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Population Genetics Of Two Sympatric Species, The Round-Tailed Horned Lizard (phrynosoma Modestum) And The Greater Earless Lizard (cophosaurus Texanus), In The Indio Mountains Of West Texas

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POPULATION GENETICS OF TWO SYMPATRIC SPECIES, THE ROUND-TAILED HORNED LIZARD (*PHRYNOSOMA MODESTUM*) AND THE GREATER EARLESS LIZARD (*COPHOSAURUS TEXANUS*),

IN THE INDIO MOUNTAINS OF WEST TEXAS

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Dedication

I dedicate this work to my grandfather. I would not have learned that every living thing has its own equally important place in the world. I may never have learned that violence is not the correct reaction to something you are afraid of. Certainly, I would never have developed the love I have for the misunderstood creepy-crawlies of the world had it not been for you. Thank you for teaching me so much in such a short time.

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by

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1 Introduction

1.1 Background

Microevolution is the change in genetic makeup of a population over time (Merilä et al. 2001), specifically in understanding how evolutionary process such as genetic drift, gene flow, mutation, and selection contribute to changing allelic frequencies. These factors may be combined with other considerations such as ecology, geography, and demography to investigate the interplay between the genetic makeup of a population and its environment (Manel and Holderegger 2013, Lowe et al. 2017). Coupling molecular and ecological data can help identify which environmental processes might influence the persistence or extinction of a population (Segelbacher et al. 2010, Amos et al. 2012). Not only does landscape genomics help test evolutionary hypotheses, but it also provides important information for the conservation of species (Luikart et al. 2003, Allendorf et al. 2010).

In his book, *My Thoughts on Biological Evolution*, Motoo Kimura (2020) describes population genetics as using an interbreeding group of individuals, or population, to investigate the forces involved in genetic structuring. Population genetics began as a largely theoretical field, advanced by scientists such as Fisher, Haldane, Wright, Kimura, and Crow until advances in molecular study methods revolutionized the field (Lewontin 1985, Charlesworth and Charlesworth 2017). The introduction of technology such as electrophoresis for allozyme detection and subsequent nucleotide sequencing methods brought the field into an empirical realm of study by allowing researchers to test and refine the previous mathematical models

(Casillas and Barbadilla 2017). The latest technological revolution has been high-throughput sequencing methods that have spawned the sub-field of population genomics (Begun et al. 2007).

Population genomics takes genomic technologies and concepts and applies the same evolutionary questions of population genetics across the genome rather than several genes (Black IV et al. 2001). Population genomics has a clear benefit in studies estimating population size, migration rates, and phylogenetic relationships; as well as identifying adaptive variation using the large data sets produced. However, it can also be used to better inform conservation actions (Luikart et al. 2003). Applications in conservation can be found using adaptive variation identification, determining which populations have high genetic variability, and potentially identifying what geographic features may impede persistence of a population (Allendorf et al. 2010, Amos et al. 2012). However, these tools have yet to be applied to many systems due to the previously prohibitive costs of high-throughput sequencing methods and computational limitations (Davey and Blaxter 2010).

The costs of high-throughput sequencing methods have become more affordable for application in genome-scale population studies of non-model organisms – i.e. organisms without a reference genome (Garvin et al. 2010, Davey and Blaxter 2010). One such method that has experienced significant growth in population genomics studies is RAD sequencing (Andrews and Luikart 2014). Double-digest restriction-site associated DNA sequencing (ddRADseq) is a modified approach to this original RADseq method (see Davey and Blaxter 2010). In short, DNA sequences are fragmented using two restriction enzymes simultaneously, eliminating the costs of random shearing and DNA end repair caused by RADseq and eliminating several high-DNA-loss steps allowing for lower DNA concentrations in starting samples. Precise, tunable size selection of genomic fragments is another improvement over the original RADseq protocol. These

capabilities permit the flexibility to choose library sizes of hundreds to hundreds of thousands of genomic regions. The use of unique barcodes attached at each end of the fragments allows for indexing of samples into exponentially larger pools (Peterson et al. 2012). The affordability, flexibility, and *de novo* analysis of this method makes it widely-applicable to non-model organisms (Peterson et al. 2012, Andrews and Luikart 2014).

Squamates are an understudied group of animals that population genomics tools and concepts could be used to study and better understand. Despite being the most diverse group of terrestrial vertebrates (~11,000 described extant species; Uetz, P. and J. Hošek 2022), much has been unresolved regrading evolutionary questions in this group ((Simões and Pyron 2021) Here, I apply a population genetics framework to determine the effects of geographic features (e.g. mountains) on the population genetic structuring of two species of lizards found in the Chihuahuan Desert, *Phrynosoma modestum* and *Cophosaurus texanus*. Specifically, I studied how the geographic features found in the Indio Mountains of western Texas may influence migration rates, and discuss the conservation implications. My study will help better understand the taxonomy and evolutionary history of squamates, while providing critical information to be applied to future conservation decisions for both the focal species and closely related species are that are of conservation concern in the state of Texas (Diniz-Filho et al. 2009).

1.2 Study Area

This study was undertaken at the Indio Mountains Research Station (IMRS) owned by the University of Texas at El Paso and situated within the northern Chihuahuan Desert. IMRS is located approximately 40 km southwest of Van Horn, Texas, and encompasses the majority of the southern spur of the Eagle Mountains, known as the Indio Mountains. The property contains

approximately 16,000 hectares and varies in elevation from 900 to 1,600 meters (Worthington et al. 2019). Average rainfall in the Chihuahuan Desert is 235 mm, with the majority of the rainfall occurring during the hottest months of the year, June-September (Schmidt 1979). Schmidt (1979) also found the annual mean temperature to be 18.6 °C, noting the homogenous nature of the climate across the desert.

The Indio Mountains began forming during the Cretaceous period when a shallow sea was filled via sediment deposition. This gave rise to the 3,210 m section of Cretaceous rocks in the mountains presently (Price et al. 1985). Price et al. (1985) also noted that during the Late Cretaceous to the Early Tertiary deformation occurred, primarily due to thrust faulting, pushing older rock above younger rock and volcanism during the Oligocene deposited ash-forming tuffs and trachyte. According to the Natural Resources Conservation Services, Redlight and Terlingua soils are the predominant soils on the research station, formed from limestone and igneous rock respectively (NRCS, 2013).

The floral and faunal diversity at the research station is typical of the Chihuahuan Desert. Though specific studies of the vegetation at the IMRS have not been undertaken, 375 species of plants have been identified on the property thus far and can be generally described as Chihuahuan Desert Scrub (Henrickson and Johnston 1983). The fauna identified on the property includes six amphibian species, 38 non-avian reptile species (including 15 lizards), 144 bird species, and 38 mammal species. Invertebrate diversity is unknown as no formal surveys have been conducted; however, over 500 species have been identified on the property which certainly represents a small subset of the actual diversity (Worthington et al. 2019).

Figure 1: Round-tailed horned lizard, *Phrynosoma modestum*.

1.3 Study Species

The round-tailed horned lizard, *P. modestum* (Figure 1), and the greater earless lizard, *C. texanus* (Figure 2), both belong to the family Phrynosomatidae and live in sympatry throughout most of the Chihuahuan desert including IMRS. However, these two species differ greatly in terms of ecology. While *P. modestum* primarily uses crypsis as an antipredator mechanism (Cooper and Sherbrooke 2010, 2012), *C. texanus* uses rapid speed to escape predators (Bulova 1994). Foraging behaviors also differ, members of *P. modestum* are primarily myrmecophagous (Pianka and Parker 1975, Barbault and Maury 1981) and sit and wait predators (Shaffer Jr. and Whitford 1981, Verwaijen and Damme 2008). In contrast, individuals of *C. texanus* have a more generalist diet (Barbault and Maury 1981, Maury 1995) with a substantial increase in foraging movements (Perry 1999, Verwaijen and Damme 2008). All of these differences may contribute to the discrepancies in home ranges and microhabitats utilized by each species (Perry and Garland Jr. 2002). Specifically, average home range of *P. modestum* ranges from 1,355 m^2 to 4,101 m^2 depending on sex (Munger 1984), while the home range of *C. texanus* is 194 m^2 to 263 m^2 for males and females respectively (Engeling 1972). Flatter areas with rocks of similar size to *P. modestum* are the preferred microhabitats for this species (Cooper and Sherbrooke 2010, 2012), while a much wider array of habitat types as well as elevations are utilized by *C. texanus* with a slight preference being found for steeper habitats (Osmanski 2014). Additionally, *C. texanus* is among the most abundant lizards at IMRS, while *P. modestum* has been found to be one of the least abundant (Mata-Silva Unpublished Data, Johnson 1998). *Phrynosoma modestum* has been found to have a higher Environmental Vulnerability Score than it's IUCN status would suggest (EVS = 12; medium vulnerability, IUCN; Least Concern) (Wilson et al. 2013) and as

such, may need conservation actions in the near future. Wilson et al. (2013) also found that *C. texanus* had a similar disparity between EVS (14) and IUCN status (Least Concern). These differences could collectively inform if and how landscape features influence the population structure of these two species.

Figure 2: Male Greater earless lizard, *Cophosaurus texanus*.

1.4 Goals and Predictions

The goal of this study was to investigate the role that geographic features play in influencing gene flow in two sympatric species of lizards, the round-tailed horned lizard (*Phrynosoma modestum*) and the greater earless lizard (*Cophosaurus texanus*), in the Indio Mountains. To achieve this goal, I investigated genetic structure within *P. modestum* and *C. texanus* in the Indio Mountains. First, I expect distinct differences in genetic structuring will be observed between the two species due to varying ecologies. This could be explained due to differences in ecological factors influencing evolutionary processes in so called eco-evolutionary dynamics (Siepielski et al. 2016, Lowe et al. 2017). Among the two species, I expect intraspecific structuring for *Phrynosoma modestum* due to their specialized diet (Pianka and Parker 1975, Barbault and Maury 1981), microhabitat preferences (Cooper and Sherbrooke 2010, 2012), and generally smaller population size in the study area (Mata-Silva Unpublished Data, Johnson 1998) that can result in strong founder effects (Farleigh et al. 2021). In contrast, the more generalist behavior and larger continuous population of *Cophosaurus texanus* is more likely to not exhibit additional population structure (Barbault and Maury 1981, Maury 1995, Osmanski 2014).

Table 1: Samples collected with GPS Coordinates and species identification

ID	Species	Latitude	Longitude
LMH1	*Phrynosoma modestum*	30.75531	-105.00413
LMH2	*Cophosaurus texanus*	30.77139	-105.01147
LMH3	*Cophosaurus texanus*	30.77622	-105.01576
LMH4	*Phrynosoma modestum*	30.73256	-104.99129
LMH5	*Cophosaurus texanus*	30.73227	-104.99100
LMH6	*Cophosaurus texanus*	30.76484	-105.00745
LMH7	*Cophosaurus texanus*	30.77250	-105.01116
LMH8	*Cophosaurus texanus*	30.77249	-105.01183
LMH9	*Cophosaurus texanus*	30.77657	-105.01699
LMH10	*Cophosaurus texanus*	30.77716	-105.01552
LMH11	*Cophosaurus texanus*	30.77108	-105.01110
LMH12	*Phrynosoma modestum*	30.77569	-105.01660
LMH13	*Phrynosoma modestum*	30.77094	-105.01204
LMH14	*Phrynosoma modestum*	30.75779	-105.00269
LMH15	*Phrynosoma modestum*	30.77654	-105.01529
LMH16	*Phrynosoma modestum*	30.72956	-104.98694
LMH17	*Phrynosoma modestum*	30.78544	-105.02334
LMH18	*Phrynosoma modestum*	30.77225	-105.01387
LMH19	*Cophosaurus texanus*	30.77131	-104.99858
LMH20	*Phrynosoma modestum*	30.75592	-105.00408
LMH21	*Cophosaurus texanus*	30.73187	-104.97482
LMH22	*Phrynosoma modestum*	30.74624	-105.00567
LMH23	*Cophosaurus texanus*	30.73454	-104.97644
LMH24	*Cophosaurus texanus*	30.73752	-104.97584
LMH25	*Phrynosoma modestum*	30.77185	-105.00322
LMH26	*Phrynosoma modestum*	30.77048	-105.01076
LMH27	*Phrynosoma modestum*	30.71953	-104.97188
LMH28	*Phrynosoma modestum*	30.75491	-105.01018
LMH29	*Phrynosoma modestum*	30.77445	-105.01509
LMH30	*Cophosaurus texanus*	30.78151	-104.99483
LMH32	*Phrynosoma modestum*	30.73216	-104.98586
LMH33	*Phrynosoma modestum*	30.72985	-104.98555
LMH34	*Cophosaurus texanus*	30.78664	-104.98828
LMH35	*Phrynosoma modestum*	30.77171	-105.01297
LMH48	*Cophosaurus texanus*	30.72991	-104.98910
LMH49	*Cophosaurus texanus*	30.72569	-104.98145
LMH57	*Cophosaurus texanus*	30.70313	-104.96832
LMH58	*Cophosaurus texanus*	30.75738	-105.00526

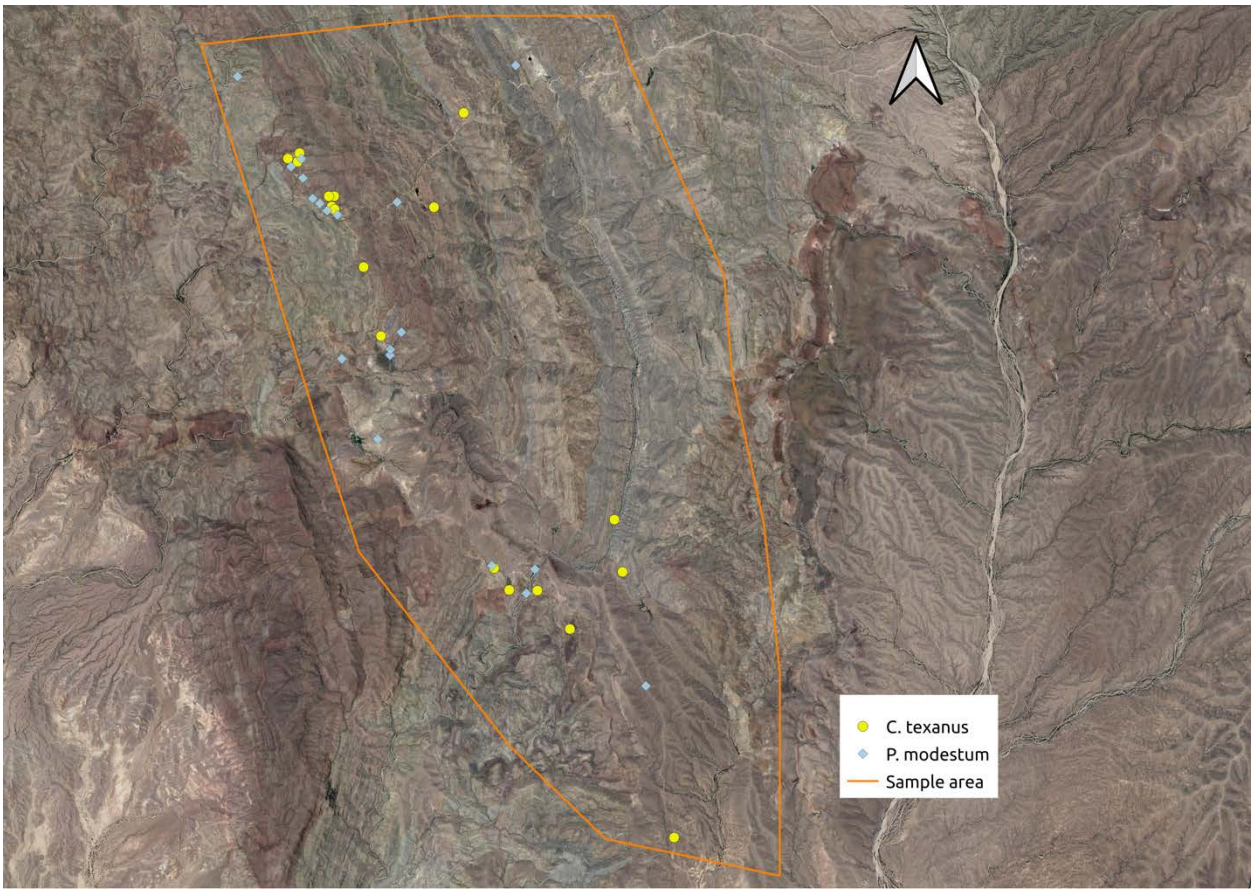

Figure 3: Map of sampled area with *Cophosaurus texanus* samples represented by yellow circles, *Phrynosoma modestum* samples represented by blue diamonds, and the sample area bounded by an orange line.

2 Materials and Methods

2.1 Sampling, DNA Extraction, and ddRADseq

Lizards of each species were collected across the property (n = 37, Table 1), particularly along the sides of Indio Mountains (Figure 3). Pitfall traps consisting of a five-gallon bucket with four running boards directing lizards to the trap and covered by a board (Figure 4) were used to catch the target species as well as opportunistically hand-catching specimens. In addition to tissue and/or blood samples, I collected morphological data for each captured lizard, including snout-vent length, tail length, weight, and sex. Not only were toe clippings used as an identification method to prevent double sampling individuals with minimal effect to the individual lizard (Ferner 2007), these were used as viable tissue samples for genetic analyses as well (Gonser and Collura 1996). Toe clips were placed in 95% ethanol and stored at -20 °C until DNA was extracted. Blood was drawn from each lizard and kept in blood buffer. DNA extractions were performed using DNeasy blood and tissue kits following the manufacturer's instructions (Qiagen). Next, DNA quality based on the presence of high molecular weight bands visualized using gel electrophoresis with a 1% agarose gel, and quantified DNA concentration using a Qubit 3 Flourometer (Invitrogen, Carlsbad, CA) to ensure a minimum concentration of 20 ng/µL.

Figure 4: A pitfall trap at the Indio Mountains Research Station (Photo by Vicente Mata-Silva).

Next, I followed procedures presented by Lavretsky et al. (2015), but with fragment size selection following Hernandez et al. (2021) to create multiplexed ddRAD-seq fragment libraries. Briefly, ~1 µg of genomic DNA was digested in 10 U of SbfI and EcoRI restriction enzymes. Next, Illumina-compatible adapters with barcodes used in de-multiplexing were ligated to the sticky ends of the fragments. The fragments were size selected using AMPure XP beads (Beckman Coulter, Inc.). Size-selected fragments were subsequently amplified using PCR. Phusion high-fidelity DNA polymerase (Thermo Fisher Scientific) was used for PCR and products were purified using magnetic AMPure XP beads (Beckman Coulter, Inc.). DNA quantification was done using the Qubit dsDNA assay kit (Thermo Fisher Scientific) and

samples with unique barcodes were pooled in equimolar concentrations. Multiplexed libraries were sent to Novogene and sequenced on an Illumina Highseq.

2.2 Demultiplexing and Preparation

Raw Illumina reads were processed using the pipeline presented by Dacosta and Sorenson (2014) available at http://github.com/BU-RAD-seq/ddRAD-seq-Pipeline. The raw reads were demultiplexed by assigning each read to an individual sample via the barcode sequences present in the adapters. Each sample underwent filtering of low-quality reads and identical sequences from the same individual were condensed into a single data line and the number of identical reads and the highest quality score among them were retained. The condensed and filtered reads were then clustered using the program USEARCH (Edgar 2010). Representative sequences from each cluster were then BLASTed against a *Scleoporus undulatus* genome. Finally, the reads in each cluster were aligned using the program MUSCLE (Edgar 2004). The genotypes for each individual at each loci were then inferred using the RADgenotypes.py script (DaCosta and Sorenson 2014). Per sample alleles we based on a minimum sequencing depth coverage of 5X (i.e. 10X per genotype) and quality per base PHRED scores of ≥30 to be retained. Finally, loci with >80% representation of samples were retained. After generating fasta files using scripts provided in the DaCosta and Sorenson (2014) pipeline, I then used additional scripts (Lavretsky et al. 2020; https://github.com/jonmohl/PopGen/) to extract bi-allelic single nucleotide polymorphisms (SNPs) from the datasets. Additionally, I used PLINK v. 1.90 (Purcell et al. 2007) to ensure that singletons (i.e., minimum allele frequency [maf] = 0.05) and any SNP missing >20% of data across samples were excluded in each dataset.

Additionally, I identified independent SNPs by conducting pair-wise linkage disequilibrium (LD) tests across ddRAD-seq autosomal SNPs (--indep-pairwise 2 1 0.5) in which 1 of 2 linked SNPs are randomly excluded if we obtained an LD correlation factor (r^2) > 0.5. These steps were done on independent datasets for each *P. modestum* and *C. texanus*, thereby generating two sets of output files. I conducted all analyses without *a priori* information on population or species identity.

2.3 Genetic Analysis

To visualize population structure for *P. modestum* and *C. texanus*, I used their respective independent bi-allelic SNP datasets to run a Principal Component Analyses in R v. 4.2.1 using the "prcomp" function and following the approach outlined in Novembre and Stevens (2008). Next, the program ADMIXTURE v. 1.3.0 (Alexander and Lange 2011) was used to find individual assignment probabilities in a maximum-likelihood framework for *K* 1-10. Each run was completed using a quasi-Newton algorithm (Zhou et al. 2011) and 5-fold cross-validation (CV). Each run was terminated when convergence was reached, defined as log-likelihood increase <0.0001 between iterations (Alexander et al. 2009). The optimal *K* was determined by CV error values as well as manual inspection of further *K*'s for structure resolution. Then,

Next, to investigate and visualize migration rates across the sampled area the program Estimated Effective Migration Surfaces (EEMS) was used (Petkova et al. 2015). A genetic dissimilarity matrix was created using the bed2diffs.py script included with the EEMS program. An outline of the sampled area was created in Qgis v. 3.26.1 (QGIS Development Team 2022) and then converted into a list of GPS coordinates for use as a required input in the EEMS

program. Next, the runeems_snps function was ran for each species separately. The number of demes for each run was set to 300 and Monte Carlo Markov Chain iterations were set to 8,000,000 with a burn-in period of 1,000,000 iterations. This was repeated several times from random seeds to ensure convergence. These outputs were then visualized using the R package "rEEMSplots" included in the EEMS github repository available at https://github.com/dipetkov/eems.

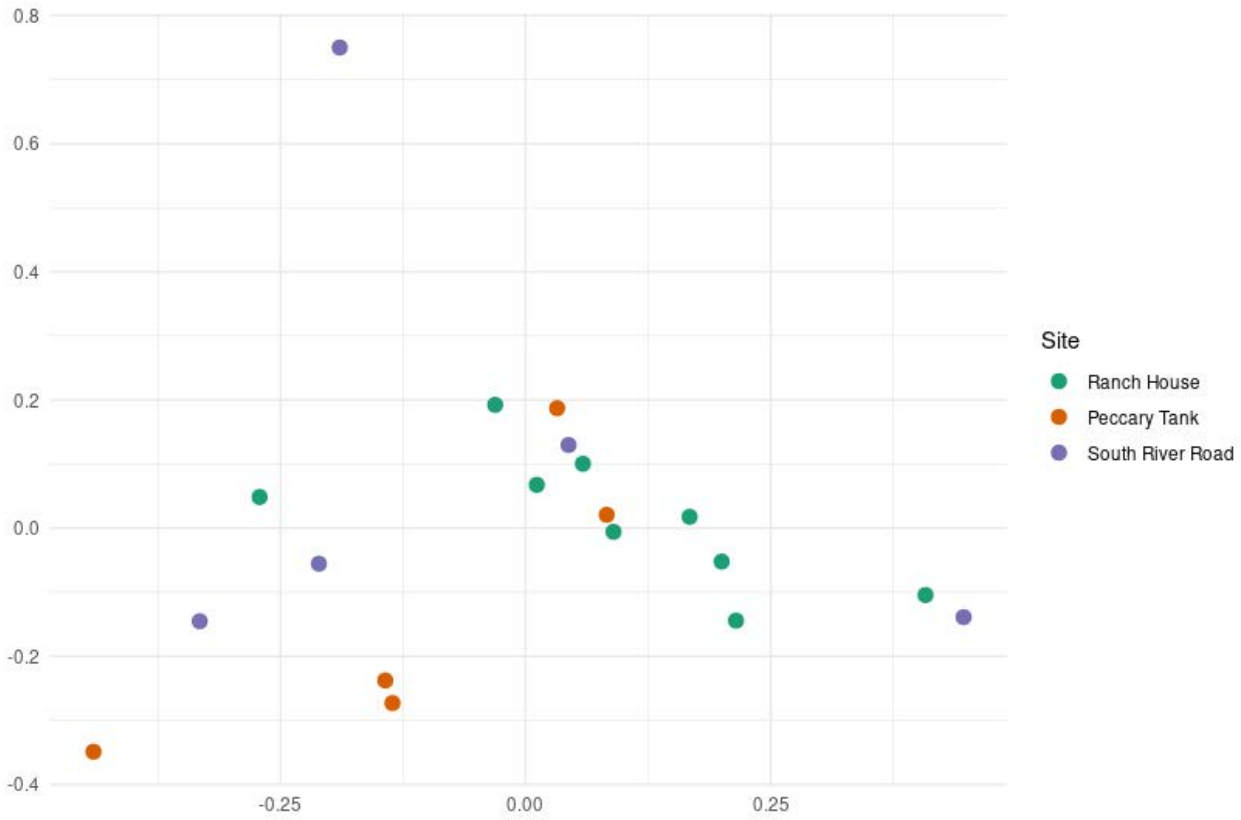

Figure 5: Scatter plot of PC1 (x-axis) and PC2 (y-axis) with 8,062 biallelic SNPs for *Phrynosoma modestum* (PC1 proportion of variance = 6.42 & PC2 proportion of variance = 6.24).

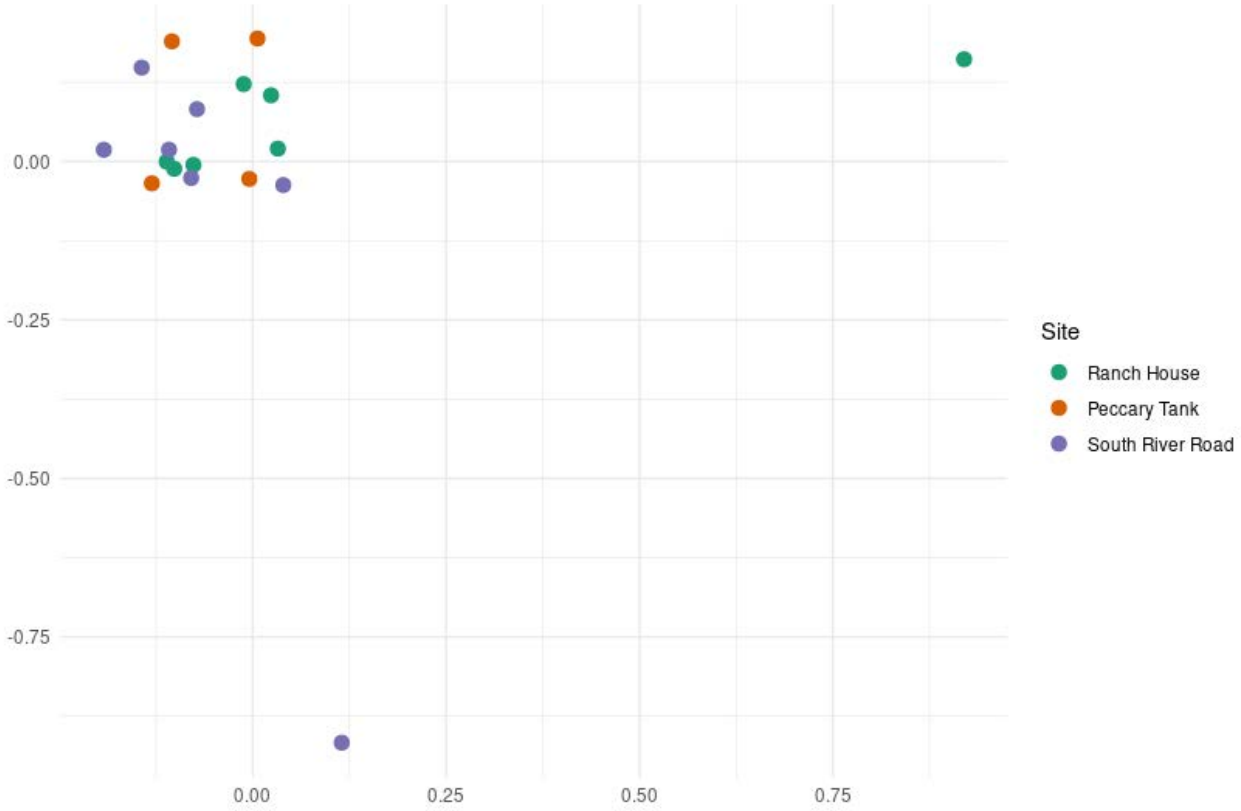

Figure 6: Scatter plot of PC1 (x-axis) and PC2 (y-axis) with 8,416 biallelic SNPs for *Cophosaurus texanus* (PC1 proportion of variance = 6.96 & PC2 proportion of variance = 6.61).

3 Results

3.1 Population Structure

First, a total of 8,063 ddRADseq loci were recovered after filtering the *P. modestum* data set. These loci included a total of 9,757 single-nucleotide polymorphisms (SNPs). These loci had an average sequencing depth of 87 reads per locus per individual. PCA and ADMIXTURE analyses were based on the SNP dataset. Plotting the first two principal components in the PCA did not recover any population structuring (Figure 5), and this was corroborated with ADMIXTURE analyses recovering an optimum population *K* of one based on CV error values (Figure XXX). While I explored additional *K* values (Janes et al. 2017), no additional structuring was found within *P. modestum*.

Next, a total of 8,416 ddRADseq loci were recovered after filtering the *C. texanus* data set that included a total of 9,843 SNPs. These loci had an average sequencing depth of 83 reads per locus per individual. Again, analyzing the SNP data also found no discernable structuring in the PCA and ADMIXTURE (Figure 6). As with *P. modestum*, ADMIXTURE analyses recovered an optimum population *K* value of one. While additional *K* values were once again explored, no structuring was revealed for *C. texanus*.

3.2 Estimated Effective Migration Surfaces

Estimated effective migration surfaces were calculated for each species separately. The resulting migration maps created highlighted areas of relative high migration in blue and areas of relative low migration in red. Areas of relative low migration for *P. modestum* closely followed

the mountainous areas of the sampling location, while flatter areas showed the highest levels of migration (Figure 7). Migration maps generated for *C. texanus* showed a nearly identical pattern of low migration rates in mountainous areas and high migration in flatter areas; however, patterns of low migration were lower in intensity (i.e. low migration areas were much lighter in color as compared to *P. modestum*; Figure 8).

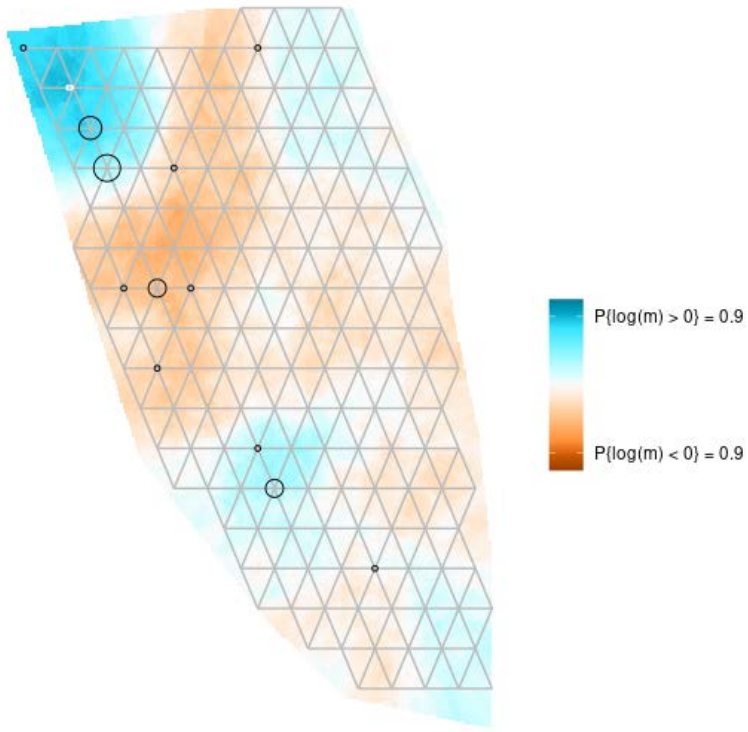

Figure 7: Contour maps of effective migration (m) for *Phrynosoma modestum*, areas of higher than expected migration under isolation-by-distance (IBD) are shown in blue and areas of lower than expected migration under IBD are shown in red.

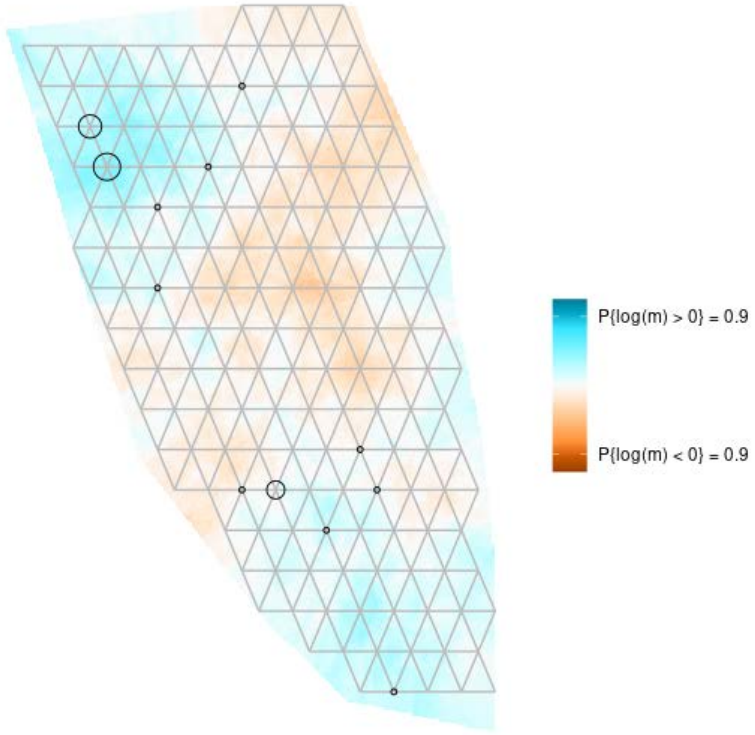

Figure 8: Contour maps of effective migration (m) for *Cophosaurus texanus*, areas of higher than expected migration under isolation-by-distance (IBD) are shown in blue and areas of lower than expected migration under IBD are shown in red.

4 Discussion

PCA and ADMIXTURE analyses failed to present a clear picture of structuring in either of the study species. This could be due to the small spatial scale of the study, which has been known to cause biases in both types of analyses (Novembre and Stephens 2008, Zheng and Weir 2016). ADMIXTURE failed to produce any discernable structuring, while the PCA seemed to perform slightly better, particularly for *P. modestum* (Figure 5 & 6). This may be due to uneven sampling which is known to heavily affect ADMIXTURE analyses (Wang 2022). While previously PCA's have been noted to suffer from uneven sampling (Petkova et al. 2015, Bradburd et al. 2016), some studies have since found PCAs to be relatively robust in regards to sampling schemes (House and Hahn 2018). Genetic structuring being slightly more apparent in *P. modestum* lends some support to the idea that *P. modestum* could be more strongly affected by the mountainous area due to some ecological considerations as compared to *C. texanus* (Siepielski et al. 2016, Lowe et al. 2017). However, a wider sampling range may be needed to better understand the degree to which structuring occurs within these species and what the potential causes may be.

The migration maps appeared to perform much better at the smaller spatial scale of this project. Despite only limited evidence of genetic structuring in each species, EEMS provided a clearer picture of how genetic differentiation occurs across a heterogenous environment. Unlike the PCA and ADMIXTURE analyses, which likely suffered from uneven sampling to various degrees, EEMS has been found to be robust to sampling gaps and has been noted for being particularly efficient at identifying geographic barriers to gene flow such as those presented here (House and Hahn 2018). Both data sets found very similar results, despite having non-identical sampling ranges. This could lend further evidence that uneven sampling did not meaningfully

impact the results. The migration surfaces accurately outlined the entire mountainous areas of the study as having below average migration, while flatter more accessible areas had higher than average migration (Figure 7 & 8). This was true for both species, but showed a stronger effect on *P. modestum*, further supporting the hypothesis that ecological factors may play a role in how geographic barriers affect genetic structuring. However, some studies have found that environmental factors may play a larger role than geographic barriers in genetic structuring in other desert squamates (Myers et al. 2019). This study did not include environmental factors, but future studies comparing the effects of geographic barriers, or isolation by resistance (Petkova et al. 2015), and the effects of environmental factors, or isolation by environment (McRae 2006, Wang and Bradburd 2014), should be conducted.

While many similar studies have found environmental factors to be most important in genetic structuring (Myers et al. 2019, Mahtani-Williams et al. 2020), many others have found evidence that geographic barriers can have strong effects on migration rates and genetic diversity (Richmond et al. 2017, Bouzid et al. 2022). Myers et al. (2019) and Mahtani-Williams et al. (2020) both found strong evidence that climatic factors influence genetic structuring in arid-dwelling snakes. However, Mahtani-Williams et al. (2020) also found that geographic barriers did influence gene flow, albeit less so than climate. Richmond et al. (2017) found evidence that large historical lakes acted as barriers to gene flow in an endangered lizard, while Bouzid et al. (2022) found evidence for both geographic barriers and environmental barriers to gene flow in a lizard species closely related to both focal species of this study. The findings of this study suggest that geographic barriers do affect migration rates and genetic diversity, despite little evidence for genetic structuring. This has been found in other similar studies of fine geographic scales (Kudla et al. 2021). Kudla et al. (2021) found that despite very little structuring in the

Michigan state-endangered snake species, *Sistrurus catenatus*, measures of migration rates and diversity followed geographic patterns of a heterogenous environment similar to the findings of this study.

Many of these similar studies have focused on species of conservation concern and attempted to use their findings to inform future conservation plans (Richmond et al. 2017, Jones et al. 2021, Kudla et al. 2021). Understanding how heterogeneity in a species environment may influence population persistence and genetic patterns could help inform future conservation plans, particularly in cases of loss of gene flow, low genetic diversity, and inbreeding depression (Allendorf et al. 2010b, Frankham 2015, Jones et al. 2021). One of the focal species of this study, *P. modestum*, has been found to have a higher EVS score than its IUCN designation would suggest (Wilson et al. 2013), and a closely-related species, *P. cornutum*, is protected in the state of Texas. Future conservation efforts for *P. modestum* may be needed, and an understanding of how geographic barriers affect the genetic diversity of this species can help direct these potential plans (Diniz-Filho et al. 2009). These findings may also potentially be extrapolated to closely-related species with similar ecological niches such as *P. cornutum*, in the context of conservation planning.

In conclusion, examination of geographic barriers revealed genetic differentiation and differential migration rates at a fine scale, despite limited evidence of genetic structuring in two species of lizards in the Indio Mountains. These findings combined with ecological and environmental factors may be used to form more holistic conservation planning in vulnerable species (Diniz-Filho et al. 2009, Allendorf et al. 2010b).

Literature Cited

Alexander, D. H., and K. Lange. 2011. Enhancements to the ADMIXTURE algorithm for individual ancestry estimation. BMC Bioinformatics 12:246.

Alexander, D. H., J. Novembre, and K. Lange. 2009. Fast model-based estimation of ancestry in unrelated individuals. Genome Research 19:1655–1664.

Allendorf, F. W., P. A. Hohenlohe, and G. Luikart. 2010a. Genomics and the future of conservation genetics. Nature Reviews Genetics 11:697–709.

Allendorf, F. W., P. A. Hohenlohe, and G. Luikart. 2010b. Genomics and the future of conservation genetics. Nat Rev Genet.

Amos, J. N., A. F. Bennett, R. Mac Nally, G. Newell, A. Pavlova, J. Q. Radford, J. R. Thomson, M. White, and P. Sunnucks. 2012. Predicting landscape-genetic consequences of habitat loss, fragmentation and mobility for multiple species of woodland birds. PLoS ONE 7.

Andrews, K. R., and G. Luikart. 2014. Recent novel approaches for population genomics data analysis. Molecular Ecology 23:1661–1667.

Barbault, R., and M. E. Maury. 1981. Ecological organization of a Chihuahuan desert lizard community. Oecologia 51:335–342.

Begun, D. J., A. K. Holloway, K. Stevens, L. W. Hillier, Y.-P. Poh, M. W. Hahn, P. M. Nista, C. D. Jones, A. D. Kern, C. N. Dewey, L. Pachter, E. Myers, and C. H. Langley. 2007. Population Genomics: Whole-Genome Analysis of Polymorphism and Divergence in Drosophila simulans. PLoS Biology 5:e310.

Bouzid, N. M., J. W. Archie, R. A. Anderson, J. A. Grummer, and A. D. Leaché. 2022. Evidence for ephemeral ring species formation during the diversification history of western fence

lizards (Sceloporus occidentalis). Molecular Ecology 31:620–631.

Bradburd, G. S., P. L. Ralph, and G. M. Coop. 2016. A Spatial Framework for Understanding Population Structure and Admixture. PLoS Genetics 12:e1005703.

Bulova, S. J. 1994. Ecological correlates of population and individual variation in antipredator behavior of two species of desert lizards. Copeia 1994:980–992.

Casillas, S., and A. Barbadilla. 2017. Molecular Population Genetics. Genetics 205:1003–1035.

Charlesworth, B., and D. Charlesworth. 2017. Population genetics from 1966 to 2016. Heredity 118:2.

Cooper, W. E., and W. C. Sherbrooke. 2010. Crypsis influences escape decisions in the Round-tailed Horned Lizard (Phrynosoma modestum). Canadian Journal of Zoology 88:1003–1010.

Cooper, W. E., and W. C. Sherbrooke. 2012. Choosing between a rock and a hard place: Camouflage in the round-tailed horned lizard Phrynosoma modestum. Current Zoology 58:541–548.

DaCosta, J. M., and M. D. Sorenson. 2014. Amplification Biases and Consistent Recovery of Loci in a Double-Digest RAD-seq Protocol. PLoS ONE 9:e106713.

Davey, J. W., and M. L. Blaxter. 2010. RADSeq: next-generation population genetics. Briefings in Functional Genomics 9:416–423.

Diniz-Filho, J. A. F., J. C. Nabout, M. Pires de Campos Telles, T. N. Soares, and T. F. L. V. B. Rangel. 2009. A review of techniques for spatial modeling in geographical, conservation and landscape genetics. Genetics and Molecular Biology 32:203–211.

Edgar, R. C. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research 32:1792.

Edgar, R. C. 2010. Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26:2460–2461.

Engeling, G. A. 1972. Ecology of the Iguanid lizard Cophosaurus Texanus (Troschel) in Comal County, Texas. Southwest Texas State University.

Farleigh, K., S. A. Vladimirova, C. Blair, J. T. Bracken, N. Koochekian, D. R. Schield, D. C. Card, N. Finger, J. Henault, A. D. Leaché, T. A. Castoe, and T. Jezkova. 2021. The effects of climate and demographic history in shaping genomic variation across populations of the Desert Horned Lizard (Phrynosoma platyrhinos). Molecular Ecology 30:4481–4496.

Ferner, J. 2007. A review of marking and individual recognition techniques for amphibians and reptiles. Herpetological Circulars 35.

Frankham, R. 2015. Genetic rescue of small inbred populations: meta-analysis reveals large and consistent benefits of gene flow. Molecular Ecology 24:2610–2618.

Garvin, M. R., K. Saitoh, and A. J. Gharrett. 2010. Application of single nucleotide polymorphisms to non-model species: A technical review. Molecular Ecology Resources 10:915–934.

Gonser, R., and R. Collura. 1996. Waste Not, Want Not: Toe-Clips as a Source of DNA. Journal of Herpetology 30:445–447.

Henrickson, J., and M. Johnston. 1983. Vegetation and community types in the Chihuahuan Desert. Pages 20–39 Invited papers from the Second symposium on resources of the Chihuahuan Desert region: United States and Mexico.

House, G. L., and M. W. Hahn. 2018. Evaluating methods to visualize patterns of genetic differentiation on a landscape. Molecular Ecology Resources 18:448–460.

Janes, J. K., J. M. Miller, J. R. Dupuis, R. M. Malenfant, J. C. Gorrell, C. I. Cullingham, and R.

L. Andrew. 2017. The K = 2 conundrum. Molecular Ecology 26:3594–3602.

Johnson, G. W. 1998. Diversity and Seasonal Growth Patterns of Lizards at Indio Mountain Research Center.

Jones, M. R., Daniel, E. Winkler, and R. Massatti. 2021. Demographic modeling informs functional connectivity and management interventions in Graham's beardtongue. Conservation Genetics 22:993–1003.

Kudla, N., E. M. McCluskey, V. Lulla, R. Grundel, and J. A. Moore. 2021. Intact landscape promotes gene flow and low genetic structuring in the threatened Eastern Massasauga Rattlesnake. Ecology and Evolution 11:6276–6288.

Lewontin, R. C. 1985. Population Genetics. Annual Review of Genetics 19:81–102.

Lowe, W. H., R. P. Kovach, and F. W. Allendorf. 2017. Population Genetics and Demography Unite Ecology and Evolution. Trends in Ecology & Evolution 32:141–152.

Luikart, G., P. R. England, D. Tallmon, S. Jordan, and P. Taberlet. 2003. The power and promise of population genomics: From genotyping to genome typing. Nature Reviews Genetics 4:981–994.

Mahtani-Williams, S., W. Fulton, A. Desvars-Larrive, S. Lado, J. P. Elbers, B. Halpern, D. Herczeg, G. Babocsay, B. Lauš, Z. T. Nagy, D. Jablonski, O. Kukushkin, P. Orozco-terWengel, J. Vörös, and P. A. Burger. 2020. Landscape Genomics of a Widely Distributed Snake, Dolichophis caspius (Gmelin, 1789) across Eastern Europe and Western Asia. Genes 11:1218.

Manel, S., and R. Holderegger. 2013. Ten years of landscape genetics. Trends in Ecology & Evolution 28:614–621.

Mata-Silva, V. (n.d.). Long-term recapture data. Unpublished data.

Maury, M. E. 1995. Diet Composition of the Greater Earless Lizard (Cophosaurus texanus) in Central Chihuahuan Desert. Journal of Herpetology 29:266.

McRae, B. H. 2006. ISOLATION BY RESISTANCE. Evolution 60:1551–1561.

Merilä, J., B. C. Sheldon, and L. E. B. Kruuk. 2001. Explaining stasis: Microevolutionary studies in natural populations. Genetica 112–113:199–222.

Munger, J. C. 1984. Home ranges of horned lizards (Phrynosoma): circumscribed and exclusive? Oecologia 62:351–360.

Myers, E. A., A. T. Xue, M. Gehara, C. L. Cox, A. R. Davis Rabosky, J. Lemos-Espinal, J. E. Martínez-Gómez, and F. T. Burbrink. 2019. Environmental heterogeneity and not vicariant biogeographic barriers generate community-wide population structure in desert-adapted snakes. Molecular Ecology 28:4535–4548.

Novembre, J., and M. Stephens. 2008. Interpreting principal component analyses of spatial population genetic variation. Nature Genetics 40:646–649.

Osmanski, A. B. 2014. On the Use of Unmanned Aerial Vehicles to Rapidly Assess Microhabitats of Two Texas Lizard Species, Cophosaurus Texanus and Aspidoscelis Gularis. Angelo State University.

Perry, G. 1999. The evolution of search modes: Ecological versus phylogenetic perspectives. American Naturalist 153:98–109.

Perry, G., and T. Garland Jr. 2002. Lizard Home Ranges Revisited: Effects of Sex, Body Size, Diet, Habitat, and Phylogeny. Ecology 83:1870.

Peterson, B. K., J. N. Weber, E. H. Kay, H. S. Fisher, and H. E. Hoekstra. 2012. Double Digest RADseq: An Inexpensive Method for De Novo SNP Discovery and Genotyping in Model and Non-Model Species. PLoS ONE 7:e37135.

Petkova, D., J. Novembre, and M. Stephens. 2015. Visualizing spatial population structure with estimated effective migration surfaces. Nature Genetics 9:94–100.

Pianka, E. R., and W. S. Parker. 1975. Ecology of Horned Lizards: A Review with Special Reference to Phrynosoma platyrhinos. Copeia 1975:141.

Price, J., C. Henry, A. Standen, and J. Posey. 1985. Origin of silver-copper-lead deposits in Red-Bed sequences of Trans-Pecos Texas: Tertiary mineralization in Precambrian, Permian, and Cretaceous sandstones.

QGIS Development Team. 2022. QGIS Geographic Information System.

Richmond, J. Q., D. A. Wood, M. F. Westphal, A. G. Vandergast, A. D. Leaché, L. R. Saslaw, H. S. Butterfield, and R. N. Fisher. 2017. Persistence of historical population structure in an endangered species despite near-complete biome conversion in California's San Joaquin Desert. Molecular Ecology 26:3618–3635.

Schmidt, R. H. 1979. A climatic delineation of the 'real' Chihuahuan Desert. Journal of Arid Environments 2:243–250.

Segelbacher, G., S. A. Cushman, B. K. Epperson, M. J. Fortin, O. Francois, O. J. Hardy, R. Holderegger, P. Taberlet, L. P. Waits, and S. Manel. 2010. Applications of landscape genetics in conservation biology: Concepts and challenges. Conservation Genetics 11:375–385.

Shaffer Jr., D. T., and W. G. Whitford. 1981. Behavioral Responses of a Predator, the Round-Tailed Horned Lizard, Phrynosoma modestum and Its Prey, Honey Pot Ants, Myrmecocystus spp. American Midland Naturalist 105:209.

Siepielski, A. M., A. Nemirov, M. Cattivera, and A. Nickerson. 2016. Experimental evidence for an eco-evolutionary coupling between local adaptation and intraspecific competition.

American Naturalist 187:447–456.

Simões, T. R., and R. A. Pyron. 2021. The Squamate Tree of Life. Bulletin of the Museum of Comparative Zoology 163:47–95.

Verwaijen, D., and R. Van Damme. 2008. Wide home ranges for widely foraging lizards. Zoology 111:37–47.

Wang, I. J., and G. S. Bradburd. 2014. Isolation by environment. Molecular Ecology 23:5649–5662.

Wang, J. 2022. Fast and accurate population admixture inference from genotype data from a few microsatellites to millions of SNPs. Heredity.

Wilson, L. D., V. Mata-Silva, and J. D. Johnson. 2013. A conservation reassessment of the reptiles of Mexico based on the EVS measure. Amphibian and Reptile Conservation 7:1–47.

Worthington, R. D., J. D. Johnson, C. S. Lieb, and W. Anderson. 2019. Natural Resources and Physical Environment of Indio Mountains Research Station (IMRS) Southeastern Hudspeth County, Texas: A Handbook for Students and Researchers.

Zheng, X., and B. S. Weir. 2016. Eigenanalysis of SNP data with an identity by descent interpretation. Theoretical population biology 107:65–76.

Zhou, H., D. Alexander, and K. Lange. 2011. A quasi-Newton acceleration for high-dimensional optimization algorithms. Stat Comput 21:261–273.

Curriculum Vitae

L. Miles Horne graduated with a Bachelor of Science in Organismal Biology from Auburn University in 2019. He started in the University of Texas at El Paso's Master's program in Biology the following semester. During his time at Auburn, Miles studied physiology, evolution, ecology, and behavior in a variety of systems including gopher tortoises, cane toads, eastern indigo snakes, American alligators, whiptail lizards, southeast Asian coral snakes, etc. He was an undergraduate teaching assistant for a Herpetology course and spent summers doing fieldwork and tutoring for a variety of subjects. He also worked as a field assistant for the Alabama Natural Heritage Program monitoring protected herpetofauna in the state. At UTEP, Miles was a graduate teaching assistant for Topics in the Study of Life lab and worked as a graduate research assistant in the Office of the Provost helping research roadblocks causing students to stop out and helping inform curricular reformation. He was also active in outreach with the UTEP Biodiversity Collections, presenting both insects and herpetofauna to varying audiences in classrooms, state parks, zoos, and museums.

He plans to begin his PhD in the Fall of 2022 in Dr. Brett Seymoure's lab at UTEP studying microevolution, behavior, and physiology in phototactic insect systems. He also plans to continue research in education. After completion of a PhD, Miles hopes to continue in academia with a post-doctoral position and eventually a tenure-track faculty position at a research university.

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