1). Using these location assignments, we then used "gl.impute" in package "dartR" to impute missing data in our genetic matrix; under this approach, average allele frequencies within sampling localities are used to impute missing information (Gruber *et al.* 2018). We used this

imputed dataset for all population genetics and genetic clustering analyses, but used a more rigorously filtered dataset for GEA and relatedness inference (see below).

We then identified spatial clusters in genetic variation using two methods: "tess3r", a spatially-explicit clustering approach (Caye *et al.* 2016) and spatial non-negative matrix factorization (sNMF), a highly-efficient non-spatial estimator of ancestry coefficients (Frichot *et al.* 2014). In each case, we ran $K = 1$ to 8, and evaluated optimal clustering solutions for each algorithm based on inflection points in estimates of cluster fit. Following identification of clusters, we characterized genetic diversity as observed heterozygosity (H_o) and genetic differentiation (F_{ST} and F_{IS}) between clusters and sampling localities using packages "adegenet" and "dartR" (Gruber *et al.* 2018).

As an initial attempt to identify putatively adaptive SNPs, we used PCADAPT, an approach that uses principal components analysis (PCA) to control for population structure before scanning for particularly divergent SNPs (Luu *et al.* 2017), and identify SNPs that may be under divergent selection throughout the Mojave Desert. Unlike GEA approaches (described below), this approach infers putative outlier loci based solely on the relative magnitude of divergence relative to background genomic variation (Luu *et al.* 2017). We ran an initial PCA and inspected variance explained by each principal component (PC) using a screeplot and visualizations of genetic variation along each axis; after selecting the PCs that explained the most variance, we corrected *p*-values using a Benjamini-Hochberg correction, and used a false discovery rate of 0.05 to infer putatively adaptive SNPs (Luu *et al.* 2017; Rödin-Mörch *et al.* 2019; Byer *et al.* 2021).

ENVIRONMENTAL ASSOCIATIONS WITH ADAPTIVE AND NEUTRAL GENETIC VARIATION

In order to characterize environmental associations with genetic variation, we leveraged a panel of edaphic and climatic covariates previously used for habitat modelling efforts for this species (Table 1). We sampled each of these environmental layers at each of the 697 sample locations and used the resulting environmental dataset for downstream analyses. We first leveraged this environmental dataset to identify putatively adaptive outlier loci using GEA. In order to avoid potentially biasing GEAs due to imputation, we more rigorously filtered our non-imputed SNP dataset to include only individuals with < 20% missing data. We then inferred putatively adaptive genetic variation along environmental gradients using two approaches. The first of these approaches was Latent Factor Mixed Modelling (LFMM), which uses latent factors to control for broad-scale population structure when scanning for univariate associations between dimensionally-reduced environmental gradients and each SNP (Frichot & François 2015). Since this is a univariate approach, we first used PCA to reduce our environmental covariates to a smaller number of synthetic ordination axes; we retained the first two PCs to represent the primary environmental gradients throughout our study area. In addition, LFMM requires users to set the number of latent factors used to represent underlying genetic structure; we used the consensus number of clusters identified across our two genetic clustering approaches as the

number of latent factors. We then used 4000 iterations across three chains, with the first 2000 discarded as burn-in, and calculated z-scores from these runs. We then corrected these z-scores for the Genomic Inflation Factor, and used the resulting adjusted *p*-values (with a False Discovery Rate of 0.05) to identify putatively adaptive loci associated with each PC axis.

The second approach was partial redundancy analysis (pRDA), a robust ordination-based approach that identifies SNPs that are particularly differentiated along environmental gradients while accounting for multivariate population structure (Capblancq & Forester 2021). Unlike traditional redundancy analysis (RDA), pRDAs allow the user to partial out the effects of sets of variables that may confound underlying GEAs (Capblancq *et al.* 2018; Capblancq & Forester 2021). A variety of approaches have been advocated regarding the constraining terms to use in pRDAs aimed at identifying putatively adaptive loci, including accounting for either spatial proximity or neutral genetic population structure (Capblancq *et al.* 2018; Capblancq & Forester 2021). We opted to include the first three PCs of an unconstrained PCA of our genetic matrix to control for underlying population genetic structure. Thus, our pRDA model statement included all environmental covariates as predictors, our imputed and filtered genetic matrix as the response, and conditioning terms based on these first three PCs to describe population genetic structure. After running this pRDA, we selected SNPs that were +/- 3 standard deviations away from the mean loading for all SNPs along the first three constrained ordination axes (RDA1, 2, and 3). After identifying putatively adaptive SNPs across PCADAPT, LFMM, and pRDA, we subset the overall SNP dataset to include only these SNPs; hereafter, we refer to this dataset as our adaptive or putatively adaptive dataset.

Table 1: Environmental predictors used for various environmental association analyses.

Predictor	**Units**	**Description**
1. Winter Mean Precipitation	Millimeters (mm)	30 year (1993-2022) normal of winter precipitation for the winter months calculated using CMIP5
2. Winter Minimum Temperature	Degrees Celsius (°C)	30 year normal of winter minimum temperature for the winter months calculated using CMIP5
3. Fall Mean Temperature	Degrees Celsius (°C)	30 year normal of fall temperature for the fall months calculated using CMIP5

4. Spring Mean Temperature	Degrees Celsius (°C)	30 year normal of spring temperature for the spring months calculated using CMIP5
5. Summer Maximum Temperature	Degrees Celsius (°C)	30 year normal of summer maximum temperature for the summer months calculated using CMIP5
6. Summer Mean Precipitation	Millimeters (mm)	30 year normal of summer mean precipitation for the summer months calculated using CMIP5
7. Percent Silt	Percent (g/100g)	Proportion of silt fragments layer obtained from https://soilgrids.org/
8. Percent Clay	Percent (g/100g)	Proportion of clay fragments layer obtained from https://soilgrids.org/
9. Percent Sand	Percent (g/100g)	Proportion of sand fragments layer obtained from https://soilgrids.org/
10. Depth to Bedrock	Centimeters (cm)	Depth to bedrock layer obtained from https://soilgrids.org/
11. Coarse Fragments	cm3/100cm3 (volume %)	Volumetric fraction of coarse fragments layer obtained from https://soilgrids.org/
12. Bulk Density	kg/dm3	Bulk density of the fine earth fraction layer obtained from https://soilgrids.org/
13. Ruggedness Metric	radians/m	Topographic ruggedness layer obtained from Dilts et al.

SPATIAL PATTERNS IN GENE FLOW AND CONNECTIVITY

We investigated gene flow and connectivity across two scales: Mojave-wide and between-cluster. For the Mojave-wide approach, we calculated Nei's D between all pairs of individuals and sample locations using the function "stamppNeisD" in package "stampp" (Pembleton *et al.* 2013), followed by characterizing the spatial extents of genetic autocorrelation using Mantel's correlograms. For the between-cluster analysis, we used BayesAss3-SNP (Mussmann *et al.* 2019) to estimate gene flow. This program estimates rates of generational migration between user-specified populations; in this case, we provided cluster assignments for each individual as the input populations. First, we assigned dominant cluster of origin to each sample, and used clusters as population assignments in a branched version of our imputed SNP dataset. We used "gl2structure" to convert this SNP dataset to a Structure file, and "PGDspider" to convert this Structure file to an IMMANC file for use with BA3-SNP. We then used the function "BA3-SNPS-autotone.py" to optimize mixing parameters for use with this IMMANC file; we retained these parameter combinations for our BA3-SNP run. From this final run, we extracted estimates of asymmetrical pairwise migration rates and posterior estimates of variance around these rates.

ASSOCIATION BETWEEN ADAPTIVE DIVERGENCE AND GENE FLOW

We then conducted several analyses to associate the spatial distribution of adaptive genetic variation and gene flow. From our putatively adaptive dataset, we first calculated H_o for each individual for both the neutral and adaptive datasets. We also quantified the mean Nei's D for each individual as an indicator of the strength of gene flow. Finally, we used linear regression to relate heterozygosity and genetic distance, with heterozygosity as the response and genetic distance as the predictor. We fit separate models for adaptive and neutral heterozygosity, assessed significance of these models using Analysis of Variance (ANOVA), and compared slopes between the neutral and adaptive models to assess the relative strength of gene flow-diversity relationships across datasets. Finally, to visually represent spatial patterns in diversity and gene flow, we used Triangulated Irregular Networks (TINs; implemented in qGIS 3.2.1) to interpolate adaptive and neutral H_o and average population-level F_{ST} across the Mojave.

Results

VARIANT CALLING AND FILTERING

After contaminant cleaning, the Illumina NovaSeq run generated a total of 1,602,023,294 reads, averaging approximately 2,223,260 reads per individual. Following reference assembly in ipyrad, these reads were organized into 1,028,928 SNPs across 607,831 loci, with a dataset-wide missing data percentage of 73.61% and an average coverage per locus and individual of 21x. After post-assembly filtering (MAF > 0.05, iterative locus-level missing data filtering, and one SNP/locus), this produced a dataset of 19,131 SNPs across 697 individuals, with an overall

missing data percentage of 24.5%. Unless otherwise noted, this dataset was used in all downstream analyses.

POPULATION STRUCTURE AND CLUSTERING

After imputation, our genome-wide SNP dataset had an average H_o of 0.1826, a global F_{ST} of 0.1471, and F_{IS} of 0.2702. Regardless of clustering algorithm used ("tess3r" or sNMF), the best supported clustering solution was five clusters ($K = 5$), similar to previous studies of genetic structure in this species (Sánchez-Ramírez *et al.* 2018; Fig. 2). The population genetic clusters corresponded to the following general portions of the Mojave Desert: north, east, south, west, and central. A PCA revealed two primary groupings amongst these clusters along PC1: one comprised of the two clusters towards the northern Mojave (gold and green colors), and the other comprised of the clusters to the south and west (dark and lighter blue), with samples in the central Mojave appearing intermediate to these two primary groupings (dark red; Fig. 2). Inspection of a screeplot of percent variance explained along these PCs revealed a sharp drop-off in variance explained after 5 PCs; thus, we retained PC1-PC5 for use in PCADAPT. Using this approach, we identified 225 SNPs that were putatively adaptive.

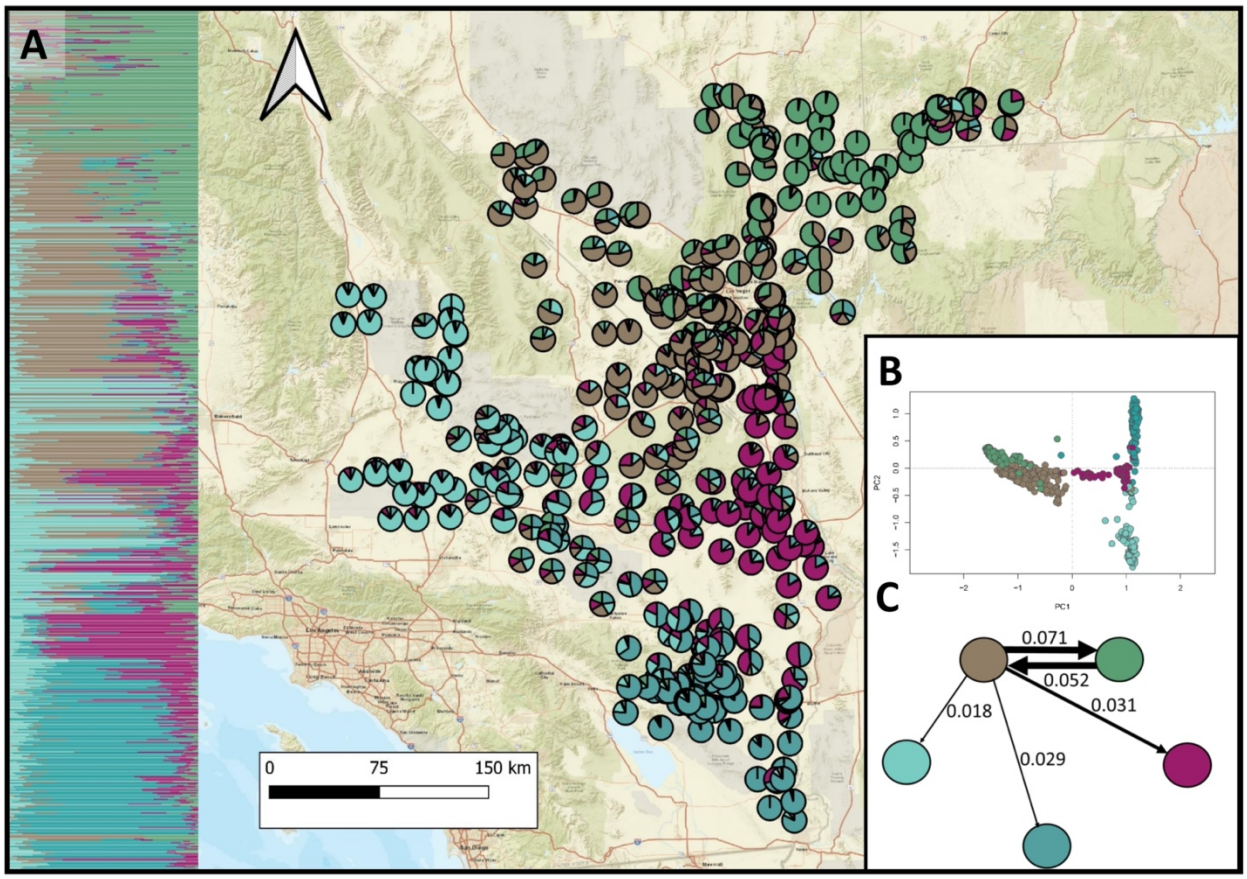

Fig. 2: (A) Spatial patterns in the best-supported number of genetic clusters ($K = 5$) based on sNMF. Each pie chart represents individual-level admixture proportions, with barplots of individual-level admixture displayed on the left. (B) Plot of a PCA of our imputed allele frequency matrix, with points colored to match the primary genetic cluster of origin identified through sNMF ($K = 5$). (C) Generational migration rates between the five primary genetic clusters, as estimated by BayesAss3. Line size is scaled to the magnitude of estimated migration, and only migration rates > 0.01 are displayed.

GENOTYPE-ENVIRONMENT ASSOCIATION ANALYSES

After removal of individuals with more than 20% missing data, our non-imputed dataset comprised 379 individuals across 19,131 SNPs with 14.2% missing data; this dataset was used for outlier detection and GEA analyses. Prior to running our GEAs, an initial PCA of associations between our environmental covariates revealed two coherent environmental gradients along the first two PCs: the first axis (PC1) primarily contrasted hotter, drier regions of the Mojave Desert from cooler, wetter areas, whereas the second axis (PC2) primarily contrasted sandier areas with higher bulk density soils from areas with lower bulk density soils, typically associated with more silt and clay (Fig. 3). Our first GEA – LFMM – was run with five latent factors to approximate the spatial population structure indicated by our clustering analyses, and indicated 31 loci significantly differentiated along PC1 and 29 loci significantly differentiated along PC2, for a total of 60 loci identified as putatively adaptive from this approach.

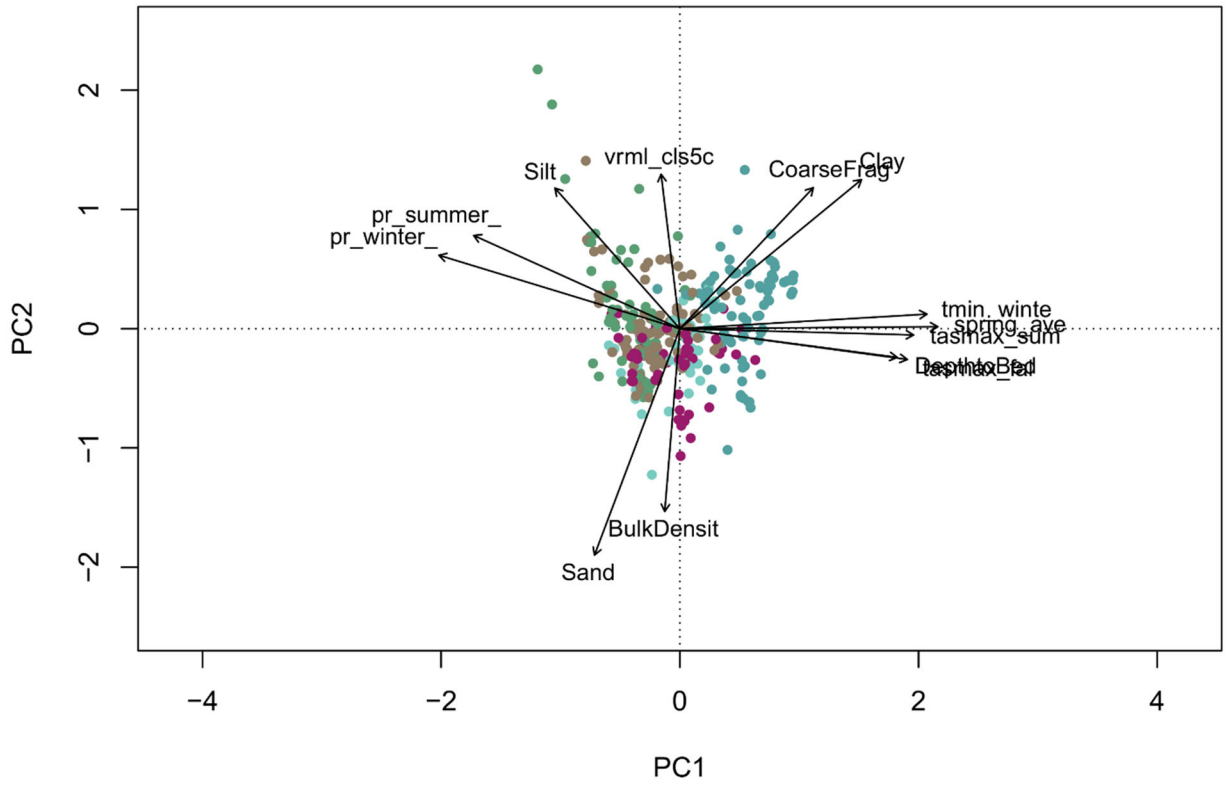

Fig. 3: A PCA of environmental associations across sampled individuals, with colors designating cluster identities.

Prior to our second GEA – pRDA – we used RDA without conditioning terms to visually explore gradients associated with genome-wide variation. This RDA revealed associations with summer precipitation and percent silt for the northern and northeastern clusters, and notable associations with higher seasonal temperatures (spring, summer, and winter) and percent clay for the southern cluster (Fig. 4). After running a pRDA while conditioning for neutral population genetic structure, we detected 131 SNPs strongly differentiated along RDA1, 144 SNPs strongly differentiated along RDA2, and 138 SNPs strongly differentiated along RDA3, for a total of 403 putatively adaptive SNPs documented by this approach. Inspection of covariate loadings along each of these three redundancy axes indicated that RDA1 was primarily associated with precipitation covariates, RDA2 with soil (percent silt, clay, and sand), and RDA3 with temperature covariates (spring, summer, and fall temperatures) (Table 2). After consolidating loci across all three outlier detection approaches (PCADAPT, LFMM, and pRDA), this produced a dataset of 632 putatively adaptive SNPs that we hereafter refer to as our putatively adaptive dataset. The remaining 18,499 SNPs are hereafter referred to as our neutral dataset.

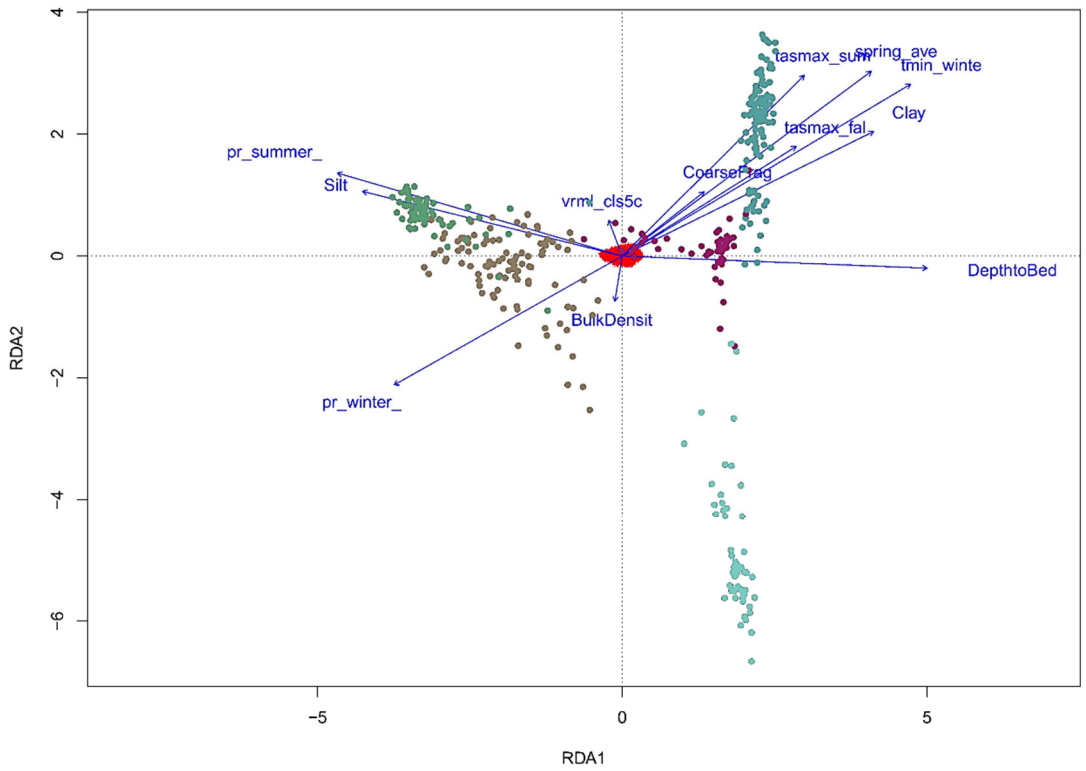

Fig. 4: Plot of a RDA, with axes representing environmental associations with genome-wide genetic variation. Arrows denote loadings of environmental variables in multivariate space.

Table 2: Correlations of environmental variables along the pRDA axes used for identification of putatively adaptive loci. Note that these variable correlations are not reflective of those displayed in Fig. 4, as that previous RDA did not include conditioning terms to control for genetic structure included in the pRDA.

Variable	RDA1	RDA2	RDA3
Minimum winter temperature	-0.096	-0.320	0.066
Maximum summer temperature	0.187	-0.219	**0.662**
Maximum fall temperature	0.128	-0.163	**0.656**
Average spring temperature	-0.025	-0.350	**0.497**
Winter precipitation	**0.409**	-0.343	-0.355
Summer precipitation	**0.502**	-0.320	-0.245
Ruggedness Metric	0.120	-0.114	-0.186
Silt	0.230	**-0.502**	-0.114
Clay	**-0.442**	**-0.488**	0.018
Sand	0.182	**0.633**	0.051
Bulk Density	0.253	0.165	0.115
Depth to Bedrock	-0.381	0.166	0.381
Coarse Fragments	-0.302	-0.061	0.127

GENE FLOW

At the Mojave-wide scale, Nei's D was positively related to geographic distance both at the individual-level ($r = 0.7048$, $p = 0.001$) and sampling location-level (0.7055, $p = 0.001$; Fig. 5a), and Mantel correlograms indicated positive and significant correlations between geographic and genetic distances out to approximately 180 km (Fig. 5b). Our cluster-level analysis with BayesAss3-SNP indicated unexpectedly high rates of generational migration from the cluster ranging from the Mojave National Preserve north through Beatty, Nevada to all other clusters; in turn, this cluster received an unexpectedly high proportion of migrants from the cluster ranging from Lake Mead, Nevada northeast to Utah (Fig. 2c).

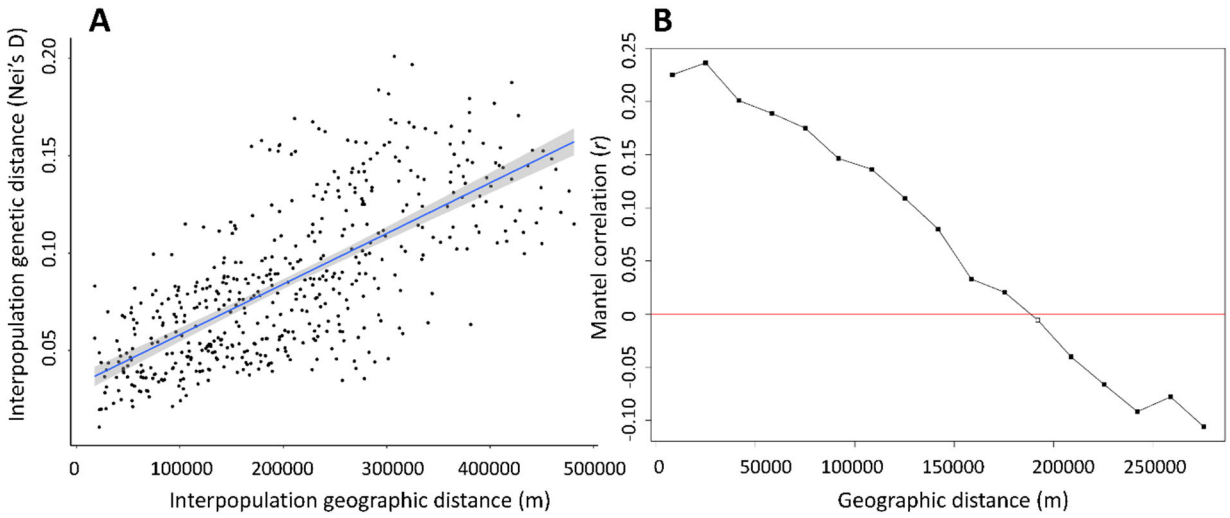

Fig. 5: (A) Inter-population geographic distance plotted against inter-population genetic distance. (B) a Mantel correlogram, describing spatial trends between inter-individual genetic distance and geographic distance. Mantel correlation coefficients were significant for every distance category other than ~19,000 m.

ASSOCIATIONS BETWEEN GENE FLOW AND DIVERSITY

Linear regressions of heterozygosity and inter-individual genetic distance were significant for both the putatively adaptive (Chisq = 455.96, $p < 0.001$) and neutral datasets (Chisq = 1626.7, $p < 0.001$). Although slopes for these relationships were negative for both datasets, the slope was marginally greater for the putatively adaptive dataset ($\beta_{adaptive}$ = -3.95; 95% CI = -4.31 to -3.59) compared to the neutral dataset ($\beta_{neutral}$ = -3.59, 95% CI = -3.76 to -3.41, difference in slopes P = 0.07), indicating that samples that were more connected with gene flow had particularly pronounced adaptive genetic variation compared to the neutral background (Fig. 6). Inspection of spatial footprints from interpolations of population-level genetic divergence indicated stronger mean divergence towards the west and northeast (Fig. 7a). Similar interpolations of heterozygosity indicated that neutral diversity was highest towards the west and central portions of the Mojave Desert (Fig. 7b), whereas adaptive diversity was highest towards the central and northeastern portions of the Mojave (Fig. 7c). This suggests the following: 1) neutral genetic diversity is strongest in the central Mojave, where genetic divergence is low and gene flow is higher, b) adaptive genetic diversity partially mirrors neutral-level associations with gene flow, but with noticeably high adaptive heterozygosity in the strongly divergent northeast Mojave. Clusters in that region also appear to be strongly connected by migration as well (Fig. 2c), indicating complex interactions between gene flow and adaptive divergence across scales.

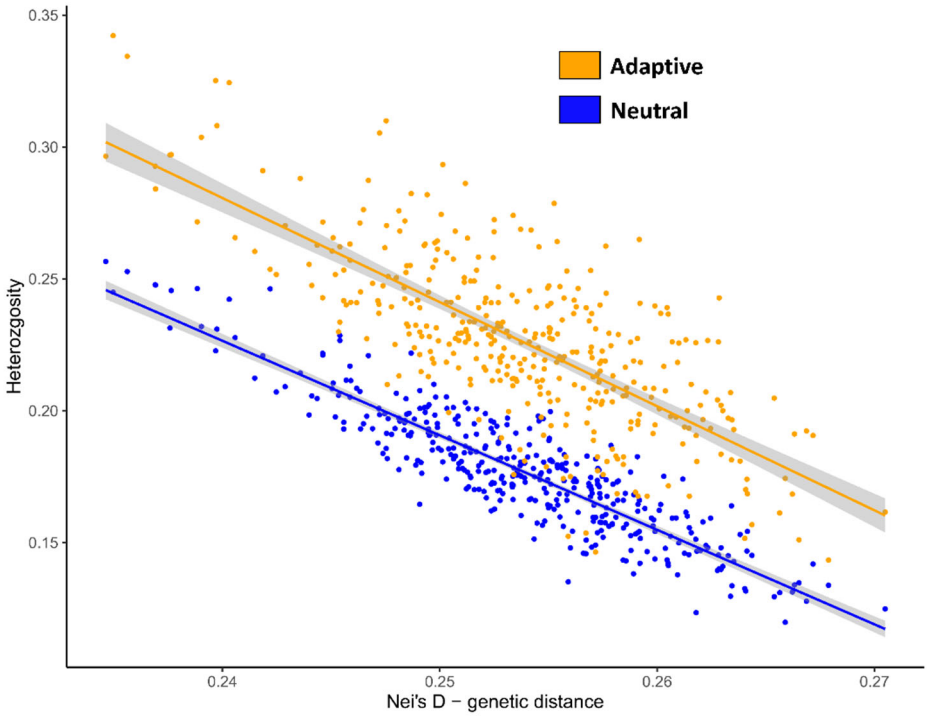

Fig. 6: Regressions between both adaptive and neutral heterozygosity and inter-individual genetic distances.

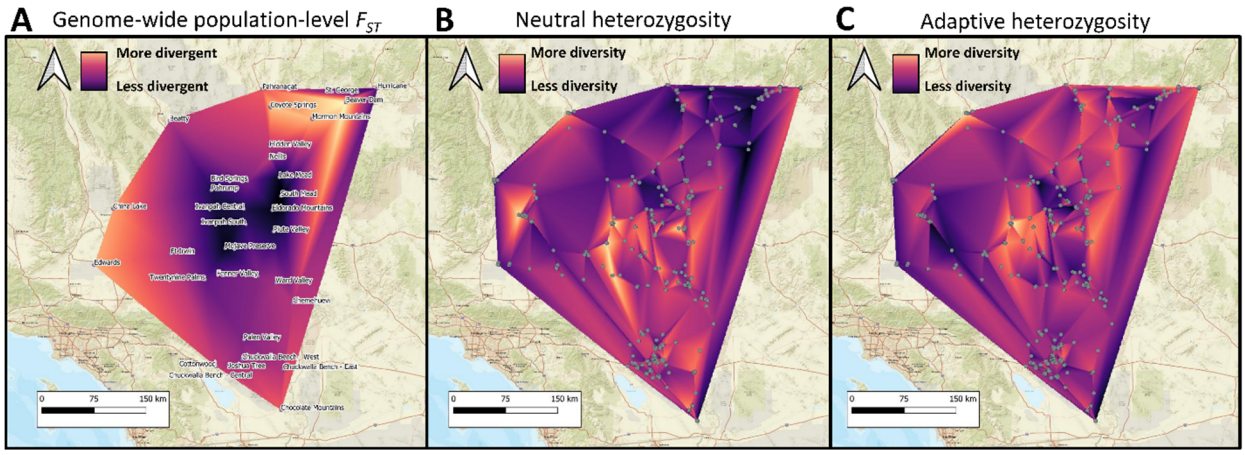

Fig. 7: (A) Triangulated irregular network surface of average population-level F_{ST} across the Mojave Desert, (B) individual-level neutral heterozygosity, and (C) individual-level adaptive heterozygosity.

Conclusions

CHARACTERIZE NEUTRAL AND ADAPTIVE GENETIC DIVERGENCE

Across the range of the Mojave Desert Tortoise we found the best supported clustering solution was five clusters, that corresponded to the north, east, south, west, and central Mojave Desert, with high levels of admixture, particularly among the north and east clusters. We used these genetic clusters to represent underlying genetic structure and were able to characterize neutral and putatively adaptive genetic divergence by consolidating loci across multiple outlier detection approaches (PCADAPT, LFMM, and pRDA), producing two datasets: one with 632 putatively adaptive SNPs and the other with the remaining neutral SNPs. This then allowed us to focus on analyses of adaptive and neutral genetic variation.

IDENTIFY PREDOMINANT ENVIRONMENTAL GRADIENTS DRIVING DIVERGENCE

We found that among our five main genetic clusters there are two primary groupings: one comprised of the two clusters toward the north, and the other comprised of the two clusters to the south and west, with the central cluster appearing intermediate. These groupings align fairly well with our GEA results that revealed environmental gradients that contrasted hotter, drier regions from cooler, wetter areas, and sandier soil conditions from those associated with silt and clay. Interestingly, our realized Mojave Desert Tortoise habitat model (Phase I, Task 4) predicted that climate variables, particularly winter minimum temperature, summer maximum temperature, and percent sand were important in determining habitat suitability, which partially converges with these findings related to temperature and edaphic gradients. Temperature gradients have been found to produce genomic signatures of adaptation and may influence life-history shifts (Cayuela et al. 2021). Our results indicate that temperature, along with edaphic conditions, are important drivers of genetic variation in Mojave Desert Tortoises and likely very important factors for continued persistence of Mojave Desert Tortoises.

IDENTIFY THE SPATIAL SCALES AT WHICH GENE FLOW AND DISPERSAL OCCUR

We found patterns of isolation-by-distance along with unexpectedly high rates of generational migration. Migration was highest in association with the central cluster through the east and north clusters. We found admixture within our five genetic clusters, indicating gene flow especially within the north and east clusters. Tortoise populations are at risk of increasing genetic isolation, with recent detection of fragmentation documented (Latch *et al*. 2011; Dutcher *et al*. 2020). Anthropogenic disturbance that degrades tortoise habitat has been estimated to occur within 1 km of 66-70% of their habitat (Carter *et al*. 2020). Because disproportionately large losses to connectivity relative to dispersal can create a critical threshold where population extinction is predicted to be high (Hand *et al*. 2014), we reiterate the recommendation of creating a connected network across the range of the Mojave Desert Tortoise, including critical habitat units, National Park Service lands, and military installations to ensure that some level of gene

flow and dispersal can persist at multiple spatial scales and across heterogeneous habitats (Averill-Murray et al. 2013, Averill-Murray et al. 2021).

CHARACTERIZE HOW DIVERGENCE IS RELATED TO GENE FLOW

This work provides a better understanding of gene flow among desert tortoise populations. Linear regressions of heterozygosity and inter-individual genetic distances were significant for both the adaptive and neutral datasets, with the adaptive dataset exhibiting a steeper slope for these relationships, indicating that higher levels of gene flow correlates with pronounced adaptive genetic variation. We found evidence for stronger neutral genetic diversity in the central Mojave Desert, where genetic divergence is low and gene flow is high, along with strong adaptive genetic diversity in the northeast Mojave, where genetic divergence and migration rates are high. These results highlight that tortoise habitat in the central Mojave is likely of great importance to genetic connectivity, serving as critical linkage areas across the range, while areas closer to the edge of the range, especially the northeast, west, and to a lesser extent south, may be of key importance to adaptive potential for the species. The complex interactions between gene flow and adaptive divergence are not surprising given the evidence that gene flow may sometimes benefit and sometimes hinder local adaptation (Sexton *et al*. 2011). Adaptation to environmental conditions across heterogeneous environmental conditions may vary along with levels of gene flow; genetic divergence can be strengthened by some gene flow (Polechova & Barton 2015; Oomen & Hutchings 2022). Our results illustrate that both adaptive divergence and gene flow can be high for tortoises, and that maintaining genetic connectivity from the core of the species range through to edge habitats is likely critical to preserving the species under current and future environmental conditions.

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