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(*Phrynosoma mcallii*) in California's Imperial Valley

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Fine-scale Genomic Assessment and Monitoring of the Flat-tailed Horned Lizard (*Phrynosoma mcallii*) in California's Imperial Valley

By

CRAIG G. FISCHER
DISSERTATION

Submitted in partial satisfaction of the requirements for the degree of

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in

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of the

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Dedication

I dedicate this dissertation to my parents, Jenny and Dean. At every stage of my life, they encouraged and nurtured my curiosity. Mom always encouraged me to read, to absorb the life experience and knowledge of others, and to embrace my gentle side. Dad fostered in me a genuine sense of adventure and a love for the maligned critters of the world. He got me out of my comfort zone and taught me to find a new comfort zone in the embrace of the unknown. In pursuit of this unknown I found work in some of the most remote reaches of this country, far away from home. Their continual support, no matter my distance, has allowed me to seek a personal and professional life in the desert southwest that has brought me tremendous peace and happiness. The work below should serve as some evidence of what that support means to me.

Abstract

The flat-tailed horned lizard (*Phrynosoma mcallii*) is a Colorado Desert endemic species with a long history of conservation and active management. Ongoing monitoring has relied on traditional mark-recapture data to inform management action. I used reduced representation genotype-by-sequencing techniques to generate a large single nucleotide polymorphism data set to assess fine-scale *P. mcallii* population structure, dispersal, landscape barriers to gene flow, and genetic diversity in California's Imperial Valley with the overall goal to inform ongoing management and monitoring of the species.

In my first chapter, over 500 individual *P. mcallii* DNA samples were genotyped to assess fine-scale population structure across the Imperial Valley. Using sparse non-negative matrix factorization paired with geographic capture data and discriminant analysis of principal components, I detected population structure across the east-west axis of the Imperial Valley, which had been described using mitochondrial DNA markers. I observed less pronounced but significant population structure between West Mesa and Yuha Basin populations which may indicate contemporary barriers to migration. I detailed estimates of contemporary genetic diversity, finding generally lower than expected heterozygosity, low inbreeding, and little variation in nucleotide diversity. These results indicate management-relevant population structure that aligns with existing management units.

In my second chapter I developed genetic pedigrees to identify sex-biased patterns of dispersal, isolation-by-distance and landscape barriers to gene flow. I calculated pairwise relatedness between individuals and measured the decay of relatedness by distance to investigate sex-biased dispersal; with these methods we detected evidence of male-biased

dispersal. I also used this data to build pedigrees that revealed the first documented instance of multiple paternity in *P. mcallii*. I tested for isolation-by-distance using the inverse proportion of shared alleles and found patterns that indicate high gene flow, and little effect of isolation-by-distance. We used Estimated Effective Migration Surfaces to identify barriers to gene flow associated with CA-98 and I-8 as well as Superstition Mountain and two BLM OHV areas. These contemporary barriers seem to reduce gene flow, but it is unclear to what extent. We recommend further landscape analyses to understand how landscape arrangement and abiotic factors may influence gene flow in the Western Imperial Valley.

In my third chapter I used samples collected from ongoing *P. mcallii* long-term monitoring plots to estimate baseline measures of genetic diversity for three management areas, estimate effective population size on each demographic monitoring plot, and test for heterozygosity fitness correlations. I found no evidence of inbreeding through estimation of genetic diversity or heterozygosity fitness correlations. Effective population sizes differed by plot but were limited in their applicability beyond the scale of sampling. I estimated an effective population size of 1,048 individuals across the western Imperial Valley. I then synthesized the results of these chapters into recommendations for ongoing management of the species. I recommend the integration of genetic monitoring into ongoing monitoring efforts including decadal reassessments of genetic diversity and effective population sizes, with additional recommendations to assemble a high-quality *P. mcallii* reference genome, to research genetic variation near range edges, to research climate change adaptation, and to investigate hybrid zone dynamics with *P. platyrhinos* and *P. goodei*.

CHAPTER 1

Fine-scale Landscape Genomics of *Phrynosoma mcallii* in California's Imperial Valley

Abstract

Population genetics has emerged as a powerful tool in wildlife conservation. Advancements have made genotyping hundreds of individuals at thousands of loci tractable for non-model species in poorly represented taxa. Reptiles are generally understudied compared to other taxa and are especially susceptible to anthropogenic landscape fragmentation and disturbance due to specialization and range-limitation. Here, we use reduced representation sequencing (ddRADseq) to assess subpopulation structure and genetic diversity for a species of conservation concern, the flat-tailed horned lizard, *Phrynosoma mcallii*, in the Imperial Valley, CA. We found evidence of population structure between Eastern and Western ends of the valley across habitat fragmented by extensive agriculture. We also identified weak population clustering within Western Imperial Valley. This differentiation is only partially attributable to isolation-by-distance, and may instead be due in part to Interstate 8 acting as an anthropogenic barrier to gene flow. We measured various parameters of genetic diversity at the population level, including expected and observed heterozygosity, nucleotide diversity, and inbreeding, and found no evidence of inbreeding or problematic genetic diversity in any sampled populations. This work provides baseline measures of genetic diversity at fine spatial scales for a species that acts as a conservation flagship for the Colorado desert and will allow us to examine temporal trends in future years as anthropogenic fragmentation of this area continues. Our findings

underscore the importance of incorporating genetic data into long-term monitoring and management of the species.

Introduction

In recent decades, population genetics has emerged as a critical tool for biodiversity conservation and management (Benestan et al., 2016; Funk et al., 2012; Hohenlohe et al., 2021). Advances in the field now allow access to much more of the genome, improving population and individual-level inference, and allowing use of more complete genomic data in non-model systems (Ekblom & Galindo, 2011; Garner et al., 2016). Advances in developing molecular markers and sequencing strategies have supported discoveries of fundamental ecological and evolutionary patterns that inform management and conservation of at-risk species (Chen et al., 2025; Vandergast et al., 2016). Genetic variation in neutral genomic regions can reveal patterns of population structure and connectivity necessary to delimiting conservation units and identifying regions or corridors of conservation priority (Balkenhol, 2016; Waits et al., 2015; Waits & Storfer, 2015). Variation in coding regions drives evolutionary adaptation, which is necessary for species persistence in the face of ongoing anthropogenic impacts, like those associated with climate change (Flanagan et al., 2018; Hoffmann & Sgrò, 2011; Seaborn et al., 2021). It is therefore critical to establish a baseline understanding of genetic variation in natural populations to formulate appropriate management actions and conservation objectives, both now and in the future.

Understanding how gene flow occurs across a landscape can improve conservation and management of species by identifying barriers to connectivity and highlighting population

structure. In non-model, free-ranging species, molecular approaches have revealed effects of anthropogenic and natural landscape barriers to genetic connectivity. For example, human-made barriers like fenced highways and canals prevented dispersal between proximal populations of desert bighorn sheep, *Ovis canadensis nelsoni*, resulting in decreased genetic diversity between populations (Epps et al., 2005). In western Australia, the Swan/Canning River systems were identified as barriers to gene flow in western tiger snakes, *Notechis scutatus occidentalis*, but researchers also found early signals of declining genetic diversity and body condition in highly urbanized sites (Lettoof et al., 2021). Wolverine, *Gulo gulo*, populations in the Rocky Mountains show genetic population structure at multiple scales influenced by separate biotic and abiotic factors; at small scale, gene flow was highly correlated with critical landscape variables like spring snow depth and terrain ruggedness, meanwhile housing density was found to influence spatial-genetic structure at broader spatial scales (Balkenhol et al., 2020). Genetic data can also be used to elucidate ecological processes, like dispersal, which require much greater effort to ascertain using traditional sampling methods (Alves et al., 2023). Despite the importance of genetic diversity, most species recovery plans and conservation efforts still do not explicitly incorporate sampling for genomic analyses (Ralls et al., 2018), particularly in data-deficient species.

Most reptiles, including those of conservation concern, have not had baseline assessments of genetic diversity or metapopulation connectivity. Since reptiles are often dispersal- and range-limited habitat specialists, they are particularly vulnerable to anthropogenic disturbance like fragmentation (Clark et al., 2010; Herrmann et al., 2017; Vandergast et al., 2016). Fragmentation divides core habitat into patches with increased edge

area, increasing risk of edge effects (Fischer & Lindenmayer, 2007; Haddad et al., 2015). Species whose core habitat has been significantly altered by human activity may exhibit demographic shifts between subpopulations (Barrows & Allen, 2009; Jackson & Fahrig, 2011), making a comprehensive understanding of the effects of habitat fragmentation critical to conservation and management (Lesbarrères & Fahrig, 2012).

The flat-tailed horned lizard, *Phrynosoma mcallii* (Figure 1.1), is a Colorado Desert endemic and flagship conservation species for the Colorado Desert and lower Colorado River region. *P. mcallii* is a dietary and habitat specialist, being more dependent on seed harvester ants as a food source than any other horned lizard species (Pianka & Parker, 1975) and being restricted to the arid sandy-soil habitats associated with the lower Colorado River basin. They are relatively sedentary and highly visually cryptic, making direct observation in the field challenging. As a range-limited habitat and dietary specialist, *P. mcallii* is considered Near Threatened by the IUCN. In recent decades, there have been multiple attempts at state and federal Endangered Species Act listing; as of now, it is considered a Species of Special Concern in California and a Tier 1 Species of Greatest Conservation Need in Arizona. A Rangewide Management Strategy (RMS) developed by state wildlife agencies and partner agencies managing extant *P. mcallii* habitat highlights the need to understand genetic variation among populations and to identify barriers to connectivity (*Flat-Tailed Horned Lizard Rangewide Management Strategy, 2003 Revision*, 2003). As a small lizard with relatively limited dispersal capabilities, short lifespan, and short generation time, we might expect that even recent landscape change could have a profound effect on sub-population structuring.

To date, molecular research of *P. mcallii* includes resolution of genus-wide *Phrynosoma* phylogeny (Gottscho et al., 2024; Hodges & Zamudio, 2004; Mulcahy et al., 2006), description of range-wide phylogeographic splits and hybridization using mitochondrial DNA markers (Mulcahy et al., 2006), range-wide phylogeography and population genetics using autosomal single nucleotide polymorphism (SNP) data (Gottscho et al., 2024), and assessment of fine-scale fragmentation across a roadway using SNPs in Yuma, AZ (Payne et al., 2025). Range-wide studies indicate that the Colorado River has a strong barrier effect on *P. mcallii* gene flow, with the Salton Sea contributing to finer-scale structure (Gottscho et al., 2024; Mulcahy et al., 2006). Payne et al. (2025) identified early signals of fragmentation in proximal populations bisected by relatively recent roadways in Yuma, AZ, suggesting that even recent anthropogenic landscape change can influence population genetic structuring in this species. No fine-scale assessments of genetic diversity and connectivity have yet been undertaken for California populations, where the highest degree of potential connectivity exists.

We sought to build on these past studies by using genomic data to examine population structure across multiple sites in California's Imperial Valley, spread across three of the six flat-tailed horned lizard interagency coordinating committee (FTHL ICC) Management Areas (MAs; East Mesa MA, West Mesa MA, and Yuha Basin MA). Our sampling design allowed us to assess the potential impacts of anthropogenic barriers (i.e. extensive agriculture, and roadways I-8 and CA-98) on patterns of genetic diversity and subpopulation structuring. We incorporated samples from over 500 individuals across the Imperial Valley and sequenced double-digest restriction site-associated DNA sequencing libraries to generate a dataset of single nucleotide polymorphisms (SNPs). We integrated multiple analytical techniques to identify distinct

population clusters, quantify fine-scale genetic structure, and assess the relationship between landscape barriers and patterns of genetic diversity.



Figure 1.1- *Phrynosoma mcallii*, the flat-tailed horned lizard in three life stages- hatchling, subadult, adult (left to right), Yuma, AZ

Materials and Methods

Sample Collection

We collected *P. mcallii* tissue samples from across the Imperial Valley (Figure 1.2) from 2021–2024 (n = 329), and collaborators at the Bureau of Land Management El Centro Field Office collected samples in 2019, and 2022–2024. Samples collected on BLM monitoring plots included East Mesa Management Area 1 (n = 49), West Mesa Management Area 1 (n = 13),

West Mesa Management Area 3 (n = 85), and Yuha Basin Management Area (n = 72).

Additionally, we collected three samples from Yuma, AZ during a training exercise in 2024.

Upon capture we recorded sex using external sex characteristics, mass to the nearest hundredth of a gram using an electronic GDEALER scale, and snout-to-vent length (SVL) to the nearest millimeter with a hard plastic ruler for each lizard. We marked lizards over 45 mm SVL with a passive integrated transponder (PIT) tag for identification in the event of recapture; smaller individuals were marked using a unique toe clipping sequence (Perry et al., 2011). Genomic DNA (gDNA) was collected via blood blot by nicking the caudal vein. Prior to DNA extraction we froze DNA blood blots on filter paper initially at -4–5° C until they could be transferred to a -20° C or -80° C freezer.

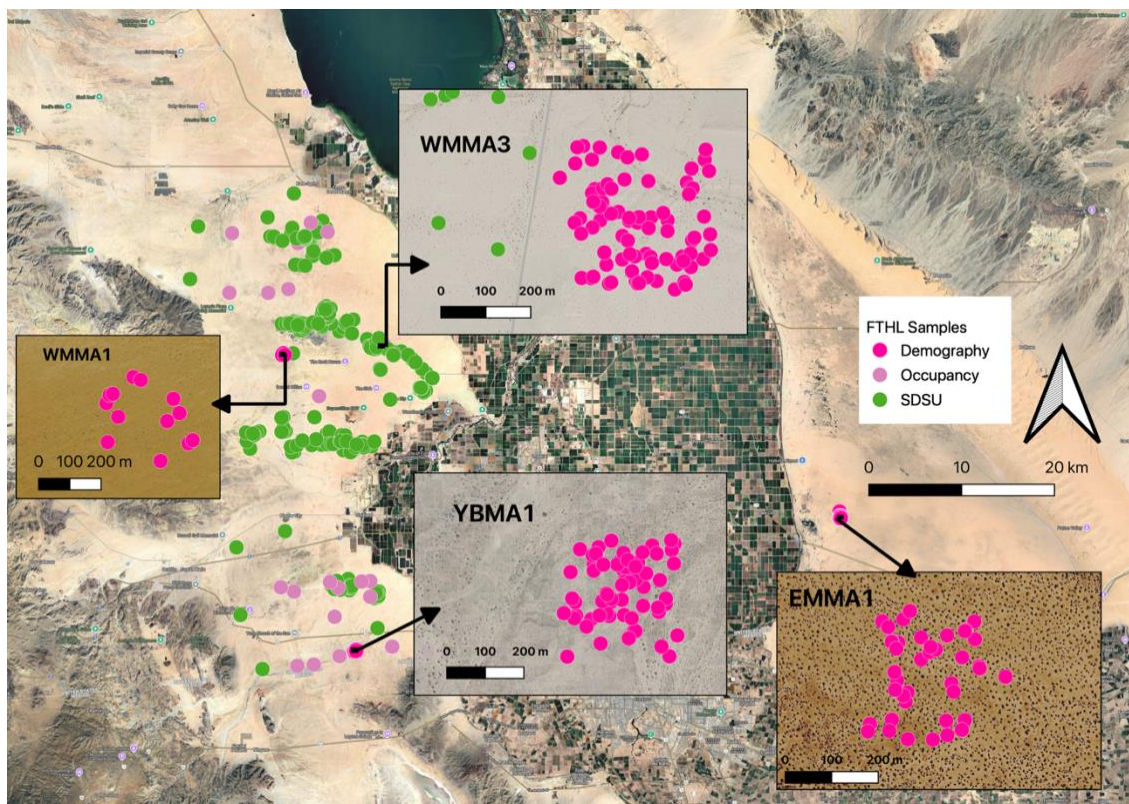


Figure 1.2 - FTHL DNA samples as colored dots across California's Imperial Valley (n = 570). Samples are colored by source- SDSU research team (green, n = 329) and BLM monitoring crews (pink, n = 241). FTHL samples collected by BLM from 2022-2024 on long-term monitoring plots, including Occupancy (n = 22). Demographic sampling plots include West Mesa Management Area plots 1 (n = 13) & 3 (n = 85), Yuha Basin Management Area plot 1 (n = 72), and East Mesa Management Area plot 1 (n = 49).

DNA Extraction, Library Preparation, and Sequencing

We extracted and purified genomic DNA using Qiagen DNeasy Blood and Tissue Kits (Qiagen, Valencia, California) in mini spin columns, with an overnight lysis step. We quantified purified DNA samples using Qubit4 (Thermo Fisher Scientific, Waltham, MA) and screened for RNA contamination using agarose gel separation. In total, we sent 6 plates representing 576 unique individual purified samples to the UC Davis DNA Technologies Core for library preparation and sequencing.

Genomic libraries were prepared at the UC Davis DNA Technologies Core using the double digest restriction site-associated DNA sequencing (ddRAD) method detailed in Peterson et al. (2012). 100ng of 576 gDNA samples digested with both MSP1 and PstHF (NEB, Ipswich, MA) restriction enzymes following the manufacturer's recommendation. These were ligated with MSP1 annealed adapters and GBS common adapters. 20 µl of each ligation product was pooled in batches of 24 samples. Each ligation pool was cleaned using a Zymo DNA clean and concentrator column (Thomas Scientific, LLC Swedesboro, NJ). We ran 5 ul of this cleaned ligation for 16 PCR cycles. The fragment size distribution of the libraries was verified via micro-capillary gel electrophoresis on a Bioanalyzer 2100 (Agilent, Santa Clara, CA) and we selected fragment sizes of approximately 350 bp. The final library was quantified by fluorometry on a Qubit fluorometer (LifeTechnologies, Carlsbad, CA). The pool was quantified by qPCR with a Lapa Library Quant Kit (Kapa Biosystems) and sequenced on an Illumina NovaSeqX 25B lane (Illumina, San Diego, CA) with paired-end 150 bp reads.

Bioinformatics Pipeline

Using the ipyrad pipeline v0.9.102 (Eaton & Overcast, 2020), sequence data was demultiplexed, trimmed, filtered, and aligned to the *P. platyrhinos* genome (Koochekian et al., 2022). We first demultiplexed each pool to individual using unique in-line adapter sequences. Then, we strictly filtered reads to remove adapters and primers as well as 3' read ends with more than 5 base calls showing low-quality Phred scores (<33). Pre- and post-filtering reads were assessed for quality using FastQC v0.12.0 (Andrews, 2010). Once sequence quality was confirmed we merged pools for ipyrad to cluster using a 90% clustering threshold. This step then maps reads to the reference genome. Ipyrad then estimates sequencing error rate and heterozygosity. Next, consensus allele sequences are estimated, and consensus sequences are clustered across samples then aligned. Lastly, final filters are applied, and output files are generated. Key parameters we used include a maximum of 5% uncalled bases (Ns) in consensus sequences, maximum 5% heterozygous bases allowed in consensus sequences, allowed a max of 10 SNPs per locus, and 10 indels per locus, and a maximum of 50% heterozygous sites per locus. We used the output VCF to further filter data.

We filtered our data using VCFTools (Danecek et al., 2011) to remove indels and keep only biallelic SNPs. We filtered at a minimum read depth of 10 (--minDP 10) and maximum read depth of 50 (--maxDP 50), and a 1% minor allele frequency cutoff (--MAF 0.01). We conservatively dropped individuals with over 80% missing data, which removed 21 individuals. We removed loci that weren't present in at least 85% of samples (--MISS 0.85). Following VCFTools filtering we used SNPfiltR (DeRaad, 2022) to filter for allele balance which can reduce

the impact of sequencing or mapping errors on the final dataset. Following allele balance conversion, we re-filtered loci at an 85% missingness cutoff.

Data Analyses

We assessed population structure using a complementary suite of approaches. Because population clustering analyses tend to over-cluster related individuals, we further filtered our data using the PLINK (Purcell et al., 2007) to remove parent-offspring, sibling, half-sibling, grandparent/grandchild, and avuncular relatives with a relatedness cutoff of 0.2 ($-rel\text{-}cutoff$ 0.2). We also pruned for linkage disequilibrium (LD) with a 100 kilobase window, stepping 10 variants. Before relatedness and LD pruning, we thinned data to only include one SNP per loci using a custom R script. First, we used the R package *adeigenet* (Jombart, 2008) to perform Discriminant Analysis of Principal Components (DAPC). DAPC maximizes between-group variation and minimizes within-group variation to identify population clusters. We used *adeigenet*'s cross-validation method for determining the appropriate number of PCs to retain for the DAPC as including too many PCs can risk overfitting the model. Using cross-validation score, we retained 200 PCs in our DAPC and visualized individual membership to three population clusters. We also used a-score optimization method which recommended using roughly 50 PCs, but we found no difference in membership assignment with either method, so we report on the model that used 200 PCs.

We further explored population structuring using R package LEA (Frichot & François, 2015) and tess3r (Caye et al., 2016) to visualize population structure and ancestry. We ran LEA's sparse non-negative matrix factorization (snmf) algorithm for $K = 1-10$, with 10 replicates for

each, and then selected the best run (lowest cross-entropy) for each of $K = 2-4$ clusters to plot Q-matrix bar graphs. Tess3r plots incorporate geospatial data to visualize spatial patterns in ancestry. We used tess3r to model spatial q-matrices for $k = 1-10$ with 10 replicates for each, then selected the best run (lowest cross-entropy) for each of $k = 2-4$ clusters to map Q-matrices over geographic sampling locations.

We also used *adegenet* to generate summary statistics including expected heterozygosity (H_E) and observed heterozygosity (H_O) and data missingness by population. We calculated Weir and Cockerham's weighted F_{ST} between sampling areas using VCFTools using a 200kb window stepping 100kb. Population F_{IS} was calculated using R package *hierfstat* (Goudet & Jombart, 2004). Nucleotide diversity (π) was calculated from variant and invariant sites using the R package *pegas* (Paradis et al., 2009) and the PHYLIP file output from the ipyrad pipeline, after filtering out loci missing in more than 30% of samples.

To investigate the influence of isolation by distance (IBD), we analyzed the pairwise effect of geographic distance on genetic distances using the inverse proportion of shared alleles (D_{PS}) between individuals. We assessed the effect size and significance of this association using a Mantel test with 9,999 permutations in the R Package *ade4* (Dray & Dufour, 2007). Because IBD can only properly reflect processes across connected populations we investigated IBD within the western Imperial Valley populations from the Yuha north through West Mesa, excluding East Mesa and Yuma samples.

Results

Genomic Data Structure

We initially received 3.2 billion reads, averaging 5.6 million reads per sample. 2.8 billion reads passed ipyrad's initial quality filtering, averaging 4.8 million reads per sample. 1.6 billion (58%) of reads mapped to the reference genome. Despite the low mapping percentage, many loci contributed to the final dataset, with a final average of 76.1 thousand loci per sample.

Our initial VCF used in downstream analyses had 2,097,293 variants across 573 individuals. Filtering individuals with over 80% missing data removed 21 individuals from the final dataset. Additionally, relatedness analysis revealed four pairs of samples with pairwise relatedness values near 1, indicating four individuals in the dataset had been re-sampled. The earlier sample from each pair was removed from the final dataset (23N3, 23N14, 23N17, 23N18). This yielded 72,914 variants across 548 samples (Table 1.1) with 8.45% missing data. This dataset was used to generate summary statistics, compute pairwise F_{ST} , and compare pairwise genetic distances (D_{PS}).

For the dataset used in population clustering analyses (DAPC, LEA and Tess3r plots), relatedness pruning removed 117 individuals from the dataset and only left one of three samples from the Yuma population, so we removed this sample to narrow the focus to California population structure (Table 1.1). This yielded 21,250 SNPs across 434 samples and an average data missingness of 8.61%.

Area	Total	Females	Males	Dataset A <i>post-filtering</i>	Dataset B <i>post-filtering</i>
WMN	290	137	153	286	233
WMS	99	44	55	98	79
Yuha	128*	62	65	119	90
EMS	49*	28	19	42	32
Yuma	3	3	0	3	0
Total	569	274	292	548	434

*Table 1.1- Numbers of samples by plot, sex, and in each dataset. Total includes all sequenced individuals, prior to any filtering. Dataset A includes all samples that made it past the first round of filtering, and a greater number of SNPs, Dataset B is further trimmed by linkage disequilibrium and relatedness for clustering analyses. *2 samples from EMS and 1 from Yuha have no sex data*

Summary Statistics

Measurements of mean expected heterozygosity by sampling area (Table 1.2) ranged from 0.140 in the Yuma population (N = 3) to 0.178 in the West Mesa North population (N = 286). Observed heterozygosity was slightly lower than expected for all California populations and nearly identical between sampling areas, ranging from 0.168 in East Mesa South (N = 42) to 0.169 in all western Imperial Valley sampling areas. Observed heterozygosity (0.164) was higher than expected (0.140) in the Yuma population, but because just three samples were present this should be interpreted with caution. Inbreeding coefficients were low across the range. In California, population-level F_{IS} ranged from 0.0371–0.0351, and as a result of higher-than-expected heterozygosity, the Yuma estimate was -0.0043. Nucleotide diversity was similar across all California populations (range 0.00056-0.00059), but greater in Yuma (0.00068). Data missingness showed no pattern across sampling areas, although East Mesa South had the highest degree of missingness at 12.85%.

Plot	N	H_E	H_O	π	F_{IS}	Missingness
WMN	286	0.178	0.169	0.00056	0.0371	8.43%
WMS	98	0.176	0.169	0.00058	0.0358	9.07%
Yuha	119	0.177	0.169	0.00059	0.0369	6.99%
EMS	42	0.174	0.168	0.00057	0.0351	12.85%
Yuma	3	0.140	0.164	0.00068	-0.0043	4.86%

Table 1.2- Summary statistics calculated using Dataset A (72,914 SNPs) include sampling area (Plot), number of lizards (N), expected heterozygosity (H_E), observed heterozygosity (H_O), nucleotide diversity (π) using variant and invariant sites, data missingness, and inbreeding coefficient (F_{IS})

Population Structure

DAPC best supports a one (BIC = 3251.9) or two-population (BIC = 3255.2) model, assigning all western Imperial Valley samples to cluster one, and all East Mesa samples to cluster two; samples from Yuha exclusively sort into a third cluster in a three-population model (BIC = 3258.9) (Figures 1.3, 1.4).

LEA ancestry coefficients reveal the same pattern. SNMF cross-entropy validation suggests the most support for K = 1 and K = 2 populations with a lowest cross entropy value of 0.467, but K = 3 (0.468) and K = 4 (0.469) had only marginally worse values. Samples show mixed ancestry across the valley, but bin as expected into East and West Mesa populations at K = 2; East Mesa, West Mesa, and Yuha Basin at K = 3; and East Mesa, West Mesa North, West Mesa South, and Yuha Basin at K = 4 (Figure 1.5). Individuals show majority ancestry for the population from which it was sampled with few exceptions at K = 4.

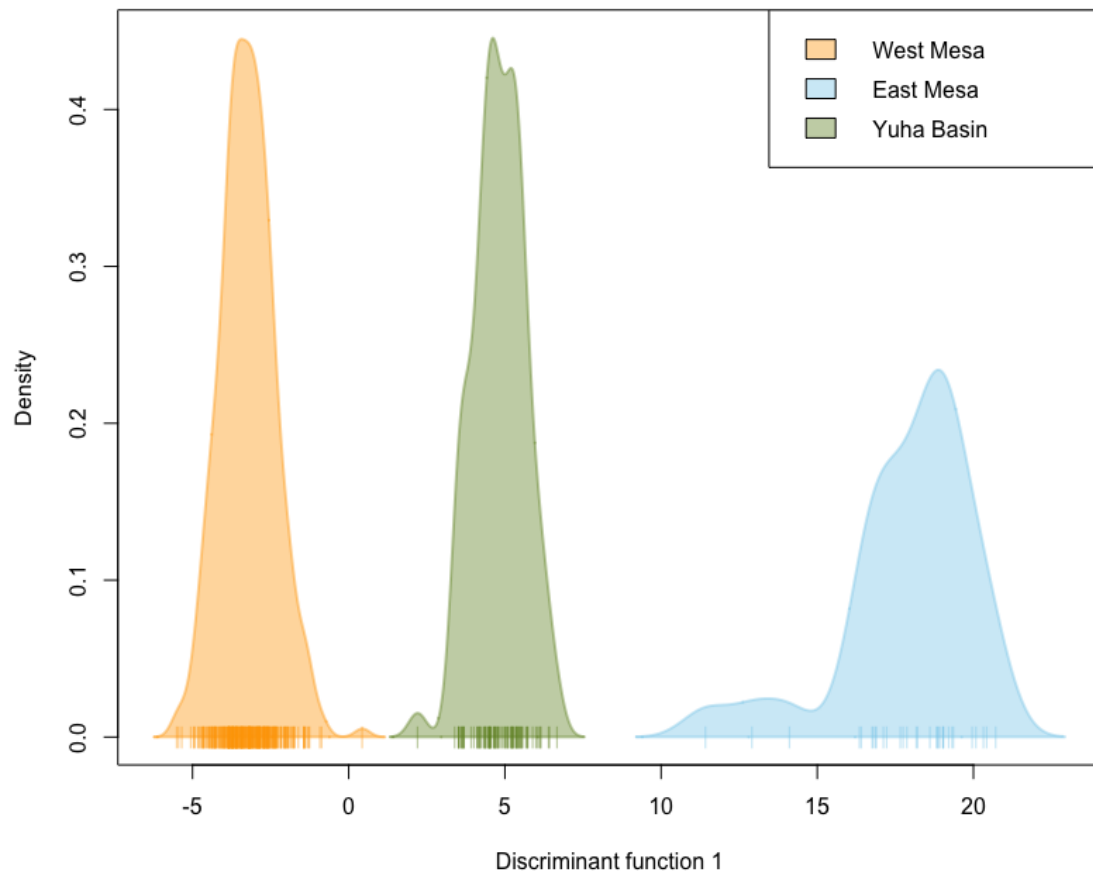


Figure 1.3- DAPC density plot in *ade4* using Dataset B. Retained 200 PCs, 3 clusters. Legend relabeled to show sampling area fully explains cluster membership- West Mesa, Yuha, and East Mesa populations all appear distinct with no membership overlap.

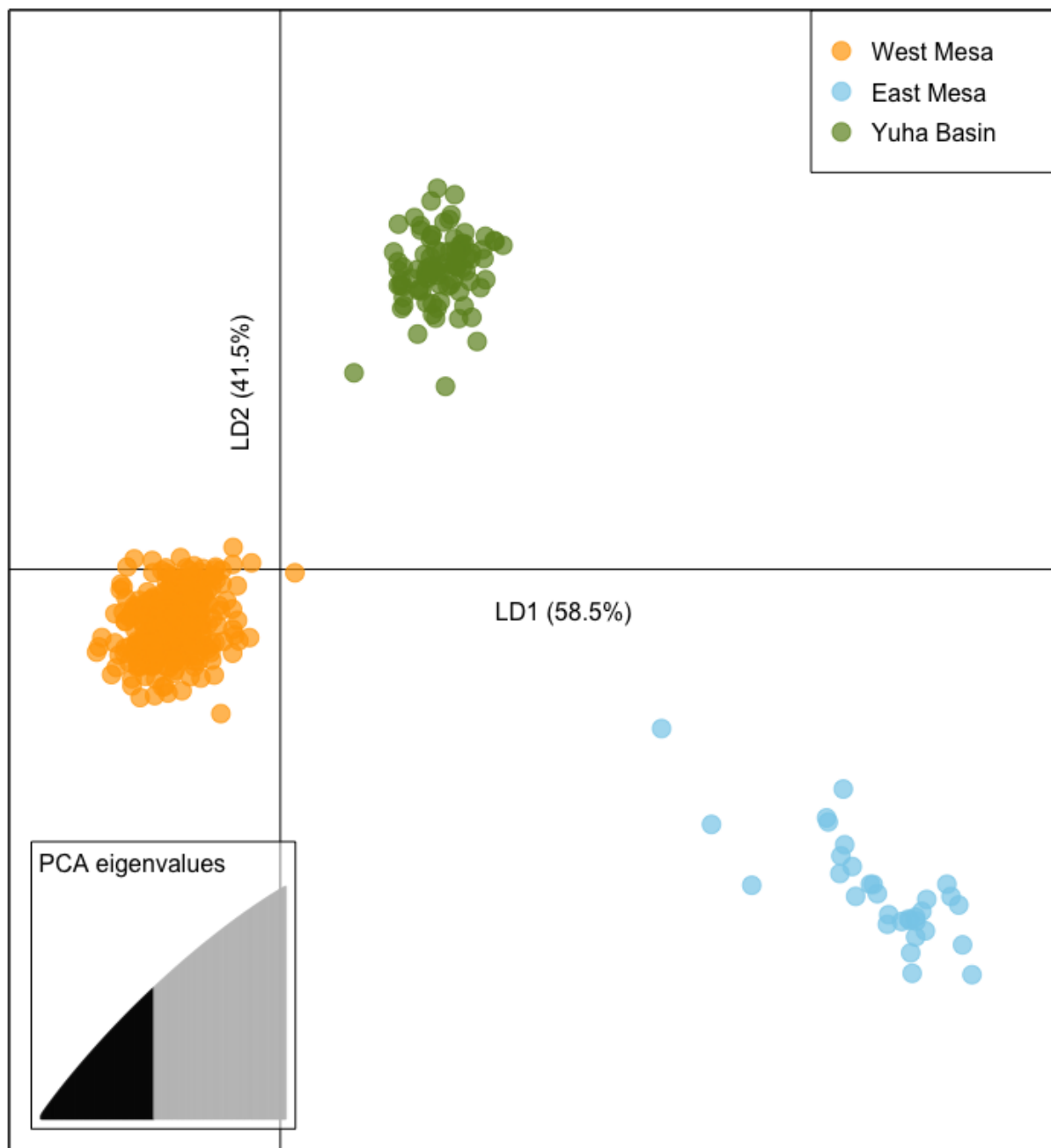


Figure 1.4- DAPC scatterplot in adegenet using Dataset B. Retained 200 PCs, 3 clusters. Legend relabeled to show sampling area fully explains cluster membership- West Mesa, Yuha, and East Mesa populations all appear distinct with no membership overlap.

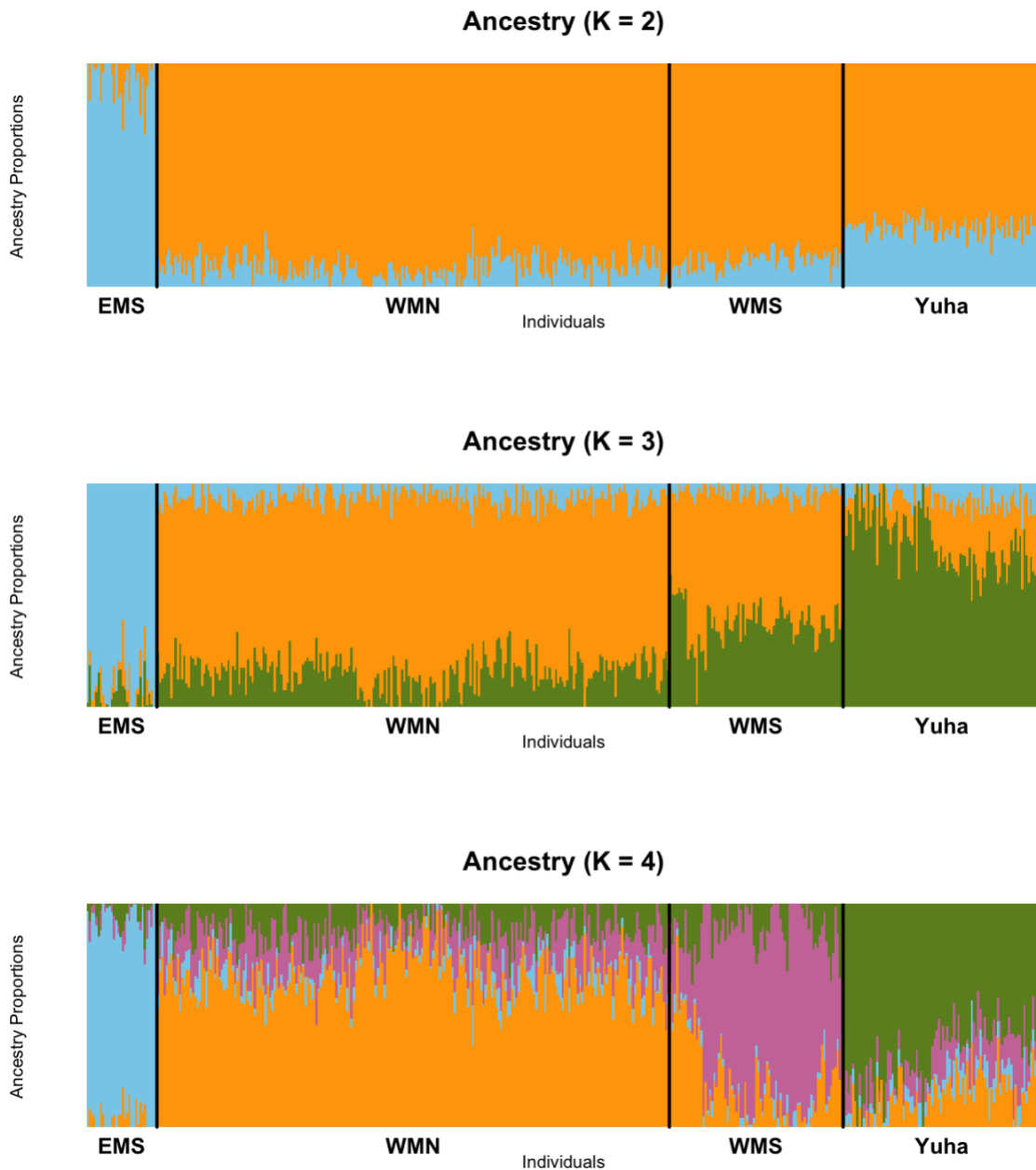


Figure 1.5- LEA structure-like ancestry plots for $K = 2-4$ using Dataset B. Each vertical bar represents a sampled individual and the colors of each bar represent the proportion of ancestry it carries from each ancestral population. Individuals are sorted on the x-axis and labeled by sampling area. $K = 2$ shows that ancestry separates across the Imperial Valley with eastern ancestry represented in blue, and western ancestry in orange. $K = 3$ further splits Yuha ancestry, represented in green, from the rest of the western Imperial valley. $K = 4$ further separates West Mesa North ancestry in orange from West Mesa South ancestry in fuchsia.

Because DAPC $K = 2$ clustered East Mesa samples separately from all western Imperial Valley samples, and $K = 3$ maintained this relationship and clustered all Yuha individuals into the third cluster, we opted to plot DAPC results for $K = 3$ as it provides more information on

subpopulation structure in the valley (Figures 1.3, 1.4). DAPC also fully clustered EMS samples and Yuha samples at $K = 3$.

Tess3r plots (Figure 1.6) show that the spatial distribution of ancestry proportions align with LEA Q-matrix bar plots. At $K = 2$ populations separate across the Imperial Valley. At $K = 3$ Yuha Basin samples cluster separately from West Mesa samples. At $K = 4$ WMN samples and WMS samples separate, but interestingly samples from western Target 103 show a higher proportion of WMN ancestry than proximal WMS samples from the adjacent Parachute Drop Zone and eastern Target 103.

Pairwise F_{ST} (Table 1.3) between sampling areas shows little overall differentiation amongst sampling areas (0.0045-0.065) with greatest differentiation between Yuma and all California populations (0.053-0.065). California populations are most different across the Imperial Valley (EMS to WMN $F_{ST} = 0.022$, EMS to WMS $F_{ST} = 0.024$, EMS to Yuha = 0.023). Yuha is marginally less different from WMN ($F_{ST} = 0.0069$) than the more proximate WMS ($F_{ST} = 0.0076$). This same pattern holds across other comparisons where WMS is also furthest removed from EMS ($F_{ST} = 0.024$) and Yuma ($F_{ST} = 0.065$).

Within the western Imperial Valley D_{PS} showed a statistically significant association with geographic distance ($r = 0.279$, $p = 0.0001$) providing evidence for a relatively minor effect of IBD at this scale (Figure 1.7).

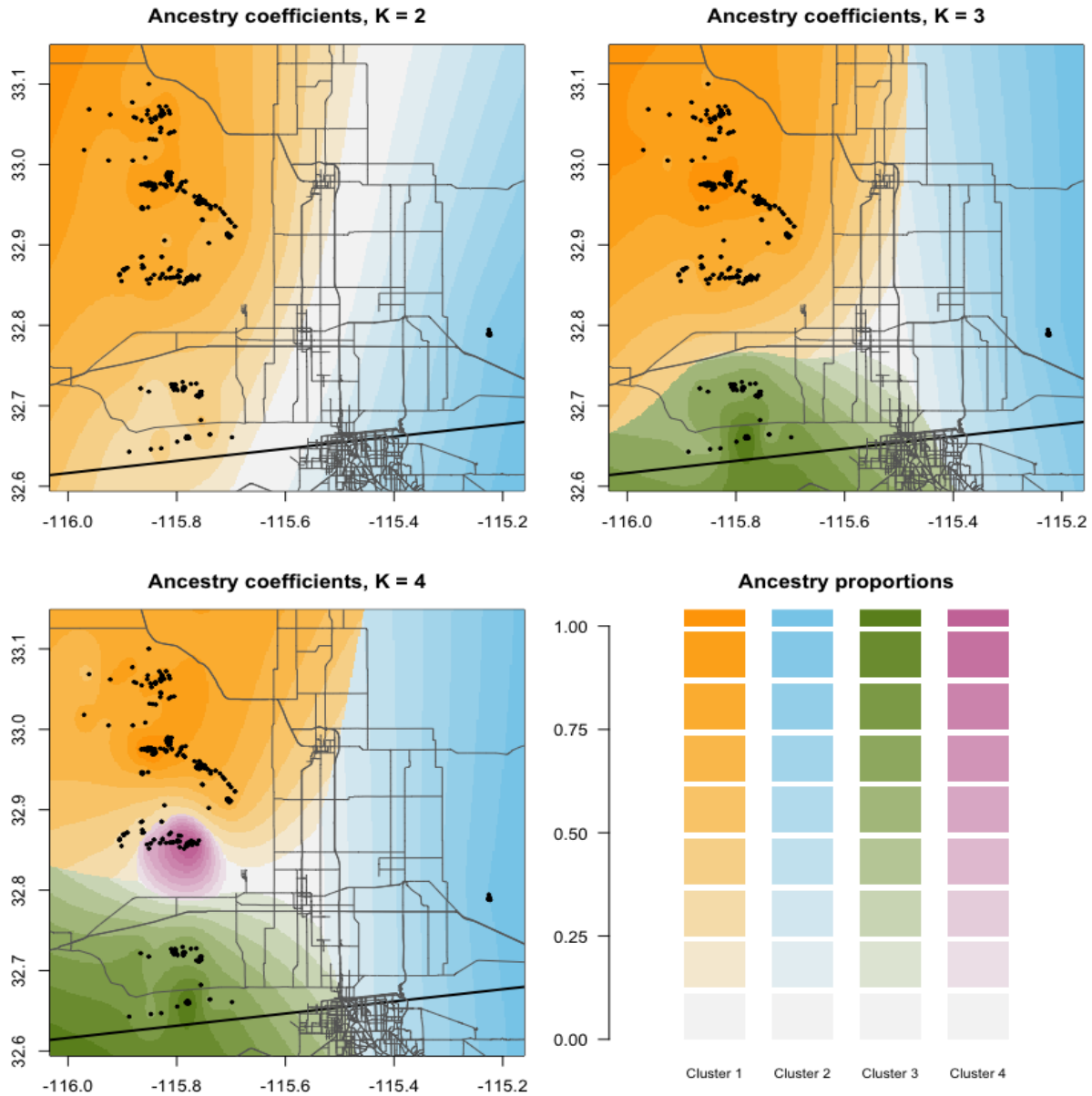


Figure 1.6- Tess3r geographic ancestry plots for $K = 2-4$, using Dataset B. Each dot represents a sampled individual mapped to the geographic coordinates of its capture. Color represents the major ancestry cluster to which each individual aligns, the diminishing gradient representing diminished proportion of that ancestry. At $K = 2$ blue represents East Mesa ancestry, and orange represents western Imperial Valley ancestry. At $K = 3$ blue represents East Mesa, orange represents West Mesa ancestry, and green represents Yuha Basin ancestry. At $K = 4$ blue represents East Mesa ancestry, orange represents West Mesa North ancestry, fuchsia represents West Mesa South ancestry, and green represents Yuha Basin ancestry. Maps were chosen via lowest cross-entropy wherein Q -matrices for each population size $k = 1:10$ were estimated 10 times apiece.

	EMS	WMN	Yuha	WMS	Yuma
EMS	NA	-	-	-	-
WMN	0.022306	NA	-	-	-
Yuha	0.023071	0.0069458	NA	-	-
WMS	0.024144	0.0044743	0.0076185	NA	-
Yuma	0.053982	0.062783	0.061599	0.06459	NA

Table 1.3- Pairwise F_{st} values calculated using VCFTools between populations from Dataset A. Differentiation is greatest between Yuma and California populations. Within California EMS is most differentiated from WMN, WMS, and Yuha. Yuha is more differentiated than WMS and WMN are from each other, but overall differences are marginal.

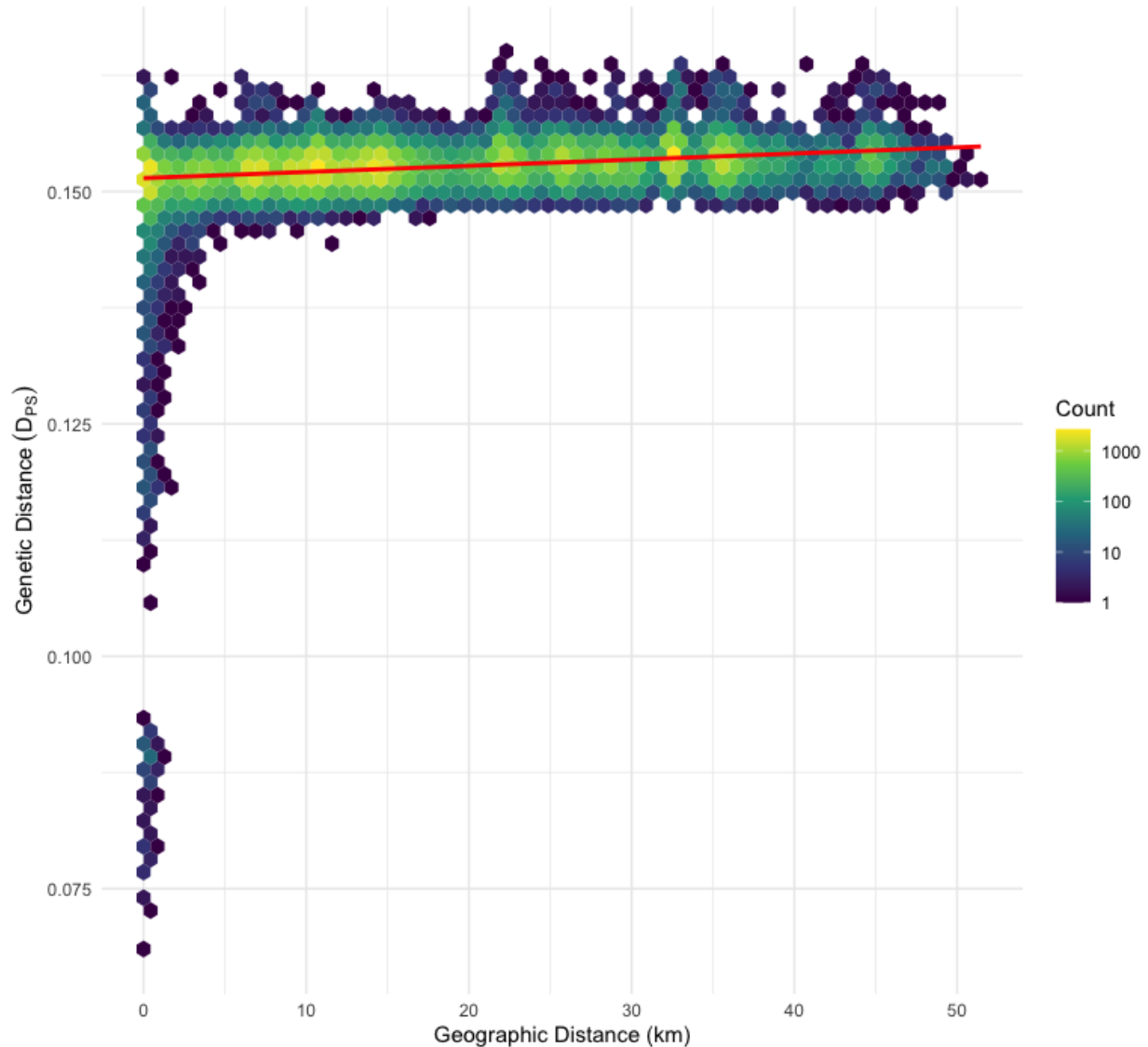


Figure 1.7- Using Dataset A, modeling pairwise inverse proportion of shared alleles over pairwise geographic distances tested with a Mantel correlation indicates a relatively weak but significant signal of Isolation by Distance across the western Imperial Valley. East Mesa and Yuma individuals were removed from this analysis since there is no connectivity between those populations and the western Imperial Valley $r = 0.279$ $p = 0.0001$.

Discussion

Our individual sampling and genotyping significantly expands on past genomic analyses for this species (Gottscho et al., 2024; Mulcahy et al., 2006; Payne et al., 2025), with a substantial increase in individual sample size representing several distinct sampling locations spread throughout the Imperial Valley. Overall, our data reinforce the findings of earlier studies, but we also identified potentially important subpopulation structure, particularly within the western Imperial Valley. These analyses revealed that, although there may be several somewhat unique genetic clusters of lizards that are forming across anthropogenic barriers in the central Imperial Valley, the overall population structure for this species is still relatively shallow, likely due to comparatively high abundance and the recency of landscape change.

Observed heterozygosity was less than expected for all California sampling areas, but only marginally. With little departure from expected levels of heterozygosity, we can conclude that overall genetic diversity across sampling areas is not impacted by overly small population sizes or other departures from Hardy-Weinberg. This is further demonstrated through low values of F_{IS} across sampling areas. Differences in expected and observed heterozygosity and resultant negative F_{IS} value at the Yuma site are likely the result of the small sample size of related individuals and not reflective of larger-scale population processes. Previously reported F_{IS} estimates from Yuma range from 0.066-0.151 (Payne et al., 2025), comparable to what we found in California, similarly indicating high levels of heterozygosity and low levels of inbreeding. Nucleotide diversity was higher than reported in Payne et al. (2025) (range 0.00039-0.00045), including our measurements from 3 individuals from the same population they

sampled ($\pi = 0.00068$). This potentially highlights the differences in parameter estimation due to marker selection, in this case restriction enzyme choice.

Population clustering with DAPC indicate genetic clustering occurs along the east-west split of the Imperial Valley by the Salton Sea and agricultural development (Figs. 3,4). While cross-entropy validation indicates strongest support for a one-population model ($K = 1$, BIC = 0.696), two and three population models ($K = 2-3$, BIC = 0.697) are only marginally less supported. This finding supports mtDNA and SNP data that identified secondary population division across the Imperial Valley (Gottscho et al., 2024; Mulcahy et al., 2006). The genetic differences that we observe in separated populations across the Imperial Valley are likely from a combination of factors including landscape arrangement influencing gene flow and historic demographics. Through the quaternary period *P. mcallii* habitat has fluctuated according to lacustrine events associated with the filling of Salton Trough and formation/receding of ancient Lake Cahuilla (Stokes et al., 1997; Waters, 1983). It is likely that there was recurrent periodic gene flow between East and West Mesa during lacustrine recessions, although mtDNA haplotype networks suggest that separation of East and West Mesa populations occurred well before anthropogenic development (Mulcahy et al., 2006). The Holocene bottleneck elucidated by Gottscho et al. (2024) would also contribute to contemporary population structure depending on the arrangement of surviving individuals on the landscape and their available migration corridors.

Considering the recency of landscape change associated with agriculture and development within California, any population structuring due to anthropogenic development is likely the product of roughly 30-50 generations of separation. Because *P. mcallii* is still a locally

abundant species with little current evidence of low genetic diversity, populations are expected to be slow to develop major changes in genetic ancestry and allele frequencies across anthropogenic barriers. However, the geographic clusters in our data are congruent with contemporary geographic separation of West Mesa and Yuha populations within the Western Imperial Valley. Clustering through DAPC, snmf, and tess3r reveal somewhat distinct populations, but the admixture in these models indicate relatively shallow differentiation. Further landscape analysis may reveal patterns associated with differences in precipitation, terrain ruggedness, or vegetation or soil composition.

Plotwise differences in genetic differentiation (F_{ST}) reveal patterns that mirror results from clustering analyses. All California populations are most differentiated from Yuma individuals, and within California differentiation is highest between East Mesa South and the Western Imperial Valley populations (WMN, WMS, Yuha). Curiously, WMS has the most distant relationship to all populations except the proximal WMN, but these differences are somewhat minor. Yuha populations appear marginally more similar to WMN ($F_{ST} = 0.007023$) than to WMS ($F_{ST} = 0.007704$), despite greater proximity to WMS. Altogether, differentiation is low but interesting patterns of differentiation might be a product of historical colonization or arrangement of dispersal corridors on the landscape, particularly during the highstands of ancient Lake Cahuilla. These minor differences could also be a product of ascertainment bias of selected markers. Broader genome coverage might reveal more expected patterns of F_{ST} within the western Imperial Valley.

The statistically significant Mantel test of pairwise D_{PS} over geographic distance supports an effect of IBD across the western Imperial Valley, but the IBD model alone does not fully

explain the subpopulation clusters we observed along a north-south axis with potential landscape barriers (east-west highways and land use) separating them. For example, if Interstate Highway 8 is preventing dispersal between West Mesa South and the Yuha then the pattern of clustering observed between these sites may be more attributable to this barrier than to IBD. It is well established that in the presence of IBD structuring algorithms can artificially identify population clusters where in fact patterns of genetic similarity are better explained by a cline (Perez et al., 2018). Alternatively in the presence of actual population clusters IBD tests can incorrectly attribute this variation to a cline (Perez et al., 2018). Furthermore, the relationship between pairwise genetic and geographic distances, while statistically significant, shows little variance and doesn't change strongly even as geographic distances extend well beyond natural dispersal distances and space use of the species. Further exploration of the population structure in this part of the valley will be necessary to disentangle the results from IBD and population structuring tests.

Our four population snmf models separated the WMS population from sites to the north (WMN) and south (Yuha). This, combined with its slightly increased difference in plotwise F_{ST} , suggests some isolation may be occurring between sites at a finer scale than previously realized, similar to findings of Payne et al. (Payne et al., 2025). To the south this may be a consequence of limited gene flow across I-8, but to the north this may be a result of natural barriers and/or land use. Superstition Mountain separates WMS from WMN, and although there is plenty of open habitat surrounding the mountain, particularly to the west, the FTHL ICC demographic plot on the southwest side of the mountain (WMMA1) generally exhibits much lower abundance and density than the other West Mesa demographic monitoring plot to the northeast (WMMA3).

Additionally, Superstition Mountain OHV Open Area is heavily visited from October through May, a time that overlaps with much of the *P. mcallii* active season; most adult lizards do not enter winter brumation until mid-late November, with some individuals becoming active again by mid-January. Since juvenile lizards sometimes overwinter above ground to take advantage of available food resources, late-season breeding events could put dispersing lizards in this area at risk. Additionally, a known mode of dispersal in the species is large egg-laying movements made by females (Pawlicki & Goode, 2026), so OHV impacts may put these females at greater risk as they move to find suitable nesting sites.

Overall, our results have revealed that this species of conservation concern can readily persist in habitats that are largely undeveloped and protected from major anthropogenic impacts, as none of our sites showed indications of extreme genetic isolation, loss of diversity, or inbreeding. This is encouraging news for the coalition of agencies that is dedicated to continually protecting this important habitat and supports the value of conservation agreements that protect large portions of this species' range. However, we did recover some worrying signs of loss of genetic connectivity across relatively recent anthropogenic barriers. Unsurprisingly, the clearest signal was the extensive agricultural development separating East and West Mesa. But even within the relatively protected West Mesa area there are indications that roadways and recreational activities may be limiting gene flow between sites. Further sampling and analysis of available data may help clarify these patterns.

An important omission to the current study is the Coachella Valley population, which has experienced substantial declines related to habitat development in recent decades (Barrows et al., 2008) and is more likely to be dealing with issues related to low genetic diversity and

potential inbreeding. Future efforts should be made to incorporate this population into ongoing genomic analyses, as our study highlights the importance of treating these fragmented populations within ICC Management and Research Areas as individual subpopulations with unique population processes and risks of extirpation and extinction.

Our genomic analyses also provide an important baseline for future comparisons that could be used to monitor changes in connectivity and genetic diversity over time. In general, the addition of genomic sampling to long-term population monitoring can provide uniquely valuable and detailed information regarding changes in population or conservation status. Several examples have demonstrated the utility of temporal genetic sampling in reptiles, including in the eastern massasauga rattlesnake, *Sistrurus catenatus catenatus* (Ray et al., 2013; Sovic et al., 2019), the San Francisco garter snake, *Thamnophis sirtalis tetrataenia* (Wood et al., 2020), and the Coachella Valley fringe-toed lizard, *Uma inornata* (Vandergast et al., 2019). *U. inornata* is sympatric with *P. mcallii* and faces similar pressures of habitat fragmentation, prolonged drought, and non-native species invasions. Their findings of increased differentiation between populations and decreased within-population allelic richness through time reflect what we might expect in *P. mcallii* populations. Incorporating genetic monitoring goals into the FTHL ICC's ongoing monitoring efforts would broaden the information available for use in management action by stakeholder agencies.

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CHAPTER 2

Genomics as a Tool to Understand Fine-scale Dispersal and Contemporary Barriers to Gene Flow in *Phrynosoma mcallii*

Abstract

Human land use has altered the arrangement and availability of wildlife habitats globally. This alteration of landscapes has fragmented formerly contiguous patches of habitat, imposing novel stressors on wildlife at all levels of ecological organization. Molecular impacts of habitat fragmentation are well-documented. Fragmentation can reduce gene flow between formerly connected populations, reduce population-level genetic diversity by removing individuals from populations and reduce overall genetic effective population size. Here, we used a large genomic dataset of single nucleotide polymorphisms to quantify fine-scale dispersal and detect molecular signals of anthropogenic habitat fragmentation and geographic distance in *Phrynosoma mcallii* in the western Imperial Valley, CA. We used multiple statistical and modeling approaches to determine the relative influence of limited dispersal and landscape arrangement on gene flow and relatedness. We detected minor signals of isolation-by-distance as well as signals of resistance across linear fragmentation features on the south end of the valley. Anthropogenic fragmentation may have more impact on this species than previously realized, even in contiguous portions of its range. This work is a valuable component of a multi-disciplinary approach to monitoring and managing *P. mcallii* populations in the USA and range-wide. Future landscape studies can help identify corridors of critical importance for maintaining genetic connectivity between management units and preventing future loss of genetic diversity.

Introduction

Human land use has drastically altered the arrangement of natural habitats across the planet. Over 50% of global land area has been converted for human use into urban, rural, and agricultural landscapes (Watson et al., 2016). The resulting fragmentation of natural habitat is one of the most prominent and persistent stressors to wildlife communities. Linear, continuous anthropogenic features like roadways, railways, and canals have divided areas of formerly contiguous habitat into smaller patches that present barriers for migration and movement (Fischer & Lindenmayer, 2007; Haddad et al., 2015). The most common and widespread form of fragmentation is the construction of roads, which affect every ecoregion in North America. Major roads in the US only cover roughly 1% of the land, but affect a much larger area (Forman, 2000; Forman & Alexander, 1998), as an estimated 83% of land in the US is within 1 km of the nearest road (Riitters & Wickham, 2003). For decades, ecologists have detailed the impact of roads on the ecology of their surrounding landscapes and have documented fundamental and profound effects on the landscape's ecological components, processes, and structures (Coffin, 2007; Grilo et al., 2021; Sukhontapatipak et al., 2025). However, roads impact species' ecology in complex ways that require assessment on a case-by-case basis.

Fragmentation effects are complex, as they can be both direct and indirect, and often vary depending on age, size, sex and other life history traits (Henle et al., 2004). Direct effects include one-time destruction or displacement of individuals during development, or continued impacts like wildlife-vehicle collisions along roadways resulting in wildlife injury or mortality (Fahrig et al., 1995; Lima Santos et al., 2017; Sukhontapatipak et al., 2025). Indirect effects include behavioral avoidance (or attraction, see (Mccardle & Fontenot, 2016; Palomino &

Carrascal, 2007; Sisson & Roosenburg, 2023)) of roadway habitat (D'Amico et al., 2026), which has been documented widely across taxa (Shepard et al., 2008). Other indirect road impacts come from known 'edge effects'—changes in physical and biological conditions at an ecosystem boundary (Fischer & Lindenmayer, 2007). Road and road-edge land usage can degrade habitat and lead to lower densities of wildlife as a consequence of road noise (Reijnen et al., 1995), habitat use (da Rosa et al., 2018), or direct mortality (Fahrig et al., 1995). These effects can profoundly influence demographic structure at the population level leading to decreased abundance (Proctor et al., 2005), sex- and age-specific outcomes (Moore et al., 2023), and genetic differentiation (Westekemper et al., 2021). Habitat specialists in highly fragmented habitats are especially at risk of negative fragmentation effects (Keinath et al., 2017; Matthews et al., 2014), underscoring the importance of maintaining intact core segments of habitat for adequate conservation (Schmidt et al., 2020).

Habitat fragmentation by anthropogenic barriers is particularly problematic in how it affects wildlife dispersal—the movement of an individual from its natal range into a breeding range or successive breeding ranges—as this process is dictated by intrinsic, individual factors, landscape characteristics, and population dynamics (Cayuela et al., 2018; Howard, 1960). Morphology, physiology, sex, or age class, or behavioral state can each contribute to the innate drivers of dispersal. Larger animals tend to disperse longer distances (Stevens et al., 2014), but in some species developing juveniles exhibit an innate “dispersal restlessness” that drives them to leave their natal area (Ritchison et al., 1992). Sex-mediated dispersal is also a common pattern, but the dispersing sex can vary across taxa; for example, most monogamous birds generally exhibit female-biased dispersal whereas mammals exhibit male-biased dispersal

(Mabry et al., 2013). Squamate reptiles do not seem to conform to one mode, with male and female-biased dispersal reported in the literature (Dubey et al., 2008; Olsson & Shine, 2003)). Dispersal is also shaped by various external conditions like landscape characteristics that facilitate or inhibit movement or population density, which can then result in individuals moving to avoid kin competition for resources or to avoid inbreeding (Bowler & Benton, 2005; Gandon, 1999). Long distance dispersal can also influence the demographics of populations through contributions to population growth via immigration or loss via emigration. Changes in effective dispersal due to habitat fragmentation can therefore have lasting impacts on populations by inhibiting gene flow and altering the genetic composition and structure of populations (Baguette et al., 2013; Rivera-Ortíz et al., 2015).

Research on the effects of habitat fragmentation has largely focused on birds and mammals (Fardila et al., 2017), even though reptiles are often range-limited habitat specialists at high risk of local extirpation from landscape modification (Cox et al., 2022; Meiri et al., 2018; Roll et al., 2017). Reptiles are especially at risk near roads due to slow movement rates, thermoregulation preferences for road surfaces, and foraging or mating behaviors (Andrews et al., 2015). Studies to evaluate specific road effects in reptiles (Brehme et al., 2018; Rytwinski & Fahrig, 2015) indicate that habitat specialization, low reproductive rates, and sensitivity to changes in temperature and precipitation put them at higher risk than other taxa (Keinath et al., 2017; Mantyka-pringle et al., 2012). Some reptile species have been found to avoid roads (Andrews & Gibbons, 2005), whereas others do not appear to show significant road avoidance (Row et al., 2007), and some are attracted to roads (Mccardle & Fontenot, 2016), so generalizations within taxa can be difficult. In the US, for example, differential mortality in

female freshwater turtles led to a male-sex bias in areas of high road density (Steen et al., 2006; Steen & Gibbs, 2004), although this same pattern was not seen in Ontario, Canada amongst four freshwater turtle species, with the exception of northern map turtles (*Graptemys geographica*) (Carstairs et al., 2018). Both studies nevertheless reported higher female mortality during the breeding season as females move to locate suitable nest sites.

From a molecular standpoint, habitat fragmentation can have functional (population-specific genetic diversity), structural (genetic structure), and compositional (gene flow; genetic effective population size) impacts on populations (Balkenhol & Waits, 2009). The growth of landscape genetics has revealed loss of critical genetic diversity in reptiles due to fragmentation (Delaney et al., 2010; Rivera-Ortíz et al., 2015; Sovic et al., 2019). Timber rattlesnake (*Crotalus horridus*) populations separated by roads in New York were found to have higher genetic differentiation and lower genetic diversity relative to populations in contiguous patches of habitat (Clark et al., 2010). Moore et al. (2008) identified fine-scale population differentiation in tuataras (*Sphenodon punctatus*) on Stephens Island associated with recent anthropogenic habitat modification. Both species have lengthy generation times and may take many years to reach reproductive maturity yet still show genomic signatures of differentiation over contemporary timescales. Because genetic differences typically take many generations to accumulate, more rapid and significant differentiation in species with shorter generation times might be expected (Fuller & Doyle, 2018), although the effect could be offset by higher population density (Zeng et al., 2012). In Los Angeles, California, a fragmented urban landscape created smaller patches of habitat, which led individuals of three relatively short-lived lizard species (*Uta stansburiana*, *Plestiodon skiltonianus*, and *Sceloporus occidentalis*) within these

patches to be more related compared to individuals at similar spatial scales in sections of core habitat (Delaney et al., 2010). This increase in local relatedness increases the likelihood of inbreeding and deleterious effects as populations approach inbreeding depression.

The flat-tailed horned lizard (*Phrynosoma mcallii*) provides a model system to investigate the interplay between dispersal and genetic connectivity in a fragmented landscape. *P. mcallii* is a Colorado Desert endemic and flagship species with a history of conservation action in the US. It is a range-restricted habitat specialist of sandy soils of lower Colorado River valleys that are fragmented by natural barriers like the Colorado River and the Salton Sea, but also by anthropogenic landscape change that includes substantial agricultural matrix, regional and local highways, and development.

Genetic population structuring of northwestern and southeastern *P. mcallii* populations was historically shaped by the Colorado River, which bisects the species' range, as well as lacustrine interval associated with the filling of the Salton trough (Gottscho et al., 2024; Mulcahy et al., 2006). Periodically, the Colorado River would empty to the northwest along the New and Alamo Rivers, filling the Salton Basin to form Lake Cahuilla instead of flowing south into the Sea of Cortez. Several well-documented highstands of Lake Cahuilla over the past two millennia reached a high water mark 13 m above sea level (Phukan et al., 2019; Rockwell et al., 2022; Waters, 1983), filling much of the Imperial Valley. As flow from the Colorado River would return south, the inland lake would slowly recede. These events routinely shaped the landscape of connectivity within the broader *P. mcallii* metapopulation west of the Colorado River.

On more contemporary time scales the Imperial Valley has been affected by multiple forms and scales of habitat fragmentation. Widespread development of these habitats

beginning in the early 20th century drastically reduced and fragmented *P. mcallii* habitat. Increased agricultural production in Colorado Desert valleys required canals and their associated infrastructure to transport water from the river. Colorado River avulsion in 1905 spilled past the Alamo Canal, filling the Salton trough and producing the current Salton Sea. By the mid 1960s, nearly 500,000 acres of farmland were established (Peterson Jr., 1963), fully separating *P. mcallii* populations on eastern and western sides of the valley. This population separation has been observed in molecular markers from mitochondrial haplotype networks (Mulcahy et al., 2006) and autosomal SNPs (Gottscho et al., 2024; Fischer, Chapter 1). It's estimated that 50% of the *P. mcallii* range in California has been converted to agriculture and other land uses (Jones & Lovich, 2009), and the remainder has been fragmented by roadways connecting regions of human development and by pervasive off-highway vehicle (OHV) use.

Two major highways bisect *P. mcallii* habitat in the western Imperial Valley. Interstate 8 (I-8) runs east-west, entering *P. mcallii* habitat just west of Ocotillo, CA and exiting habitat as it traverses the Gila Mountains east of Yuma, AZ. California State Route 98 (CA-98), also known as the Yuha Cutoff, splits south from I-8 west of Ocotillo, then heads east towards Calexico, bisecting the Yuha Basin. I-8 is a four-lane split highway across the Imperial Valley. It was a plank road in the early 20th century that became paved asphalt by the 1950s, before being completed in its current form by the late 1960s/early 1970s. Only two clustered highway underpasses facilitate wildlife dispersal between the Yuha Basin and West Mesa; both underpasses are located just east of Ocotillo. CA-98 is a two-lane divided highway. It was a gravel-asphalt road in the 1930s, then became fully paved by 1956. In 2024, sections of I-8 traversing *P. mcallii* habitat in the western Imperial Valley received between 15,900 and 22,800 vehicles per day in annual

average daily traffic (AADT) (Caltrans Traffic Census Program, 2024). In 2024 sections of CA-98 bisecting *P. mcallii* habitat in the Yuha Basin received 1,900 to 2,500 vehicles per day AADT (Caltrans Traffic Census Program, 2024). In an assessment of road risk to 166 species of California herpetofauna, *P. mcallii* ranked Very High Risk (Brehme et al., 2018).

Vehicular stressors to *P. mcallii* are not limited to paved surfaces in the western Imperial Valley, as thousands of miles of OHV trails, visited by millions of people each year, traverse much of the “intact” *P. mcallii* habitat in California. Five major designated OHV units in addition to numerous networks of OHV trails occur within large sections of *P. mcallii* habitat in the Borrego and Imperial Valleys. Hundreds of thousands of OHV riders visit OHV recreation areas in the Western Imperial Valley each year. OHV use has been shown to reduce abundance and recruitment of vertebrates in California deserts (Bury et al., 1977). It is unknown how OHV stressors, associated habitat fragmentation, or direct mortality may influence population demographic patterns in *P. mcallii* range.

P. mcallii activity and space use has been studied using traditional methods of radiotelemetry tracking across the active season (Miller, 1999; Fischer, 2021; Foley, 2002; Painter & Ingraldi, 2008; Pawlicki & Goode, 2026; Setser, 2004; Wone & Beauchamp, 2003). Findings generally concur that *P. mcallii* males use roughly twice as much space as females, travel greater distances per day, and male home ranges tend to overlap with females more than female home ranges overlap one another. Miller (1999) observed lizards in Yuma shifting the center of their home range over time, rather than utilizing one core area. This may contribute to gradual dispersal in this species. The larger space use and daily movements by males suggests a male-biased dispersal system, but Pawlicki and Goode (2026) and Setser (2004) described large

female movements just prior to oviposition, which act to disperse hatchlings from the female's primary use area. Pawlicki and Goode (2026) also recaptured 27 hatchlings in subsequent years, after natal dispersal. On average they were found 248.3 ± 118 m from their hatching location, with a range of 3–3,118 m.

Few studies to date have characterized the impact of anthropogenic fragmentation and land use in *P. mcallii* (Barrows et al., 2008; Barrows & Allen, 2009; Beauchamp et al., 1998; Gardner et al., 2004; Grant & Doherty, 2009; Payne et al., 2025) and only one study conducted at a single location has investigated molecular effects of anthropogenic habitat fragmentation. In Yuma, AZ, a heavily traveled roadway was identified as a primary barrier to gene flow, causing genetic differentiation between populations on either side of the road (Payne et al., 2025). In Texas horned lizards (*P. cornutum*), dispersal resistance was greater across a highway with a mean daily traffic volume of 3,829 annual average daily traffic (AADT) than across a highway with 1,998 AADT, revealing significant differences in fragmentation effects even at relatively low traffic volume (Wall et al., 2024). Blainville's horned lizards (*P. blainvillii*) are more widely distributed than *P. mcallii*, and habitat fragmentation has caused increased genetic population structure among smaller habitat fragments than among larger fragmented landscapes (Wenner, 2020). Given that several horned lizards are thus known to be sensitive to habitat fragmentation, further exploration of fragmentation effects is warranted in these systems, especially for species of conservation concern like *P. mcallii*.

The goals of this study are to better understand the factors that influence *P. mcallii* population structure at varying spatial scales. Our objectives were to 1) identify the scale of dispersal and look for signals of sex-biased dispersal using molecular data, 2) evaluate the

relative influence of geographic distance on genetic population structure at various scales in the western Imperial Valley, and 3) detect genomic signals of barriers to gene flow associated with anthropogenic landscape features and land use. We approached these questions using a large autosomal genomic dataset from *P. mcallii* samples gathered across the western Imperial Valley with the aim of informing conservation and management of the species.

Materials and Methods

Sample Collection

We collected *P. mcallii* tissue samples from across the western Imperial Valley in the Yuha Basin and West Mesa Management Areas (Figure 2.1) from 2021–2024 (n = 329). Collaborators at the Bureau of Land Management El Centro Field Office collected samples on BLM monitoring plots West Mesa Management Area 1 (n = 13), West Mesa Management Area 3 (n = 85), and Yuha Basin Management Area (n = 72), as well as select occupancy monitoring plots from 2022–2024. Details of lizard capture and processing, DNA extraction, library preparation, and sequencing are described in Chapter 1. Post sequencing, the same ipyrad (Eaton & Overcast, 2020) pipeline described in Chapter 1 was used to generate genetic variant calls for downstream analyses. Details of genome assembly and alignment to the *Phrynosoma platyrhinos* genome (Koochekian et al., 2022) are described in the Bioinformatics Pipeline section of Chapter 1.

Data Filtering

For these fine-scale analyses of population structure, we filtered our data using VCFTools v0.1.17 (Danecek et al., 2011) to remove indels and retain biallelic SNPs. We filtered at a minimum read depth of 10 (--minDP 10) and maximum read depth of 50 (--maxDP 50), and a 1% minor allele frequency cutoff (--MAF 0.01). We conservatively dropped individuals with over 80% missing data, which removed 21 individuals from the final dataset. We also removed loci that weren't present in at least 85% of samples (--MISS 0.85). Following VCFTools filtering we used SNPfiltR (DeRaad, 2022) to filter for allele balance which can reduce the impact of sequencing or mapping errors on the final dataset. Following allele balance conversion, we re-filtered loci at an 85% missingness cutoff. The resulting dataset is hereafter referred to as the "full dataset". Analyses that require other data filtering techniques to satisfy method-specific assumptions such as linkage disequilibrium (LD) pruning are detailed below.

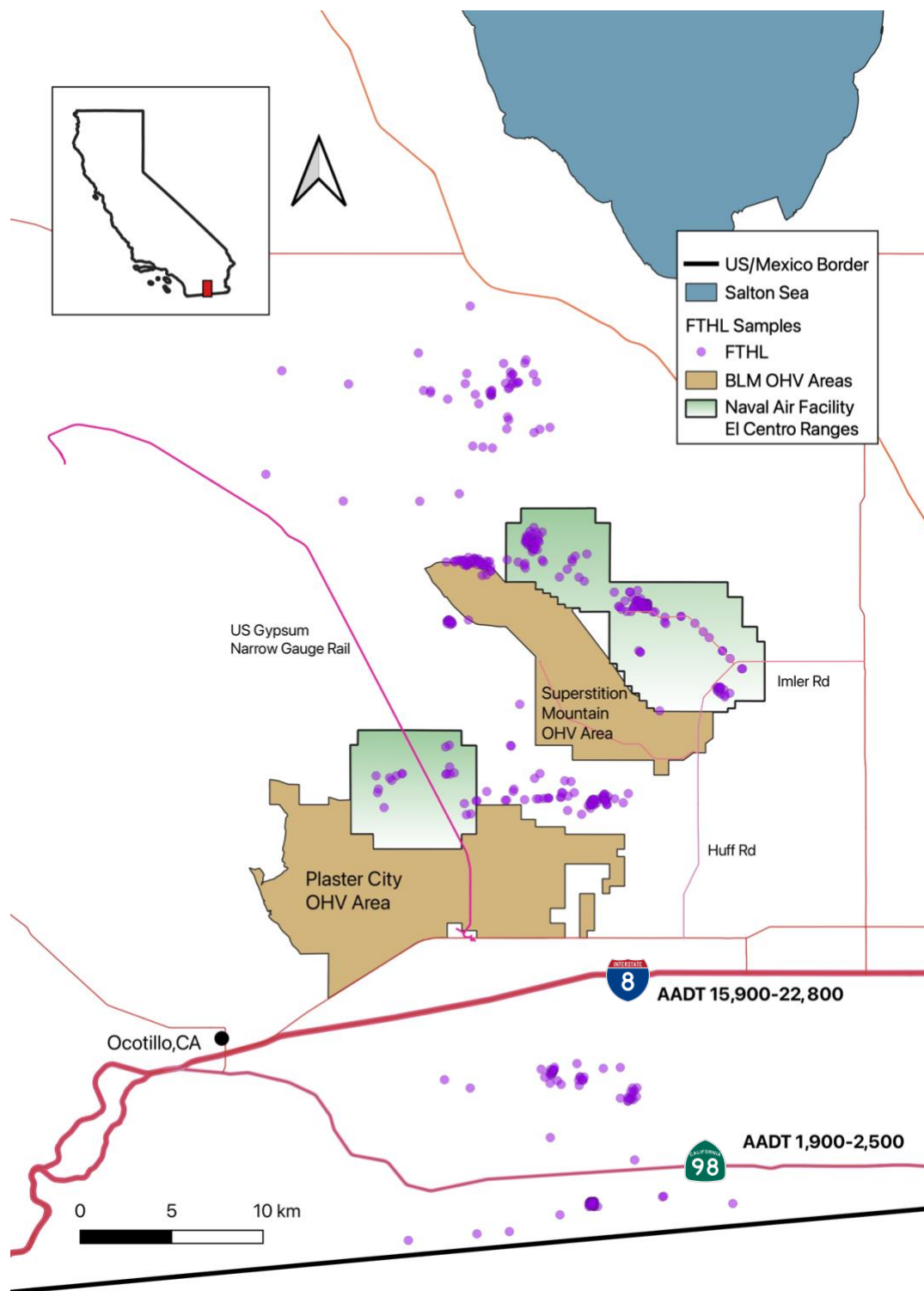


Figure 2.2- Map of flat-tailed horned lizard (FTHL) samples collected in the Western Imperial Valley, California (purple dots). The map also shows Interstate-8, a four-lane divided freeway with Average Annual Daily Traffic of 15,900-22,800 vehicles/day and California-98, a two-lane highway with 1,900-2,500 vehicles/day. Land use polygons include Plaster City OHV Area, Superstition Mountain OHV Area, Shade Tree Naval Range, and Loom Lobby Naval Range which is bisected along with Plaster City OHV Area by the US Gypsum rail line that runs to the Fish Creek Quarry

Data Analyses

Dispersal

Using the full dataset pruned for linkage disequilibrium, we used PLINK's --genome (Purcell et al., 2007) to calculate pairwise proportion of alleles shared identical-by-descent ($\hat{\pi}$, IBD). PLINK has been shown to be an effective tool to determine IBD in systems with relatively low population structure and low levels of inbreeding (McMaster et al., 2025). PLINK maximizes precision in kinship estimation with low minimum minor allele frequencies and moderate per-loci missingness (McMaster et al., 2025). We used Z-values Z0, Z1, and Z2, representing the probability that two individuals share zero, one, or two alleles identical-by-descent at a random locus to assign first-degree relationships (parent-offspring and full sibling) to sampled individuals in our dataset. The proportion of the genome shared IBD, is calculated as $\hat{\pi} = Z_2 + (0.5 \times Z_1)$. We identified first-degree relatives, built putative pedigrees based on these statistics and validated them with survey capture data, then measured the Euclidean distance between individuals to infer dispersal distances.

We calculated distances between pairs of brothers, sisters, brother-sister, father-daughter, father-son, mother-daughter, and mother-son. We then log-transformed these values and tested for significant differences in mean distance between parent-offspring groups using a one-way ANOVA followed by a Tukey multiple comparisons of means post-hoc test to identify significantly different groups. We omitted sibling relationships from statistical analysis since most sibling pairs were found as hatchlings within the same year and would naturally be found closer than other groups. We also wanted to test for evidence of maternal oviposition dispersal,

so we conducted a Welch two sample t-test on the log-transformed distances between mother-hatchling and father-hatchling pairs that were found in the same year.

We then plotted the proportion of alleles shared identical-by-descent against Euclidean geographic distance to assess the scale of dispersal. We compared this relationship for male-male, female-female, and female-male pairs to test for sex-biased dispersal. We fitted these plots with loess curves then used the loess curves to estimate δ , the distance at which the proportion of the genome shared identical-by-descent drops halfway to the mean from its maximum value, following Aguillon et al. (2017). A lower δ value indicates a more rapid decay of genetic relatedness and therefore a stronger effect of isolation-by-distance.

Landscape Genomics

We used the R package *adeget* (Jombart, 2008) to calculate pairwise proportion of shared alleles between individuals using the full dataset. We also calculated the Euclidean distance between all pairs of individuals using the geographic location of each capture. We then tested the inverse proportion of shared alleles (D_{PS}) against geographic distance with a Mantel test using the R package *ade4* (Dray et al., 2002). We tested each subpopulation separately, then subsampled within subpopulation at smaller geographic distance scales (500 m, 1.5 km, 3 km, 5 km, 10 km) to test the scale at which the Mantel correlation explained the most variation in the data (Broquet & Petit, 2009). We plotted the linear relationship between D_{PS} and geographic distance for each of the three subpopulations of interest as well as within the western Imperial Valley as a whole.

We then used the program EEMS (estimated effective migration surfaces) (Petkova et al., 2016) to detect landscape influences on migration rate and genetic diversity in the western Imperial Valley. EEMS incorporates a genetic distance matrix and a geographic distance matrix, testing these data against a null model of isolation-by-distance to create heatmaps of migration (m) and diversity (q) that help identify corridors or barriers to gene flow. Because EEMS models assume independence between individuals and SNPs, we filtered data by relatedness using PLINK to remove individuals above a relatedness value of 0.2 (`--rel-cutoff 0.2`) and filtered for linkage disequilibrium with a 100 kb window, stepping 10 variants. The final EEMS dataset contained 403 individuals and 52,054 SNPs. We overlaid a grid of 300 demes atop the western Imperial Valley sampling area then ran EEMS for 20 million steps, discarding the first 8 million as burn-in, then sampling every 25,000 steps. To resolve issues related to high acceptance rates of algorithmic parameters we changed three hyperparameters from their default values (`qSeedsProposalS2 = 10`, `mSeedsProposalS2 = 100`, `mEffctProposalS2 = 1`).

Results

Genomic Data Structure

The post-sequencing raw FASTQs contained a total of 3.2 billion reads, averaging 5.6 million reads per sample. 2.8 billion reads passed ipyrad's initial quality filtering, averaging 4.8 million reads per sample. 1.6 billion (58%) of reads mapped to the reference genome. Despite the low mapping percentage, many loci contributed to the final dataset, with a final average of 76.1 thousand loci per sample.

Our initial VCF used in downstream analyses had 2,097,293 variants across 573 individuals. Filtering individuals with over 80% missing data removed 21 individuals from the final dataset. Additionally, relatedness analysis revealed four pairs of samples with pairwise relatedness values near 1, indicating four individuals in the dataset had been re-sampled. The earlier sample from each pair was removed from the final dataset (23N3, 23N14, 23N17, 23N18). This yielded 72,914 variants across 548 samples with 8.45% missing data. LD pruning resulted in a dataset of 52,054 SNPs across 548 samples with 8.57% missing data.

Dispersal

We found 102 first degree relatives within our genomic dataset. Of these, 35 of these were sibling (SIB) relationships: 19 brother-sister, 11 sister-sister, and 5 brother-brother. Parent-offspring (PO) relationships comprised 67 first-degree relatives: 15 father-daughter, 15 father-son, 21 mother-daughter, and 9 mother-son, with 7 pairs that could not be directionally ascertained but which belonged to either father-daughter or mother-son groups. Mean distance between sister-sister was 66.4 ± 59 m, brother-brother 292 ± 470 m, brother-sister pairs 147 ± 236 m, father-daughter pairs 122 ± 114 m, father-son pairs 241 ± 222 m, mother-daughter pairs 260 ± 329 m, mother-son pairs 187 ± 132 m, and directionally unspecified parent-offspring pairs (either Father-daughter, or Mother-son) 338 ± 307 m (Table 2.1).

Distances between parents and offspring did not differ significantly by any tested groups (Figure 2.2). The one-way ANOVA on log-transformed parent-offspring distances showed no significant differences based on relationship groups ($F_{4, 61} = 1.66$, $p = 0.171$). We did not observe any signals of maternal oviposition dispersal evidenced by greater distances between mothers

and offspring compared to fathers and offspring captured within the same year ($t_{14.3} = -0.93$, $p = 0.37$).

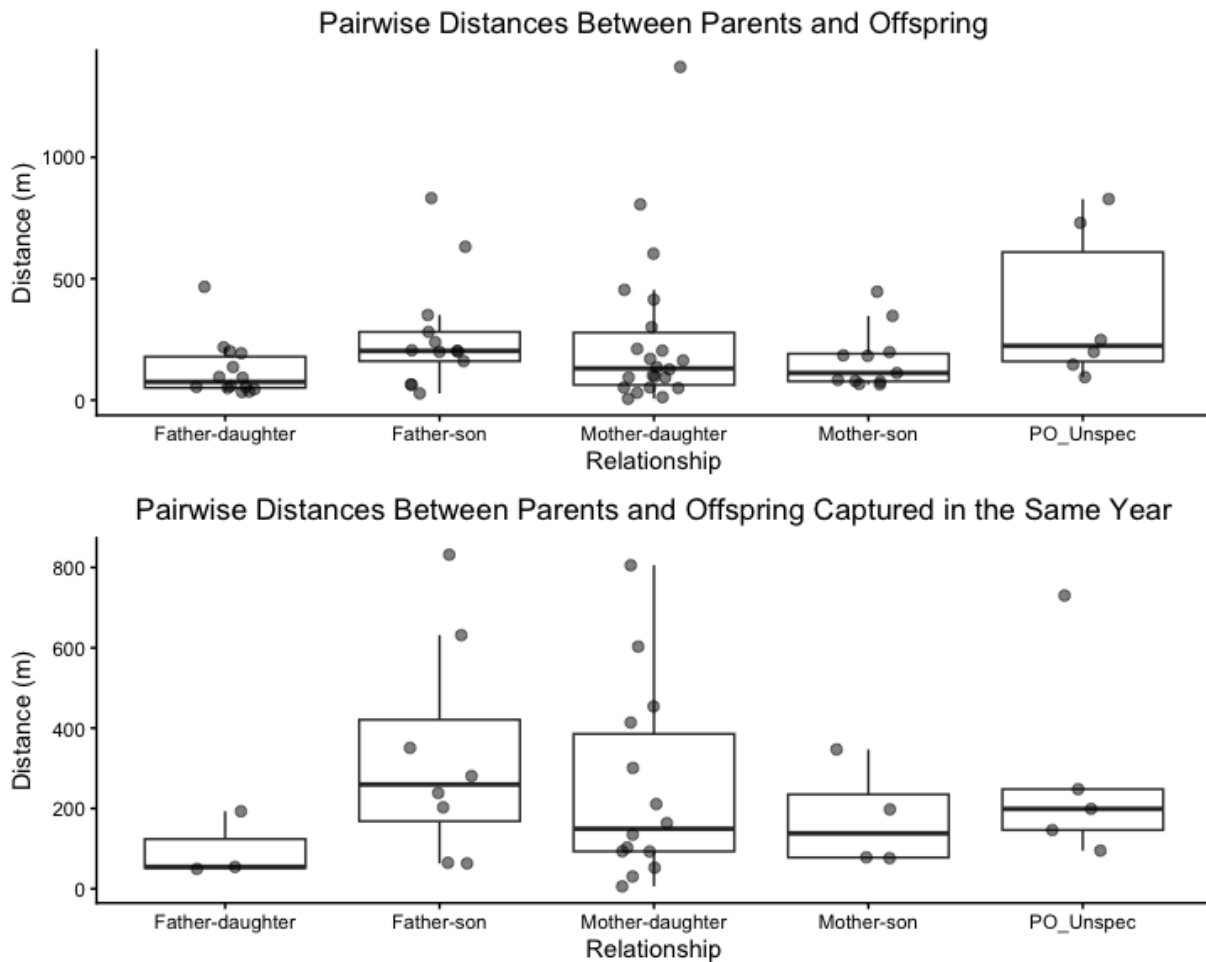


Figure 2.3- Geographic distances between all Parent-Offspring Pairs and pairs captured in the same year. Pairwise relatedness between individuals determined using PLINK –genome. Relationships validated using capture data and –genome output values π_{hat} , Z0, Z1, and Z2. PO_Unspec are parent-offspring pairs that could not be directionally validated. They are either Mother-son or Father-daughter relationships.

Demographic monitoring plot WMMA3 yielded a multi-year pedigree revealing multiple familial groups across years. The most extensive of these stems from female 22N4, who was initially captured in 2022 (Figure 2.3); this female mated with an unknown male to produce a son, 23N15, captured the next year on 8/9/2023, measuring 71 mm SVL and weighing 13 g. In

2024, her son 23N15 coupled with female 24N48 and produced at least two offspring. Hatchling female 24N10 was captured on 7/24/2024 measuring 37 mm SVL and weighing 1.3 g and female 24N36, captured on 7/29/2024 measuring 28 mm and weighing 1.1 g.

In spring 2023, female 22N4 coupled with a different male, 22N5, and on 8/11/23 2023 their offspring, hatchling female 23N16 was captured measuring 34 mm and weighing 2.05 g. Next year, its siblings 24N6, 24N35, and 24N40 were captured as adults (69-75 mm, 16-20 g). Female 24N6 hatched a daughter that year, female 24N31, who was captured as a hatchling. The distances between the capture location of hatchling female 23N16 and her adult siblings in 2024 represent the distance her siblings dispersed over the course of a year. From the capture point of 23N16, female 24N6 dispersed 52 m, female 24N40 dispersed 46 m, and male 24N35 dispersed 184 m. Male 24N35 was found 232 m and 228 m, respectively, from its adult sisters 24N6 and 24N40. Mother 22N4 was originally located 12 m from where her hatchling 23N16 was initially captured as a hatchling. Her son 24N35 dispersed the furthest of all offspring (182 m from her capture location, compared to 12-65 m for the others). This inter-generational family pedigree documented on a 300 m x 300 m monitoring plot highlights instances of reduced natal and adult dispersal distances in the species but must be combined with broader scale sampling in order to also document the longer distance movements made by some individuals (i.e. the 3 km dispersal documented by Pawlicki and Goode (2026)).

Smaller, less complete pedigrees were able to capture partial family groups, including the coupling between female 22YH8 (captured as a juvenile measuring 49 mm SVL on 8/25/22) and male 23YH16, which produced offspring female 23YH7 and male 23YH1, both captured as hatchlings in late July 2023. Female 23YH33 and male 23YH21 produced offspring male 24YH22

and female 24YH17, both captured as hatchlings in late August 2024. Other pedigrees include one parent and two siblings but lack sampling from the second parent. For example, male 24N17 and an unknown female coupled to produce males 24N34 and 24N30, both captured as hatchlings in late July 2024. Between July 27th and August 25th, 2023, a set of five hatchling siblings were captured on YBMA1, female 23YH3, male 23YH24, female 23YH25, female 23YH28, and male 23YH29. No parents responsible for this clutch were sampled.

In addition to these full siblings, we identified nineteen sets of half-siblings. We limited our inference of half-sibling relationships to hatchlings from the same year, except in the few cases where extended pedigrees with first-degree relatives allowed us to identify half-siblings that were captured in different age classes. We detected one set of half-siblings born in the same year, from the same mother, indicating some incidence of multiple paternity. Male 24N30 was captured on 7/26/24 measuring 40 mm SVL and 2.3 g, male 24N34 was captured on 7/29/24 measuring 35 mm SVL and 2 g. Both individuals were first degree relatives to female 24N17, originally captured as an adult on WMMA3 in 2023. Because of their size and the time of year they were captured, we can determine that these individuals belonged to the same clutch from female 24N17.

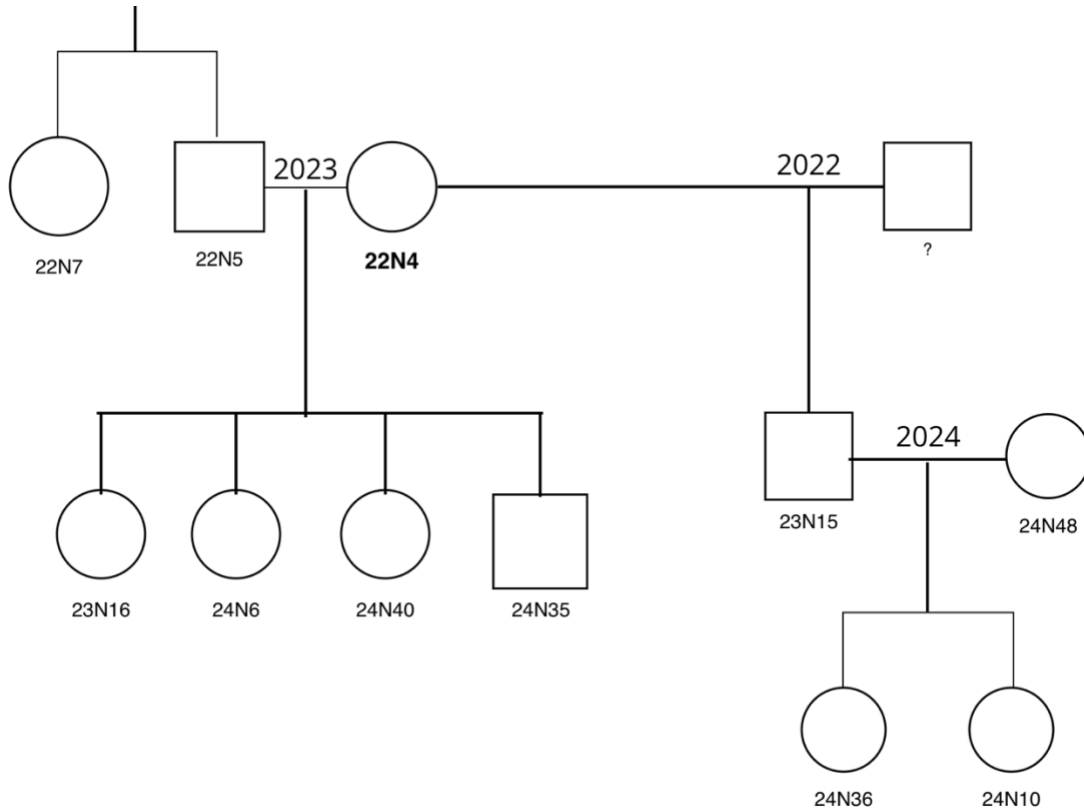


Figure 2.4- Multi-generational family pedigree centered around female 22N4 captured on demographic monitoring plot WMMA3. Mating events are labeled with the year they occurred; sample names indicate the year the individual was captured and sampled (23N16 was the 16th unique lizard captured on the Navy plot in 2023). Relatedness was determined using π_{hat} , Z0, Z1, and Z2 values output from PLINK's `-genome` function. These values confirm half-sibling relationships between 23N15 and 23N16, 24N6, 24N35, and 24N40.

Relationship	n	Mean Distance (m)	Median Distance (m)	Standard Deviation (m)	Minimum Distance (m)	Maximum Distance (m)
Sister-sister	11	66.4	45.5	59	9.85	207
Brother-brother	5	292	96.2	470	38.6	1129
Brother-sister	19	147	105	236	12	1074
Father-daughter	14	124	75.1	118	33.5	466
Father-son	13	266	203	229	27.7	832
Mother-daughter	22	252	131	323	5.83	1372
Mother-son	11	167	112	126	64.8	447
PO Unspecified	7	338	199	307	94.8	828

Table 2.1- Pairwise first-degree relatives and the mean, median, standard deviation of the mean, minimum, and maximum distances between groups of individuals

Dispersal analysis via proportion of shared alleles that are identical-by-descent revealed male-biased dispersal across the western Imperial Valley (Figure 2.4). The decay rate of relatedness was higher for female-female pairs ($\delta = 134$ m), than for male-female pairs ($\delta = 180$ m), and male-male pairs ($\delta = 228$ m). This decay rate shows that female-female relatedness decays more quickly compared to male-female and male-male pairs.

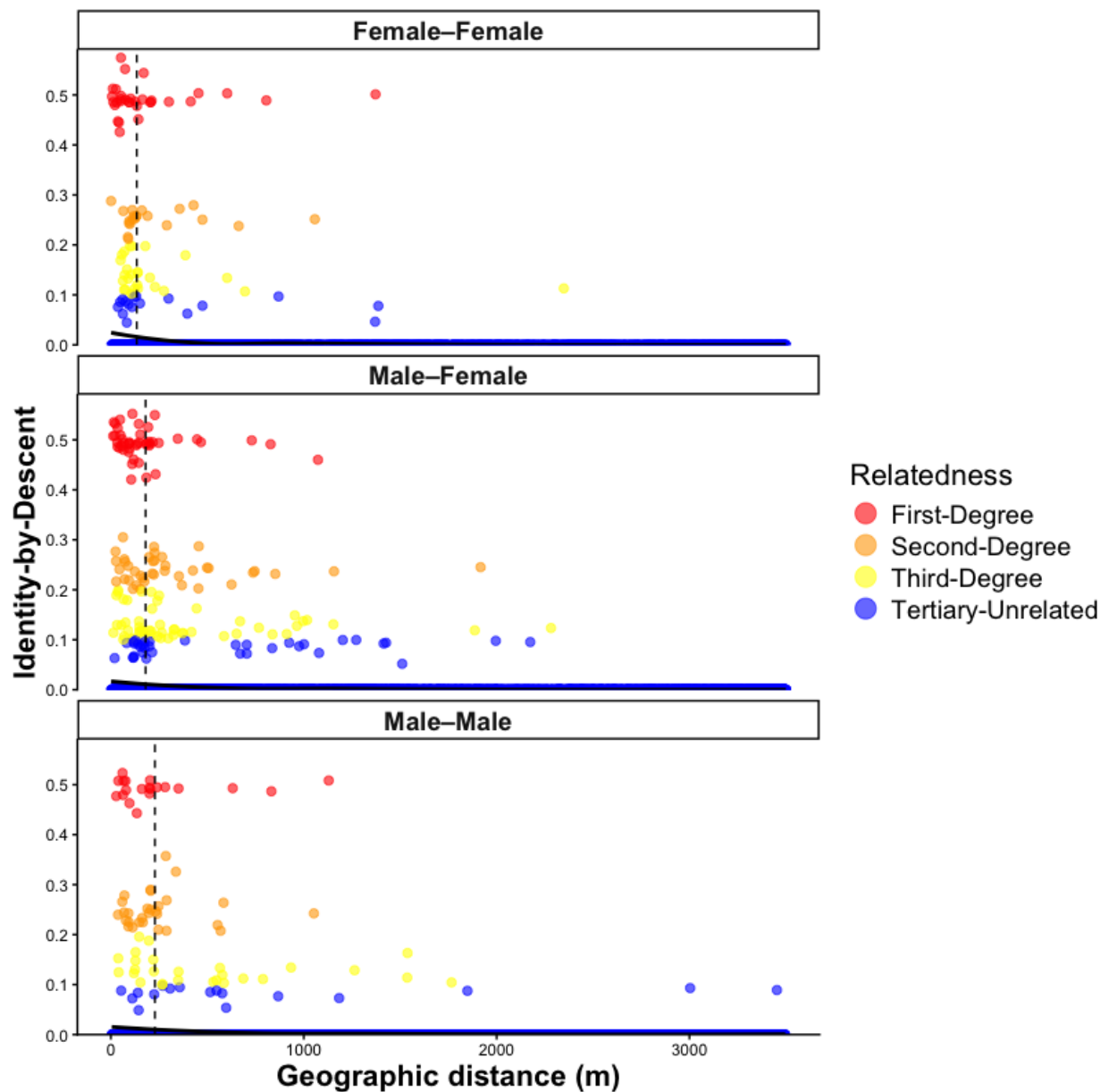


Figure 2.4- Decay of pairwise relatedness (proportion of genome shared identical-by-descent) over geographic distance reveals slightly stronger isolation-by-distance patterns between female pairs, indicating a higher degree of dispersal by males. The dashed lines indicate the distance (δ) at which the proportion of the genome shared identical-by-descent falls halfway to the

mean from its maximum value. A lower δ value indicates a more rapid decay of genetic relatedness by geographic distance. For female-female pairs $\delta = 134$ m, male-female $\delta = 180$ m, male-male $\delta = 228$ m.

Genetic Distance vs. Geographic Distance

A minor but statistically significant effect of isolation distance was detected across all western Imperial Valley sampling areas, and broadly across the entire western Imperial Valley (Figure 2.5). Subpopulation Mantel tests of genetic distance (D_{PS}) against geographic distance for West Mesa North revealed a statistically significant effect of isolation-by-distance at various spatial scales, including 500 m ($r = 0.17$, $p < 0.0001$), 1.5 km ($r = 0.17$, $p < 0.0001$), 3 km ($r = 0.17$, $p < 0.0001$), 5 km ($r = 0.16$, $p < 0.0001$), 10 km ($r = 0.16$, $p < 0.0001$), and the furthest extent between individuals 30 km ($r = 0.16$, $p < 0.0001$).

Mantel tests of genetic distance (D_{PS}) against geographic distance for West Mesa South also supported an effect of isolation-by-distance across spatial scales of 500 m ($r = 0.25$, $p < 0.0001$), 1.5 km ($r = 0.26$, $p < 0.0001$), 3 km ($r = 0.26$, $p < 0.0001$), 5 km ($r = 0.26$, $p < 0.0001$), 10 km ($r = 0.26$, $p < 0.0001$), and the furthest extent between individuals 13.8 km ($r = 0.26$, $p < 0.0001$).

Mantel tests of genetic distance (D_{PS}) against geographic distance for the Yuha Basin also supported an effect of isolation-by-distance across spatial scales of 500 m ($r = 0.21$, $p < 0.0001$), 1.5 km ($r = 0.22$, $p < 0.0001$), 3 km ($r = 0.23$, $p < 0.0001$), 5 km ($r = 0.23$, $p < 0.0001$), 10 km ($r = 0.23$, $p < 0.0001$), and the furthest extent between individuals 17.9 km ($r = 0.23$, $p < 0.0001$) (Table 2.2).

		Distance Class											
		500 m		1.5 km		3 km		5 km		10 km		Highest	
		<i>r</i>	<i>p</i> -value	<i>r</i>	<i>p</i> -value	<i>r</i>	<i>p</i> -value	<i>r</i>	<i>p</i> -value	<i>r</i>	<i>p</i> -value	<i>r</i>	<i>p</i> -value
Subpopulation	WMN	0.17	< 0.0001	0.17	< 0.0001	0.17	< 0.0001	0.16	< 0.0001	0.16	< 0.0001	0.16	< 0.0001
	WMS	0.25	< 0.0001	0.26	< 0.0001	0.26	< 0.0001	0.26	< 0.0001	0.26	< 0.0001	0.26	< 0.0001
	Yuha	0.21	< 0.0001	0.22	< 0.0001	0.23	< 0.0001	0.23	< 0.0001	0.23	< 0.0001	0.23	< 0.0001

Table 2.2- Isolation-by-distance trends across the western Imperial Valley broken down by subpopulation and distance class. Correlations between pairwise inverse proportion of shared alleles (D_{PS}) and geographic distance were tested by Mantel test at five subsampled distance classes and the highest inter-individual distance values within each plot (WMN = 30 km, WMS = 13.8 km, Yuha Basin = 17.9 km). Mantel test r statistic is reported along with p -value of correlation. The r statistic describes the strength of the correlation between genetic and geographic distance. The highest r value describing the strongest correlation is highlighted for each subpopulation to show the distance class at which isolation-by-distance shows the strongest effect.

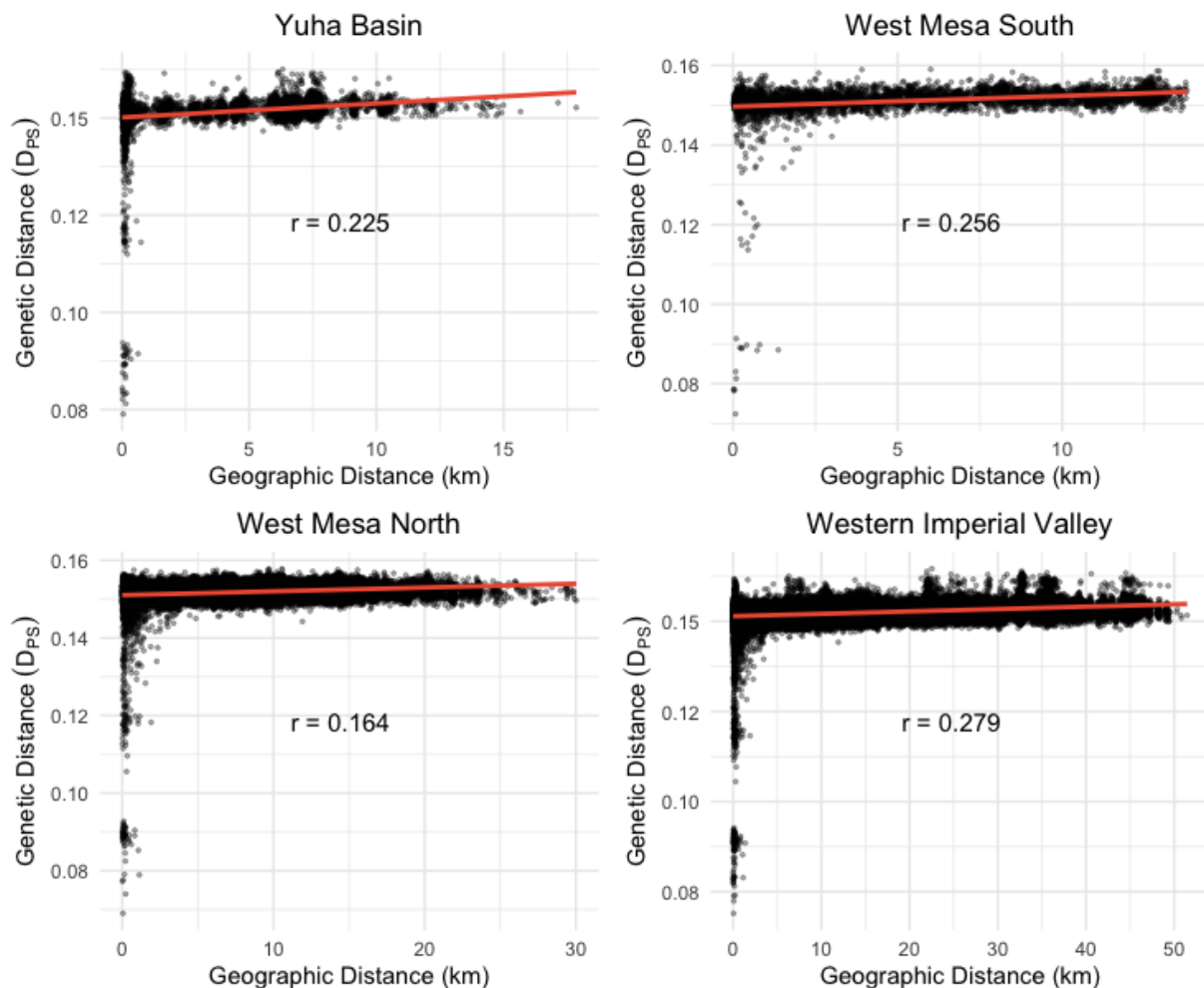


Figure 2.5- Inverse proportion of shared alleles (D_{PS}) over geographic distance for each subpopulation- Yuha Basin, West Mesa South, West Mesa North, and all samples in the Western Imperial Valley. Mantel r values are printed for each subpopulation in

its entirety. All subpopulations show a statistically significant ($p = 0.0001$ for all) but minor effect of IBD, and no subpopulation showed a significantly higher r value within smaller distance class subsets (500 m, 1.5 km, 3 km, 5 km, 10 km).

EEMS

EEMS surfaces show patchy landscape effects on migration and diversity (Figure 2.6). EEMS results show barriers to effective migration (m) in the central Yuha Basin region that is bisected by CA-98. The region containing the I-8 development corridor and Plaster City OHV Area was also associated with low connectivity, as well as to the north associated with Superstition Mountain and Superstition Mountain OHV Area. Effective diversity appears highest in the Yuha basin, with generally low effective diversity throughout West Mesa. Low diversity zones are most strongly associated with habitat edges.

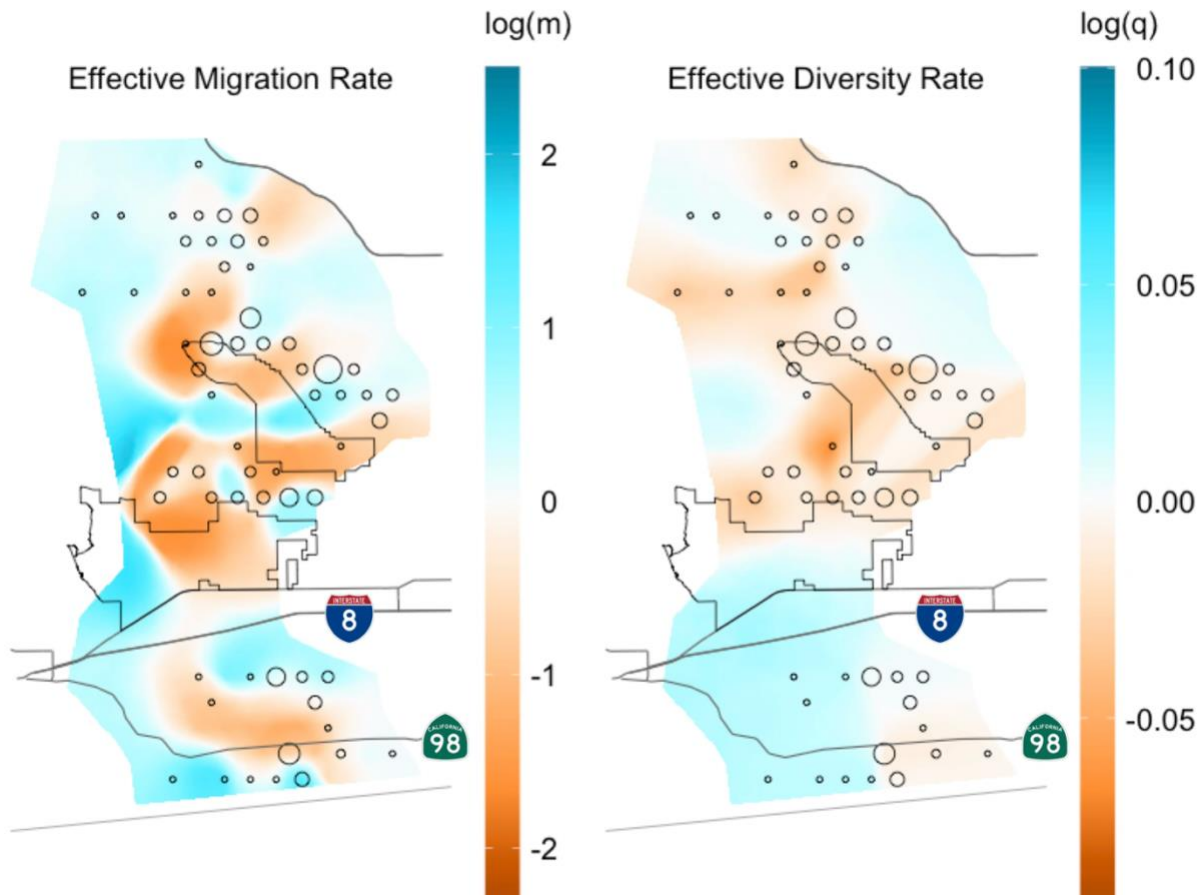


Figure 2.6- Estimated migration and diversity parameter surfaces in the Western Imperial Valley by EEMS (Petkova et al., 2016). The migration surface estimates between deme divergence, and the diversity surface estimates within deme divergence. The resultant heatmap reveals potential barriers by regions of lower-than-expected migration (or diversity) in darkening shades of red and potential corridors by higher-than-expected migration (or diversity) in darkening shades of blue, compared to a null model of isolation-by-distance. Effective migration barriers appear to be associated with CA-98 and I-8 (barrier represented north of I-8 in Plaster City OHV Area), as well as Superstition Mountain. Effective diversity appears highest in the Yuha Basin, but geographic sampling clusters may account for the apparent lack of within deme diversity throughout the western Imperial Valley. Polygons represent Plaster City OHV Area to the south and Superstition Mountain OHV Area to the north.

Discussion

This study is the first of its kind to use genomic data collected on long-term *P. mcallii* monitoring plots to infer dispersal and fine-scale connectivity across a large regional conservation area using molecular markers. We leveraged a large SNP dataset to identify dispersal distances and to detect potential genomic signals of anthropogenic landscape change and geographic landscape arrangement in the western Imperial Valley. Our analysis revealed multi-generational family groups living in close proximity, a tendency towards male-biased

dispersal, consistent isolation-by-distance effects at multiple spatial scales, and genomic signals of broader scale landscape features impeding gene flow among and within management units.

Although active management across its range in the US is focused on maintaining population persistence within largely intact patches of habitat, each management unit is subject to some degree of internal fragmentation due to variation in land use. For ongoing species management, it is critical to elucidate the drivers of population dynamics within and among management units, as differences may require unique management actions or resource allocation. Understanding how genes historically moved across the landscape and how anthropogenic changes may affect important migration corridors on contemporary timescales is essential to effective management.

Dispersal

Our data show a slight bias in male dispersal, which aligns with previous studies using radiotelemetry to quantify *P. mcallii* space use (Anderson Miller, 1999; Fischer, 2021; Setser, 2004; Wone & Beauchamp, 2003). The greatest distances between first-degree relatives were observed between a mother-daughter pair (1,372 m), and a pair of brothers (1,129 m). Sisters exhibited the lowest average distance; however, given that many of these samples were collected from hatchlings we expected proximity between siblings. Additionally, since samples were collected in different years, the distance between individuals is not always contemporaneous. For example, female 22YH8 and offspring female 23YH7 were found 170 m apart, but 22YH8 was not captured in 2023, so their contemporaneous distance is unknown.

The furthest same-year parent-hatchling pairs were between individuals we captured during the SDSU genomics study, rather than on demographic plots, which were spatially constrained to the plot dimensions (300 m x 300 m). Male Yuha 6 was found on 6/22/2023, and its hatchling male Yuha 44 was found on 10/8/2023, 632 m away. Female Yuha 8 was also initially captured on 6/22/2023 and her hatchling male Yuha 34 was found on 10/1/2023, 347 m away. Sampling dispersal primarily from the demographic plot biases the resulting parent-offspring distances by favoring capture of offspring from local females that may not have made large oviposition movements, or whose oviposition movements fell within or near the plot boundaries. A detailed assessment of dispersal could be performed by utilizing the dense sampling of individuals from ongoing monitoring efforts, expanded into the space surrounding the monitoring plot the week before or after demographic sampling.

Patterns of dispersal estimated from genetic data support the existing body of literature on *P. mcallii* spatial ecology, as well as in lizards (Perry & Garland Jr., 2002) and snakes (Todd & Nowakowski, 2021) more broadly. Males tend to move more and use larger tracts of space (Anderson Miller, 1999; Fischer, 2021; Wone & Beauchamp, 2003) throughout the year. Females in Arizona have been documented making large movements for oviposition then returning to a central core of activity (Pawlicki & Goode, 2026). Given these data it would seem *P. mcallii* dispersal is bimodal with males responsible for breeding dispersal and females responsible for natal dispersal. Little is yet known about the dispersal of neonates as they approach reproductive maturity in their first year, although Pawlicki and Goode (2026) detected movements over 3 km within the first year of life. It is unclear what conditions cause some *P. mcallii* to remain philopatric and others to disperse several kilometers outside their natal range.

If competition for resources drives dispersal, then lizard density could be a major contributor to large individual dispersal movements as lizards seek areas of greater resource availability. High annual mortality may conversely decrease local density such that hatchlings or juveniles may not need to disperse far from their natal range to access available resources.

Dispersal may also occur slowly, over the lifespan of the lizard as it shifts its range in response to environmental and social stimuli and moves genes around the landscape. Individuals have been reported shifting their center of activity through time (Anderson Miller, 1999; Setser, 2004), which gradually may disperse related individuals away from one another. These movements are probably pronounced in males as they cover more ground in search of mates, leading to the observed differences in annual movement and space use between the sexes (Anderson Miller, 1999; Fischer, 2021; Setser, 2004; Wone & Beauchamp, 2003). This is not always the case, though. Multi-generational matriarch 22N4 was initially captured on a 300 m x 300 m plot in 2022, then successively recaptured on this plot later in the year and then relocated on the same plot in 2023. The tendency for some individuals to remain philopatric for long periods of time has been observed by other researchers (Leah Carpenter, pers comm) who reliably find some males and females in the same general area in separate years. As individuals disperse in shorter timeframes or gradually over a lifetime, exposure to roadways and high-use OHV areas may inhibit gene flow across the landscape.

In addition to informing estimates of dispersal, our pedigree analysis also shed light on other aspects of the life history of this secretive species. We found multiple cases of individuals breeding the year after hatching. Female 22YH8, female 23YH22, female 24N6, and male 23YH2 were first captured as juveniles and their offspring were captured as hatchlings the following

year. Annual differences in age of first offspring likely differ year-to-year based on local precipitation and resource availability. Young (2010) observed that hatchlings did not attain adult size in a year during drought conditions, but after sufficient autumn and winter rains, hatchlings could reach adult sizes in six months. Winter rainfall in the Imperial Valley, 2022–2023, was higher than average, which probably contributed to increased body condition for lizards born in spring 2022 as well as spring 2023, allowing for first year reproduction.

We also documented the first known instance of multiple paternity in *P. mcallii*. Multiple paternity is standard in squamates (Clark et al., 2014; Fitze et al., 2005; Friesen et al., 2020; Stedman et al., 2016; Uller & Olsson, 2008), but examples from natural populations can be difficult to acquire without dedicated, long-term sampling. Multiple paternity has been documented in several lizard species, but only a few in the family *Phrynosomatidae*, and only once previously for a *Phrynosoma* spp. that we know of. Approximately 21%-28% of clutches from a captive breeding program of Texas Horned Lizards (*P. cornutum*) were polyandrous (Winter and Williams, unpublished data). We identified many half-siblings in our dataset that may originate from polyandrous clutches, but without parental data, we could not determine whether they were half siblings from polygyny or polyandry. We identified seven family groups with the necessary structure to identify polyandry, multiple siblings and a known mother. Two of these offspring out of the 16 total were half-siblings, indicating roughly 12.5% of offspring are polyandrous, but considering that we found 19 pairs of half-siblings without any maternal links, the percentage of polyandrous clutches may be slightly higher, closely reflecting the 20% found in *P. cornutum*. We did not identify related individuals from second clutches by one female in one year. Since most related pairs were captured on monitoring plots, if individuals are

producing second clutches in late summer, offspring would not be available for capture until September or October, so second-clutch offspring are unlikely to be captured by the current monitoring schedule.

Genetic Distance vs. Geographic Distance

Geographic distance explains some degree of genetic variation in many broadly distributed species, especially those with high levels of philopatry. *P. mcallii* population structure in the Western Imperial Valley described in Chapter 1 exhibited a minor pattern of isolation-by-distance. Species with small dispersal distances are often assumed to fit a classic population genetic model of isolation-by-distance where nearby individuals are more genetically similar than those further away (Wright, 1943). The simplicity of this model, however, often overlooks the complex realities of ecological systems (Meirmans, 2012). Correlations between measurements of genetic similarity and interdeme geographic distance can depend on the maximum dispersal distance of a given species as well as landscape configuration (van Strien et al., 2015).

Mantel tests indicate statistically significant relationships between genetic and geographic distance within all subpopulations in the western Imperial Valley, but this effect is minor and the data show little variance, even as scale increases dramatically. This reflects a case-II pattern indicating historically high gene flow (Hutchison & Templeton, 1999). Case-II patterns are often reflective of the genetic homogeneity of a founder group after recent colonization. This can remain a case-II pattern if too little time has passed to reach a regional equilibrium (i.e.- a strong pattern of isolation-by-distance), or if the population maintains

panmixia through gene flow. Given the recent (176–4,990 years ago) range-wide bottleneck in the species (Gottscho et al., 2024), it is plausible that not enough time has passed for these populations to have re-established a regional equilibrium, or that the geographic scale within the western Imperial Valley and historically high gene flow may not be sufficient to exhibit a strong pattern of isolation-by-distance. If the population structure detected in Chapter 1 reflects changes to gene flow due to anthropogenic features like the I-8 development corridor or CA-98, then we may observe increasing differentiation through time.

EEMS

EEMS analysis reveals three primary areas that appear to impede migration; these regions are congruent with fragmentation imposed by the I-8 development corridor and Plaster City OHV Area, the CA-98 highway, and Superstition Mountain and its associated OHV area in the central part of the valley. Decreased migration in the Yuha Basin between populations straddling CA-98 are likely a result of this high-traffic barrier, as there are no natural landscape features associated with the east-west directionality of CA-98 that would lead to this pattern.

The barrier in the south-central part of the valley is associated with I-8 to the south, as well as several smaller barriers to the east and west. The specific location of this region of low migration is not well represented by EEMS mapping because of the sampling gap between Yuha and West Mesa, but it is representative of a relative lack of gene flow between those two populations compared to other portions of the range. In addition to the highway, there is considerable OHV activity between I-8 and West Mesa South samples in the Plaster City OHV Area. OHV use here is not limited to trails and includes heavily used camping areas along Evan

Hewes Highway (north of I-8, running parallel) that have denuded these habitat patches of vegetation and undoubtedly compacted soils. The Juan Bautista de Anza National Historic Trail and the Paster City rail line run north-south along the west side of this south-central barrier; however the lack of landscape resistance around the westernmost samples in this region indicate that these barriers may not affect migration as much as the land use and fragmentation to the south. Regardless, the rail line may pose a slight physical barrier, and the well-traveled historic trail may impact lizard density along the edges. On the east side of this south-central barrier is habitat edge that abuts agricultural matrix, and Huff/Imler Road isolate one patch of habitat from the rest. Huff/Imler Road appears to be a barrier between this isolated patch and adjacent samples collected in West Mesa North.

In the north-central part of the valley, Superstition Mountain (the northwest lobe of Superstition Mountain OHV Area) appears to inhibit gene flow between populations to the north and south, even though washes of suitable habitat connect the north and south sides of the mountain. Superstition Mountain OHV Area encompasses most of the mountain, as well as land to the south, and these connecting habitat corridors are also popular riding features. The habitat against the sand fields on the south edge of the mountain is appropriate for *P. mcallii* and *Uma notata*, but this region is a high-use area during the active riding season, which begins in autumn and ends in late spring, with some use continuing through the summer by dedicated riders that operate primarily during optimal diurnal lizard activity hours before the ground temperatures climb too high. The rocky, mountainous terrain of Superstition Mountain and the high OHV land use pose unique constraints on *P. mcallii* migration in the area. Nonetheless, there appears to be a migration corridor along the southeast side of Superstition Mountain.

Most high migration areas appear to connect adjacent, mostly intact patches of *P. mcallii* habitat in the north and south Yuha, and in the northern West Mesa patches, near San Sebastian Marsh. The high migration corridor near where Evan Hewes Highway meets I-8 is approximately where washes travel beneath I-8, just east of Ocotillo, although given the lack of samples in this area, this should be interpreted with caution. However, this is also the region where tess3r ancestry coefficients between the Yuha Basin and West Mesa appear to mix (Figure 1.6), which indicates that these highway underpasses may help maintain some level of connectivity between Yuha Basin and West Mesa populations.

Effective diversity (q) appears relatively low throughout West Mesa. This trend is patchy but most strongly associated with habitat edges. Diversity appears highest in the Yuha Basin, which could be partially explained by generally lower OHV use and landscape features that may limit off-trail riding. The lowest diversity area in the Yuha Basin appears in the southeast quadrant where habitat ends against an agricultural matrix. Habitat edges can be sinks for genetic diversity due to a lack immigrating individuals from surrounding matrix. The agricultural edge also subsidizes perching raptors, which may increase predation near the edges.

Conservation and Management Implications

Genetic differentiation is a temporal process that occurs across hundreds or thousands of generations, but genomic tools can often identify barrier effects imposed by high-traffic volume roads on relatively short timescales (Delaney et al., 2010; Fusco et al., 2021; Maigret et al., 2020). Fragmentation effects imposed by vehicular traffic are likely underestimated due to the amount of OHV activity on unpaved roads across *P. mcallii* range. OHV impacts are not

limited to direct mortality (Clark et al., 2024) but a host of biotic and abiotic effects that include destruction and denuding of vegetation (Navas Romero et al., 2019), erosion and compaction of soils (Al-Dousari et al., 2019), the spread of invasive species like Sahara Mustard (*Brassica tournefortii*) (Rodriguez et al., 2024; Trader et al., 2006), and destruction of soil biocrusts which regulate soil water uptake by infiltration and retention (Belnap, 2006; Chamizo et al., 2016).

Rangewide, *P. mcallii* populations are likely at greater risk from direct vehicle mortality than from curtailed gene flow due to road avoidance, since a greater proportion of roadway fragmentation in their range is composed of unpaved OHV trails that likely do not include the same type of avoidance as high-traffic volume paved roads. Regardless, it has been shown that populations experiencing direct road mortality are more at risk of extinction than those that exhibit behavioral avoidance (Ceia-Hasse et al., 2018). Furthermore, these lizards are prone to thermoregulating on roads of mid-to-low traffic density (pers obs). Despite regular research in OHV units, observations of direct OHV mortality are few (Grant, 2005; Grant & Doherty, 2009; Setser, 2004). However, behavioral avoidance of OHVs due to noise and visual cues may cause lizards to remain close to shrub cover or in burrows during the riding season, inhibiting or reducing overall dispersal, but we have limited information on how individuals directly respond to OHV use, warranting further study on this topic.

The support presented here for geographic population structuring and the presence of barriers between management units should be of concern for ongoing management of the species. Populations within the Yuha Basin and West Mesa may continue to diverge through time, and unique, population-specific demographic dynamics and stochasticity may drive changes within separate management units. Sections of CA-78 in the Borrego Valley receive

traffic volumes comparable to CA-98 in the Yuha Basin (roughly 1,200-1,600 AADT with peaks over 2,200, Caltrans Traffic Census Program, 2024), so there may be disruption of gene flow between the western Imperial Valley and Ocotillo Wells State Vehicular Recreation Area on the north side of CA-78. These Management and Research Areas, as designated by the FTHL ICC (FTHL ICC RMS, 2003), must continue to evaluate population health and structure as well as ongoing threats to persistence of the species within and between units.

The potential signals of gene flow occurring beneath the highway underpasses east of Ocotillo, CA call for a targeted molecular study along the boundaries of I-8 between the Yuha Basin and West Mesa populations. While these underpasses are common gathering locations and riding destinations for OHV riders during the active season, their absence during the summer months may provide adequate opportunity for lizards to disperse through this corridor and provide necessary gene flow between management units. Thorough sampling south of I-8 in the Yuha Basin, and north from Ocotillo to the agricultural edge east of Plaster City and south of Evan Hewes highway may better capture genomic signatures of a highway barrier. The engineering of additional highway underpasses along I-8 could promote gene flow between more widely dispersed demes of lizards, improving overall genetic diversity for this species among others that are undoubtedly affected by this high-traffic roadway. Such a project should include molecular ecology research goals aligned with the before-after control-impact design (BACI) where genetic samples are collected from a broad community of species occupying patches adjacent to the underpass before development, after development (monitoring), and if possible, from control (unimpacted) and impacted sites to evaluate the efficacy of the project (Balkenhol & Waits, 2009; Conner et al., 2016; Gracanin et al., 2023).

While roads are the most common fragmenting linear feature across *P. mcallii* habitat, the development of a border wall along the US-Mexico border has introduced perhaps the most substantial single fragmenting feature to the species' range, and construction of a second wall began in 2026. The border wall has the potential to impact *P. mcallii* gene flow between populations in the Yuha Basin and Laguna Salada to the south, as well as populations in Yuma and Gran Desierto de Altar, although research to date is lacking. Further research could expand the efforts here, using genomic tools to elucidate the impacts of the border walls on the connectivity and dispersal of this species.

This study is a first attempt to describe the genetic effects of anthropogenic development and land use for *Phrynosoma mcallii* in California. This information serves as a baseline from which further studies may build targeted questions and hypotheses about how landscape factors continue to influence *P. mcallii* ecology. The combined impacts of anthropogenic fragmentation and ongoing land use will continue to put pressure on *P. mcallii* populations across their range. It is imperative that we document these impacts and mitigate them where possible. While the patterns documented here do not support the immediate need for interventions like assisted migration, it is clear that further research is necessary to characterize in more detail the potential differentiation arising between populations and the causes of that differentiation. Identifying gene flow corridors between management units is key to maintaining genetic diversity within and between adjacent populations. Spatial distance and landscape arrangement are responsible for only a small portion of overall genetic variation in our populations, so we must further explore the environmental, geographic, and anthropogenic influences on both neutral and adaptive genomic regions.

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CHAPTER 3

Integrating Temporal Genetic Monitoring into Ongoing Monitoring and Management of the Flat-tailed Horned Lizard (*Phrynosoma mcallii*)

Abstract

As global biodiversity declines threaten ecosystems and natural populations, there has been massive effort to conserve at-risk species through active management. Management action relies on information gathered through monitoring efforts tailored to the needs of conservation systems. Single-species conservation efforts often rely on temporal population monitoring to assess changes to population dynamics through time. Recently, molecular ecology approaches have introduced a unique suite of tools for conservation practitioners to use in assessing genetic diversity, population connectivity, inbreeding, and other genetic metrics of interest through time in natural populations. The flat-tailed horned lizard (*Phrynosoma mcallii*) is a Colorado Desert flagship species with a decades-long history of population monitoring and management. We collected genomic DNA from lizards captured on demographic and occupancy monitoring plots from 2022-2024 and created a biallelic single nucleotide polymorphism dataset using double-digest restriction site-associated DNA sequencing. We used these data to assess the effect of various genetic sampling regimes on population genetic summary statistics, then tested for heterozygosity-fitness correlations (HFCs) and estimated effective population size (N_e) on each of four demographic sampling plots. We found no evidence of inbreeding or HFCs that would indicate problematic levels of genetic diversity. We also estimated a western Imperial Valley effective population size above the recommended thresholds to maintain adaptive potential through time. We integrated these findings with those from previous chapters to make holistic recommendations for integration of genetic monitoring into existing

monitoring efforts and discuss the future of genetic research for *P. mcallii*. This case study will provide a basis for similar monitoring programs to incorporate temporal genetic monitoring as a tool within conservation and management systems.

Introduction

Wildlife Monitoring

Global biodiversity decline is one of the most pressing issues of our time (Butchart et al., 2010; Dirzo et al., 2014). Legislation like the Endangered Species Act (ESA) in the US and the myriad state-level ESAs that followed led to a proliferation of single-species conservation and recovery efforts (Greenwald et al., 2019; Schwartz, 2008). The framework central to the ESA protects imperiled species, sets recovery goals, then monitors conservation outcomes to gauge success, with the ultimate goal of de-listing species that have met recovery criteria. Though this generally successful approach centers on individual species, effective conservation considers their ecological context, incorporating community and habitat-level protection into conservation planning (Runge et al., 2019), and thus “flagship” or “umbrella” species serve as a means of protecting greater ecological communities. Wildlife monitoring is therefore an essential component of biodiversity conservation planning and management (Bottrill & Pressey, 2012).

Due to the landscape scale of conservation and management, monitoring often includes collaboration among partner groups. The interconnectedness of natural systems requires managing wildlife across political boundaries, so wildlife monitoring strategies often extend beyond individual political or management boundaries and include national (Costa et al., 2021; Kelly et al., 2024), international (Aburto-Oropeza et al., 2018), and global (Brooks et al., 2006;

Carneiro et al., 2025; Le Prestre, 2017) coordination and collaboration. Together, stakeholders must establish monitoring goals, expected outcomes, and actions.

Effective monitoring requires clearly defined objectives. Monitoring design depends directly on stated research or management objectives and must be tailored to answer central questions. A common objective is monitoring how the state of communities or populations may change through time (Pollock et al., 2002). This “surveillance monitoring” is often conducted with the goal of maintaining some benchmark of relative abundance; this basic goal then requires sampling wild populations at regular intervals to attain robust temporal estimates (Hutto & Belote, 2013). However, something even as straightforward as measuring abundance through time can require considerable resources (labor and money) that tend to be limited in conservation (Bottrill & Pressey, 2012). Researchers must select research plots of appropriate size, number, and distribution to adequately capture population processes of interest, and this sampling design must have the power to detect change. Factors of detectability, population heterogeneity, and the underlying assumptions of statistical models limit the resolution and representativeness of collected data and add uncertainty to conclusions drawn from population modeling (Pollock et al., 2002).

Once monitoring objectives are established, researchers must identify thresholds of change at which management action is required and determine realistic actions that may reverse or mitigate the observed change (Lindenmayer et al., 2013). From that point, effectiveness monitoring can determine the efficacy of management actions based on data collected with the specific aim of resolving the observed problematic change (Hutto & Belote,

2013). Without actionable trigger points, monitoring is itself insufficient to protect populations from decline (Lindenmayer et al., 2013).

Technological advancements have yielded novel tools for use in wildlife monitoring (Lahoz-Monfort & Magrath, 2021). Advancements in DNA sequencing have extended ecological research well-beyond the single-locus and modest multi-locus studies of the early genetics era (Heather & Chain, 2016; Zhang et al., 2011). Next generation whole-genome DNA sequencing techniques now allow for the genotyping of hundreds of individuals at thousands of loci, opening the doors to a wide array of ecological and evolutionary questions that can inform management of imperiled species (Ekblom & Galindo, 2011; Gilad et al., 2009). The extension of these tools to non-model systems makes them tractable for imperiled species with little genetic research history (Helyar et al., 2011; Peterson et al., 2012). Additionally, availability of powerful open-source computational tools has allowed for stakeholders to employ methods formerly relegated to academic labs. From these conditions, the birth of conservation genetics has yielded many insights into the ecological and evolutionary dynamics of natural systems (Allendorf et al., 2022; Benestan et al., 2016; Funk et al., 2019).

Conservation Genomics

The central goal of conservation genetics and genomics is to characterize patterns of genetic variation in natural populations. Genetic variation holds insights into conservation-relevant processes such as inbreeding depression, population connectivity, introgression, and adaptation, which can inform management (Allendorf et al., 2022). Individual species genetic diversity monitoring primarily targets potential loss of within and between-population genetic

diversity as well as loss of functional or adaptive genetic diversity (O'Brien et al., 2022).

Additionally, genetic information can be used to identify individuals, delimit conservation units, and assess historical and contemporary population size (Hendricks et al., 2018; Hohenlohe et al., 2021). Since genetic diversity is coupled with fitness and adaptive potential, it has become an important component of conservation and management programs aimed at maximizing population persistence (Bertola et al., 2024; Ralls et al., 2018).

Genetic diversity is key to population persistence because, like other measures of population wellbeing, it is shaped by both historic and contemporary demographic and stochastic processes that can be explored in natural populations and leveraged to inform management action. For example, historic population contraction and expansion leave unique molecular signatures through the loss of genetic diversity and shifting of allele frequencies. Evidence of drastic population losses may persist into contemporary gene pools (Frankham et al., 2002), and this loss in genetic diversity and increase in inbreeding potential can decrease individual fitness and threaten population persistence (Grueber et al., 2008). Inbreeding effects have been explored through heterozygosity-fitness correlations (HFCs), and through correlations between multi-locus heterozygosity and fitness proxies like breeding success, survival, or body condition that indicate phenotypic effects of decreased heterozygosity (Gibbs & Chiucchi, 2012; Judson et al., 2018; Rodríguez-Quilón et al., 2015).

The application of genetic and genomic tools to conservation systems has improved monitoring in several ways. Molecular markers have been used to track genetic diversity in small populations facing high inbreeding risk and to maintain genetic diversity in captive breeding programs supplementing wild populations (Chen et al., 2025; Sovic et al., 2019). Genetic

monitoring has elucidated risks associated with conservation translocations where local adaptation may influence translocation outcomes (Marshall et al., 2022; Seaborn et al., 2021). Additionally, temporal trends in diversity metrics provide insight into the efficacy of ongoing conservation actions and provide baselines against which to judge changes in diversity that may require management action (Alves et al., 2023; Arpin et al., 2024; Gajdárová et al., 2023). Genetic monitoring also improves traditional methods of population monitoring by supplementing demographic monitoring data and improving population-level inference (Hemstrom et al., 2022; Wood et al., 2020).

Genetic monitoring adheres to the same standards as traditional monitoring programs (Flanagan et al., 2018). Goals should be clear and quantifiable, and sensitive to changes in time and space (Fischer et al., 2026; Weber et al., 2004). Additionally, genetic monitoring must meet criteria to be maintained by stakeholders, namely financial constraints and usefulness of monitoring outcomes (Pärli et al., 2021). For existing monitoring programs with field crews already collecting data from individual organisms, integrating collection of tissues adequate for genomic sequencing can be straightforward, but genetic monitoring becomes more complicated during sample processing, DNA extraction and library preparation, sequencing, and analysis. Involving stakeholders with expertise in these processes improves the utility of genetic data by leveraging available laboratory and labor resources (i.e. lab equipment, graduate and undergraduate students).

Reptiles are generally understudied and poorly represented in the conservation genomics literature (Carneiro et al., 2025; Roll et al., 2017; Shaffer et al., 2015; Tingley et al., 2016). Reptiles are diverse and abundant and perform important ecological roles as predators

and prey, ecosystem engineers, and as commensal species (Böhm et al., 2013). They are often range-limited as habitat or niche specialists which makes them sensitive to landscape change and therefore useful bioindicators (Keinath et al., 2017). Few annual reptile monitoring programs exist (Erb et al., 2015; How et al., 2022; Moussy et al., 2022), and even fewer use a temporal genetic monitoring component. Application of genomics tools to reptile conservation is both practical and expands the relatively small body of ecology and evolution research dedicated to these understudied taxa.

Flat-tailed Horned Lizard Case Study

Here we return to a conservation and management flagship of the Colorado Desert (and Chapters 1 and 2), *Phrynosoma mcallii*, the flat-tailed horned lizard, which will serve as a critical case study for the incorporation of genomic tools into long-term monitoring approaches coordinated across various institutions and agencies. As a range-restricted habitat and dietary specialist, its life history traits make it vulnerable to landscape modification and environmental change. An estimated 50% of its range in California has been lost to landscape modification and development, and current land use in its remaining habitat threaten its long-term persistence (Barrows & Allen, 2009; Beauchamp et al., 1998; Leavitt et al., 2015).

In November 1993, the US Fish and Wildlife Service (USFWS) issued a proposed rule to list *P. mcallii* as threatened under the US ESA. In 1997 the USFWS decided against listing citing both inconclusive population data documenting substantial declines as well as the proactive formation of a multi-agency conservation agreement tasked with protecting the lizard on public lands (62 Fed. Reg. 37852). This conservation agreement was drafted by a group of state and

federal stakeholders who established a flat-tailed horned lizard interagency coordinating committee (FTHL ICC) with the overall goal to maintain self-sustaining populations of flat-tailed horned lizards in perpetuity (*Flat-Tailed Horned Lizard Rangewide Management Strategy, 2003 Revision*, 2003). The ICC's range-wide management strategy (RMS) details threats to long-term persistence, identifies research priorities, and sets conservation goals for the species, which includes development caps for signatories that manage *P. mcallii* habitat, risk mitigation practices, and annual monitoring across five established Management Areas (MAs) and one Research Area (RA) (Figure 3.1).

On an annual basis, stakeholders monitor *P. mcallii* populations on each MA using many widespread occupancy plots and a few closed mark-recapture demographic sampling plots. Arizona Game and Fish Department (AZGFD) scientists regularly report on Yuma Desert Management Area trends (Arnett-Romero et al., 2023a, 2023b; Grimsley-Padron et al., 2021) and additionally perform Population Viability Analysis (PVA), a common management tool using population data and an array of life history traits to predict long-term population persistence (Grimsley-Padron et al., 2017, 2019). Analysis of this survey data has detected cyclical changes in population abundance within MAs, the first temporal trends published for the species (Leavitt et al., 2015).

Previous molecular studies of *P. mcallii* have focused on resolving group (*Phrynosoma*) phylogenies and exploring phylogeography (Gottscho et al., 2024; Hodges & Zamudio, 2004; Leaché & McGuire, 2006; Mulcahy et al., 2006). Genetic markers from these studies have revealed strong population structuring at a broad scale across the Colorado River, which acts as a barrier to gene flow between southeastern and northwestern populations (Gottscho et al.,

2024; Mulcahy et al., 2006). Biogeographic studies help demystify present patterns of region-wide biodiversity and species distribution through a historic lens, but management also requires incorporating contemporary measures of existing population structure and genetic diversity. Payne et al. (2025) published the first contemporary measures of genetic diversity for a *P. mcallii* population outside of Yuma, AZ and documented molecular signals of fragmentation imposed by recent roadways. Additional assessments of range-wide genetic diversity are still necessary to support appropriate conservation and management.

Chapter 1 detailed population genetic structure congruent with existing management units; the goal of this chapter is to explicitly link genomic tools to on-the-ground monitoring efforts to provide a case study for how genomic analyses may be incorporated into multi-agency long-term species monitoring. We used genomic DNA samples collected on FTHL ICC demographic and occupancy monitoring plots, in addition to samples collected by SDSU for the first two chapters of this dissertation, to generate a SNP dataset using reduced representation DNA sequencing. We used this dataset to address the following questions and then detail a pragmatic approach to incorporating genomic sampling into the existing *P. mcallii* monitoring program:

1. What sampling scheme provides the best resolution of population-wide genetic diversity within Management Areas?
2. Does each Management Area meet the upper threshold of effective population size recommended in the conservation ecology literature?

3. Do genomic tools reveal any indications of genetic and phenotypic effects of small population size or inbreeding depression as a consequence of a recent bottleneck or other impacts (Gottscho et al., 2024)?
4. In synthesizing the results of this and other chapters of this dissertation, what recommendations can be made to improve FTHL conservation and highlight this work as a case study for making genomic tools foundational in ecological monitoring?

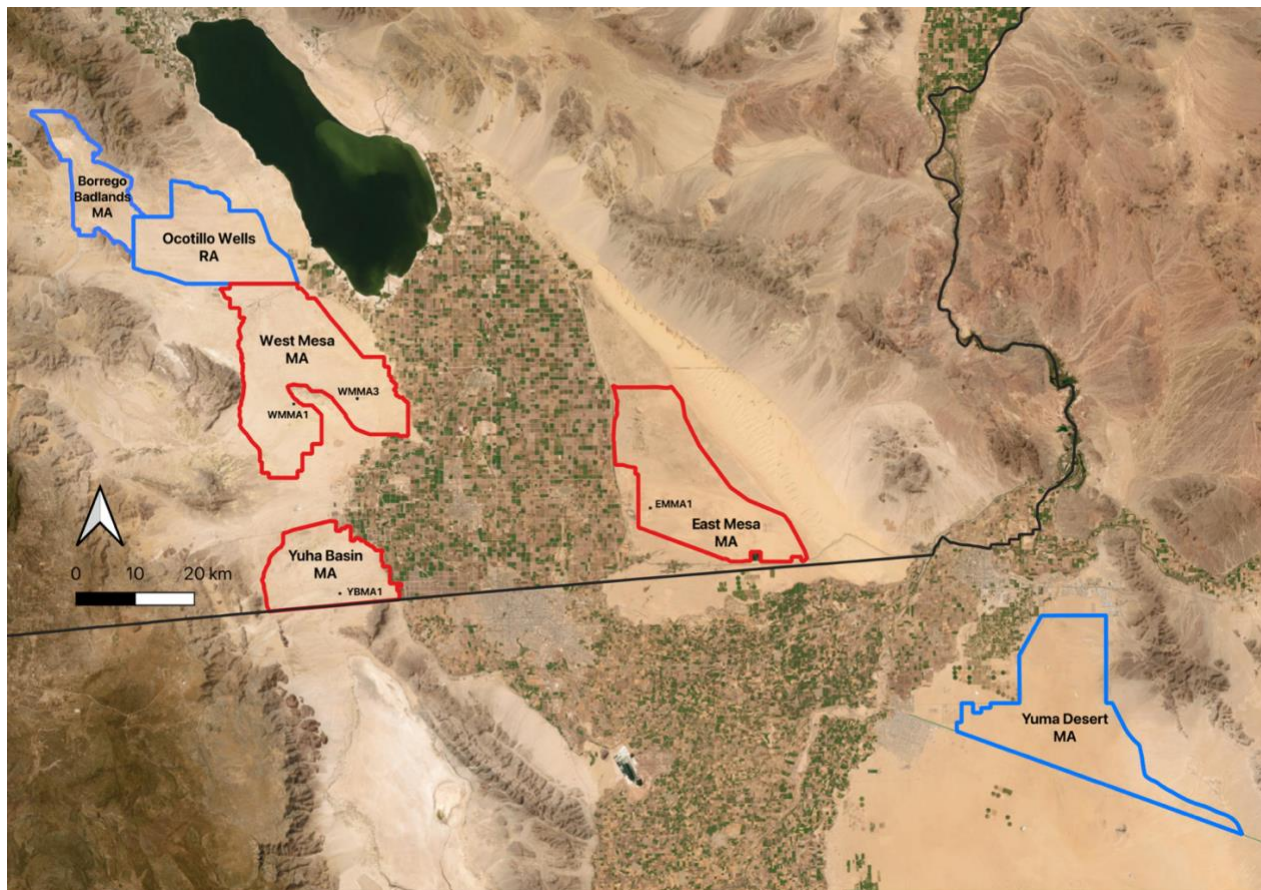


Figure 3.5- FTHL ICC Management and Research Areas. Sampled MAs are outlined in red and each of four demographic monitoring plots are labelled within MA (WMMA1, WMMA3, YBMA1, EMMA1).

Materials and Methods

Sample Collection

BLM field crews collected *P. mcallii* tissue samples on BLM demographic monitoring plots East Mesa Management Area 1 (EMMA1), West Mesa Management Area 1 (WMMA1), West Mesa Management Area 3 (WMMA3), and Yuha Basin Management Area (YBMA1) from 2022-2024. Samples were also incidentally collected from Yuha and West Mesa occupancy monitoring plots in 2023 and 2024, respectively (Figures 3.2, 3.3).

We additionally used visual encounter surveys to locate and collect tissue samples from *P. mcallii* across the western Imperial Valley, between the Yuha Basin and West Mesa (Figure 1.2). We collected genomic DNA via blood blots on filter paper that were then frozen at -20° C. On capture we measured individual lizard mass and snout-to-vent length (SVL). See chapter 1 for further details of lizard capture and processing, DNA extraction, library preparation, and sequencing. The same ipyrad (Eaton & Overcast, 2020) procedure was used to generate genetic variant data for downstream analyses. Details of genome assembly and alignment to the *Phrynosoma platyrhinos* genome (Koochekian et al., 2022) can be found in the Bioinformatics Pipeline section of Chapter 1.

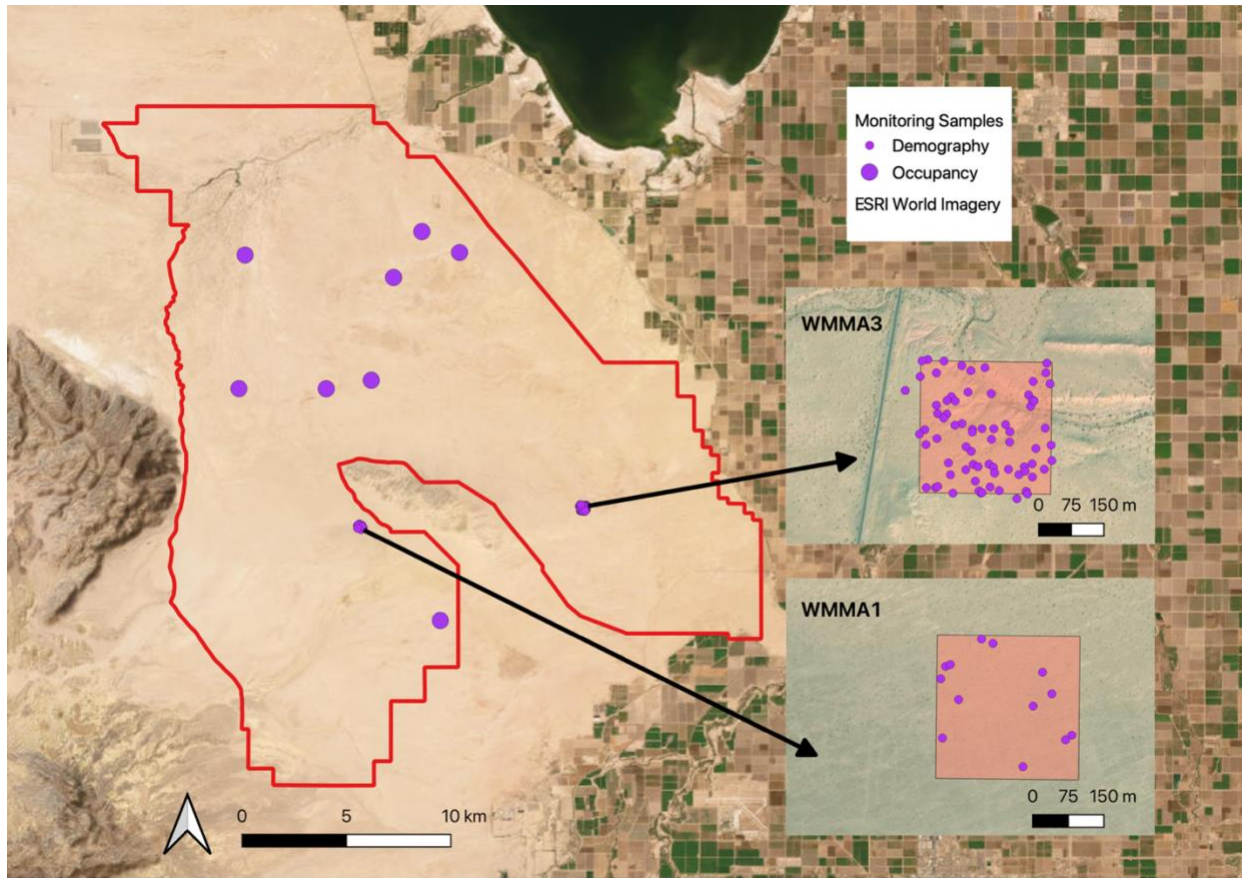


Figure 3.6- Capture locations for *P. mcallii* samples collected during West Mesa Occupancy and Demographic monitoring.

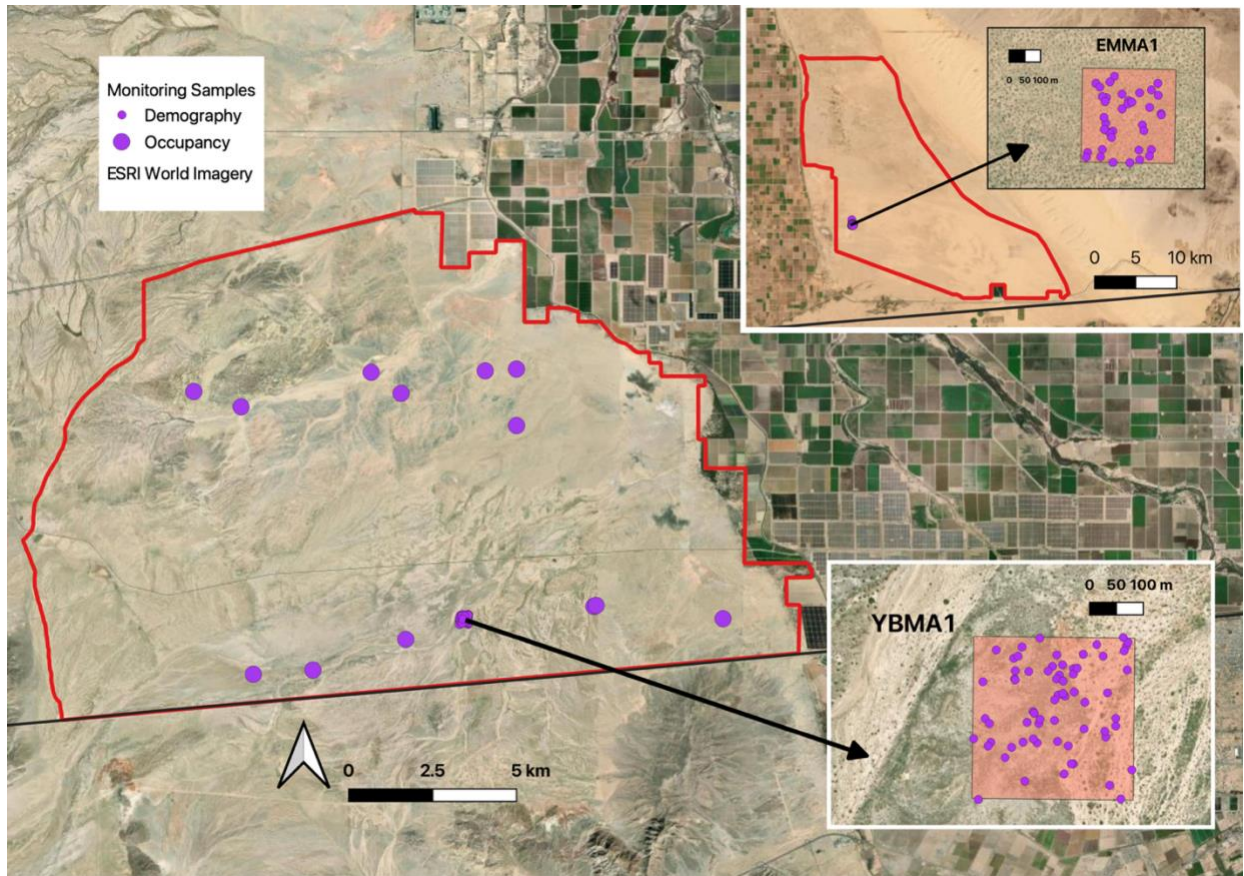


Figure 3.7- Capture locations for *P. mcallii* samples collected during Yuha Basin and East Mesa Occupancy and Demographic monitoring

Data Filtering

For these fine-scale analyses we filtered our data using VCFTools (Danecek et al., 2011) to remove indels and keep only biallelic SNPs. We filtered at a minimum read depth of 10 (--minDP 10) and maximum read depth of 50 (--maxDP 50), and a 1% minor allele frequency cutoff (--MAF 0.01). We conservatively dropped individuals with over 80% missing data, which removed 21 individuals from the final dataset. We removed loci that weren't present in at least 85% of samples (--MISS 0.85). Following VCFTools filtering we used SNPfiltR (DeRaad, 2022) to filter for allele balance which can reduce the impact of sequencing or mapping errors on the

final dataset. Following allele balance conversion, we re-filtered loci at an 85% missingness cutoff. The resultant dataset is hereafter referred to as the full dataset.

Data Analyses

Genetic Diversity

We calculated diversity metrics using several data subsets to investigate the impact of various sampling regimes on genetic diversity estimation. We sampled four FTHL ICC demographic monitoring plots from 2022-2024 including West Mesa Management Area 1 (WMMA1), West Mesa Management Area 3 (WMMA3), Yuha Basin Management Area 1 (YBMA1), and East Mesa Management Area 1 (EMMA1). We also collected samples from ICC occupancy monitoring plots in the Yuha Basin in 2023 and from West Mesa in 2024. The various sampling regimes are intended to capture variation in sample size, timeframe, and geographic coverage (Table 3.1). Occupancy samples collected in consecutive years are not represented in our final dataset but are pertinent to our discussion of future genetic monitoring. In addition to FTHL ICC monitoring sampling regimes, we included SDSU study samples for West Mesa and the Yuha Basin. SDSU West Mesa samples represent greater sample sizes, as many as 4 generations, and broad geographic coverage. SDSU Yuha captures greater sample sizes, a single temporal frame, and broad geographic coverage.

Expected and observed heterozygosity and percent missing data were calculated with the R package *adegenet* (Jombart & Ahmed, 2011) using SNPs from the full dataset, averaged by individual then across each sampling group. F_{IS} was calculated by sampling group using the R

package *hierfstat* (Goudet & Jombart, 2004). To test for a correlation between data missingness and F_{IS} we calculated Pearson's correlation coefficient.

Table 3.3- Genetic sampling regimes based on current monitoring efforts. Combined monitoring includes samples collected from both occupancy and demography monitoring plots.

Sampling Regime	Sample Size	Geographic Coverage	Temporal coverage
Demography, Single Year	Small-moderate	Limited	One generation
Demography, Consecutive Years	Moderate-large	Limited	Multiple generations
Occupancy, Single Year	Small	Broad	One generation
Occupancy, Consecutive Years	Moderate-large	Broad	Multiple generations
Combined Monitoring, Single Year	Large	Broad	One generation

Effective Population Size

Using the full dataset filtered to one SNP per locus we estimated contemporary effective population size (N_e) for each of the FTHL ICC demographic monitoring plots. We used the LD method with assumption of random mating, and estimated N_e using multiple minor allele frequency cutoffs ($P_{crit} = 0.01, 0.02, 0.05$) with NeEstimator v2.1 (Do et al., 2014). Large numbers of SNPs in LD-based N_e estimation can decrease the accuracy of confidence interval estimation (Jones et al., 2016), so we ran NeEstimator on 10 random subsets of 2,000 SNPs for each population. After subsetting data by population, we ran NeEstimator on each population's full dataset to acquire a list of monomorphic loci and then removed those loci from each subset to ensure only polymorphic loci were used in estimates. We then subset to 2,000 random SNPs and output population genepop files. Reported estimates are an average of the 10 runs for each population. We additionally plotted all 10 estimates with confidence intervals for each population.

Heterozygosity-Fitness Correlations

To test for HFCs within *P. mcallii* populations sampled in demographic monitoring plots, we used scaled mass index (SMI) as a fitness proxy since body condition has been demonstrated as a reliable indicator of fitness in lizards (Le Galliard et al., 2004). SMI is calculated with the formula $\widehat{M}_i = M_i \left[\frac{L_0}{L_i} \right]^{b_{SMA}}$ where M_i and L_i are the mass and snout-to-vent length of individual i , L_0 is the arithmetic mean SVL of all sampled individuals, and b_{SMA} is the scaling exponent estimated by the standardized major axis regression of mass on length of all sampled individuals (Peig & Green, 2009). We used the R package *smatr* (Warton et al., 2012) to acquire the slope of the standardized major axis regression of log-transformed SVL and mass, and used this as the value for the exponent, $b_{SMA} = 2.899$. In our data the arithmetic mean SVL was $L_0 = 57.92$ mm. We estimated the potential effect size on body condition for each population using the Pearson product moment correlation (r) between MLH and SMI. We then used SMI as the response variable in a series of linear models against fixed variables multi-locus heterozygosity (MLH), plot, year, and sex to best account for other factors that could potentially influence body condition. Season was not included since all samples were collected during demographic sampling efforts that occur in late summer. Initially the global model included interaction terms, but the interaction terms were non-significant, so they were removed. We then incorporated models with increasing complexity up to the global model, and used the R package *MuMIn* (Bartoń, 2026) to perform model selection, selecting the model with the lowest overall AIC. We visually observed the global model residuals for assumptions of linearity and signs of heteroscedasticity. For this analysis we used individuals across all sampling years

for each site since HFCs, if present, would affect individuals regardless of sampling year. Two females that were presumed gravid were removed from SMI scaling and subsequent analysis.

Results

Data Structure

The final dataset contained 548 samples, 72,914 SNPs, and 8.4% missing data. We used 529 samples to estimate genetic diversity parameters. We used 193 individuals from demographic sampling plots to estimate N_e : 39 from EMMA1, 13 from WMMA1, 75 from WMMA3, and 66 from YBMA1. Prior to monomorphic loci removal, this dataset contained 20,130 SNPs. Each plot differed in the number of monomorphic loci, leaving different numbers of SNPs from which to draw the 2,000 random SNPs for N_e estimation: EMMA1 had 4,075 monomorphic loci, leaving 16,055 SNPs; WMMA1 had 6,690 monomorphic loci, leaving 13,440 SNPs; WMMA3 had 1,511 monomorphic loci, leaving 18,619 SNPs; YBMA1 had 2,307 monomorphic loci, leaving 17,823 SNPs. 512 samples were used to estimate N_e across the entire western Imperial Valley. The western Imperial Valley had 5 monomorphic loci, leaving 20,125 SNPs.

Genetic Diversity

Observed heterozygosity was less than expected for all plots and timeframes with sample sizes ≥ 15 , but greater than expected for all plots and timeframes with sample sizes < 15 (Table 3.2). Data missingness was generally low (5.1–15%) and there was no correlation between data missingness and F_{IS} ($r = 0.11$, $p = 0.64$). Inbreeding coefficient was highest on

YBMA1 in 2024 ($F_{IS} = 0.0569$) and EMMA1 in 2024 ($F_{IS} = 0.0526$), showing slight heterozygote deficiency, but was otherwise generally low (F_{IS} range = 0.0185–0.0569). Nucleotide diversity showed little variation across all plots and sampling regimes (range = 0.00053–0.00062).

Plot	Year	n	H_E	H_O	π	F_{IS}	Missingness
WMMA1	2023	8	0.163	0.173	0.00054	0.0185	10%
	2024	5	0.152	0.169	0.00053	0.0219	8.7%
	All	13	0.168	0.171	0.00054	0.0283	9.5%
WMMA3	2022	11	0.168	0.171	0.00058	0.0321	8.4%
	2023	12	0.170	0.172	0.00057	0.0386	9%
	2024	52	0.175	0.170	0.00055	0.0384	12.1%
	All	75	0.176	0.170	0.00056	0.0376	11.1%
YBMA1	2022	11	0.168	0.172	0.00058	0.0302	6.7%
	2023	27	0.171	0.170	0.00056	0.0312	9.0%
	2024	21	0.171	0.166	0.00057	0.0569	11.4%
	All	59	0.175	0.169	0.00057	0.0434	8.6%
EMMA1	2023	20	0.171	0.168	0.00056	0.0185	11.5%
	2024	15	0.169	0.167	0.00058	0.0526	15%
	All	35	0.173	0.168	0.00057	0.0474	13%
Yuha Occupancy	2023	13	0.170	0.171	0.00060	0.0461	5.1%
WM Occupancy	2024	8	0.166	0.173	0.00062	0.0389	12%
Yuha Monitoring	2023	40	0.176	0.169	0.00058	0.0458	8.1%
WM Monitoring	2024	65	0.177	0.171	0.00056	0.0404	11%
SDSU Yuha	2023	42	0.175	0.170	0.00059	0.0428	5.4%
SDSU WM	2021-2024	284	0.178	0.169	0.00056	0.0498	7.8%

Table 3.4- Diversity metrics across FTHL ICC demographic monitoring plots (WMMA1, WMMA3, YBMA1, EMMA1), occupancy plots in the Yuha Basin and West Mesa, combined demographic and occupancy monitoring within one year for West Mesa and the Yuha Basin, and SDSU sampling efforts. Average expected (H_E) and observed (H_O) heterozygosity and data missingness were calculated using R package *adegenet*. Population level inbreeding coefficient (F_{IS}) was calculated using R package *hierfstat*. Nucleotide diversity (π) was calculated from variant and invariant sites using R package *pegas*. All summary statistics generated using the full dataset with the exception of π which was generated using a full alignment PHYLIP file filtered for loci present in at least 70% of individuals then subset into the above groups.

Effective Population Size

Average estimation of effective population size on all demographic plots ranged from 29–117 individuals (Table 3.3, Figure 3.4). WMMA1 had the lowest N_e at 29 ($8-\infty$), and EMMA1

had the highest 117 (48– ∞), but both had unbounded upper confidence intervals. WMMA1 UCI is likely due to sample size, but EMMA1 may be caused by large populations surrounding the sampled individuals. N_e estimated from samples across the entire western Imperial Valley, including the Yuha Basin, was 1,048 (827–1,404).

Plot	N_e (LCI-UCI)		
	$P_{crit} \ 0.05$	$P_{crit} \ 0.02$	$P_{crit} \ 0.01$
WMMA1	29 (8– ∞)	53 (12– ∞)	53 (12– ∞)
WMMA3	69 (45–123)	79 (53–135)	88 (60–151)
YBMA1	62 (43–102)	69 (47–113)	76 (52–125)
EMMA1	117 (48– ∞)	143 (55– ∞)	179 (63– ∞)
WIV	1,048 (827–1,404)	1,074 (846–1,444)	1,063 (833–1,441)

Table 3.3- Average effective population size estimates for each ICC Demographic monitoring plot, under three different minor allele frequency thresholds (P_{crit}). Estimates are averaged from 10 independent sets of 2,000 SNPs randomly generated from a larger SNP dataset.

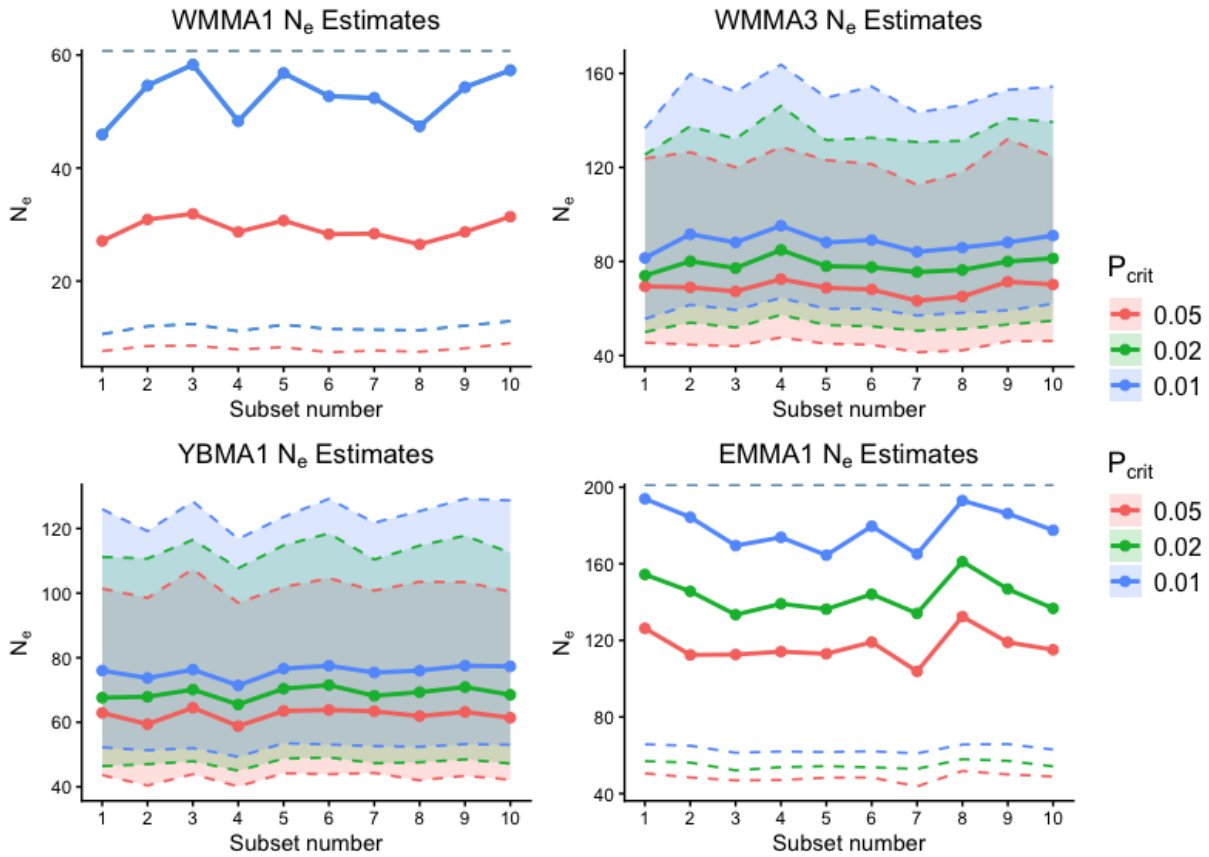


Figure 3.4- Effective population size estimates using 3 different P_{crit} values (0.01, 0.02, 0.05) for each of 10 2,000 SNP subsets by ICC Demographic sampling plot. Upper and lower N_e confidence intervals are bounded in the color of the associated P_{crit} value. EMMA1 and WMMA1 upper confidence limits were infinite. WMMA1 estimates for P_{crit} values of 0.01 and 0.02 are identical and overlap.

Heterozygosity-fitness correlations

None of the monitoring plots exhibited significant correlations between MLH and body condition (EMMA1 $r = 0.032$, WMMA1 $r = -0.106$, WMMA3 $r = -0.033$, YBMA1 $r = -0.215$) (Table 3.4). EMMA1 was the only plot to demonstrate a positive correlation between body condition and heterozygosity, but this correlation was not statistically significant ($p = 0.858$). Linear modeling of SMI revealed no statistically significant associations with any of our dependent variables in the global model ($SMI \sim MLH + Plot + Sex + Year$, $AIC = 688.4$). The simplest model including only MLH was selected as the most informative ($SMI \sim MLH$, $AIC = 679.5$).

Plot	Mean MLH	HFC Effect Size (<i>r</i>)	P-value
EMMA1	0.167	0.0319	0.858
WMMA1	0.170	-0.106	0.757
WMMA3	0.170	-0.0325	0.782
YBMA	0.168	-0.215	0.102

Table 3.4- Heterozygosity-fitness correlations by demographic monitoring plot using Pearson product moment correlation. Mean multi-locus heterozygosity (MLH) was calculated as the proportion of heterozygous loci per individual, averaged by plot. HFC effect size is the Pearson correlation *r*-value between body condition (SMI) and MLH for each plot along with the correlation's *p*-value to assess significance. No demographic monitoring plot exhibits a significant correlation between body condition and heterozygosity.

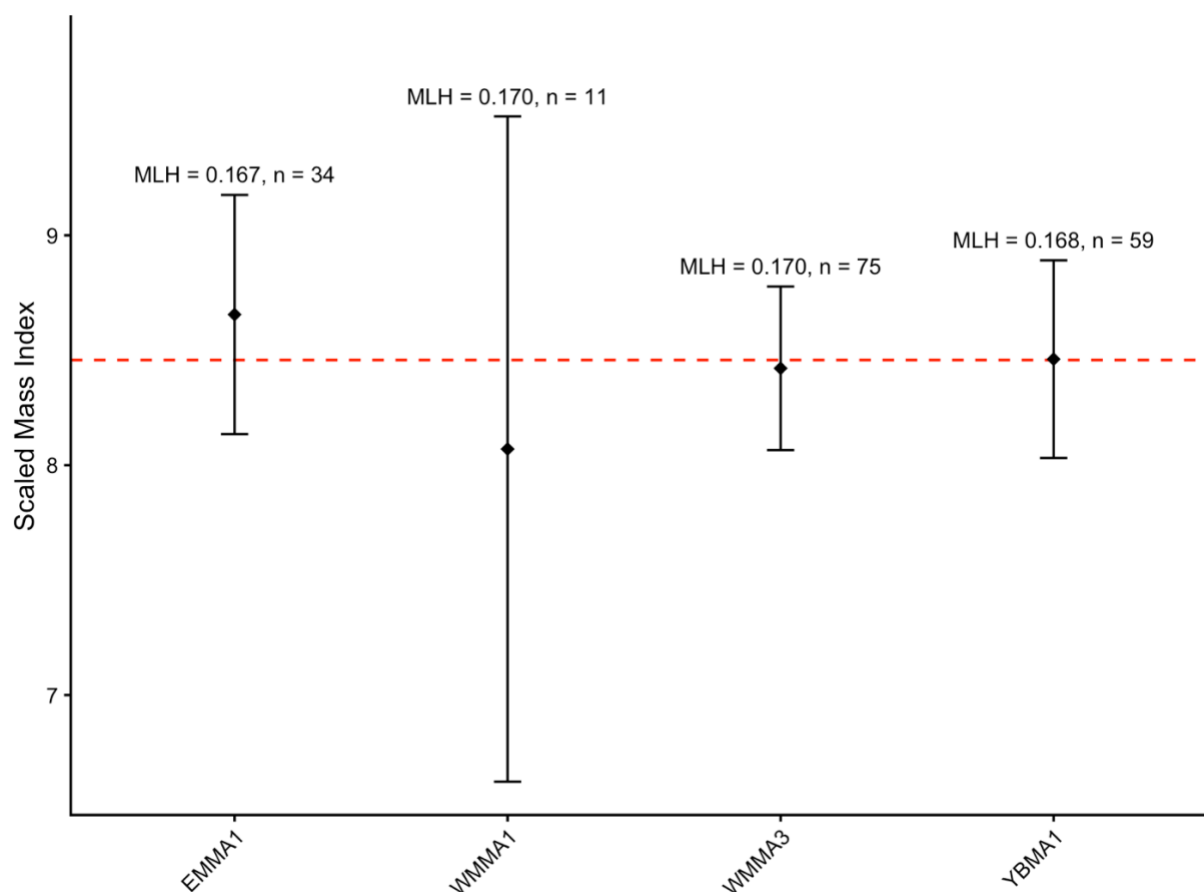


Figure 3.5- Mean body condition (filled diamond) with 95% confidence interval (black bars) by ICC demographic plot. Labels include average multi-locus heterozygosity and sample size. The dashed line indicates the mean scaled mass index (8.458 g). None of the sampling plots reveal a significant heterozygosity-fitness correlation or fitness effect by plot. The large confidence interval of WMMA1 is due to small sample size and a pair of large males (over 80 mm SVL) with relatively low mass (15.8, 17 g).

Discussion

Genetic Diversity

We measured genetic diversity across an array of sampling regimes and found generally high overall observed heterozygosity (range 0.166–0.173) and low inbreeding across all sampled areas. Higher observed heterozygosity (H_O) than expected (H_E) emerged in populations with few samples (< 15 individuals). Estimates of F_{IS} calculated using *hierfstat* do not reflect the expected heterozygote excess from these values because *hierfstat* calculates within-population gene diversity, H_S , using Nei's corrected estimator which corrects for small sample sizes. When estimated with 15 or more samples H_O was always smaller than H_E . Thus, future sampling efforts should aim to collect at least 20 samples for adequate estimation of genetic diversity. H_O values are similar, but higher than reported in Payne et al. (2025) (range 0.109–0.120), and higher than those reported in 2017 for a sympatric lizard in a more highly fragmented habitat, the Coachella Valley fringe-toed lizard (*Uma inornata*) (range 0.089–0.112) (Vandergast et al., 2019).

Nucleotide diversity only slightly varied by sampling area or regime (range 0.00053–0.00062). Nucleotide diversity in Yuma populations (range 0.00039–0.00045) was slightly lower (Payne et al., 2025). This is surprising given the preferred hypothesis that *P. mcallii* range expanded to the northwest (Gottscho et al., 2024; Mulcahy et al., 2006). Mulcahy et al. (2006) detected greater haplotype and nucleotide diversity in populations southeast of the Colorado River, which is often the case in ancestral populations. In Chapter 1 our few Yuma samples had much higher nucleotide diversity than California populations, so the difference between our estimates and Payne's is likely due to marker choice (i.e. restriction enzymes).

Inbreeding appears to be low on all demographic sampling plots (range 0.019–0.057), with higher values appearing on YBMA1 (2024, $F_{IS} = 0.057$) and EMMA1 (2024, $F_{IS} = 0.053$). Inbreeding measurements on demographic sampling plots in consecutive years generally increase through time; these differences may be due to sampling bias (sampling more siblings and family groups) or could be a legitimate signal of decreased individual level heterozygosity that may emerge more clearly in future genetic sampling. Given short but overlapping generations, this is worth further investigation.

The data presented here and by Payne et al. (2025) are encouraging measures of genetic diversity, although these measurements come from core sections of their range and cannot be assumed to represent rangewide population dynamics. Range edge population dynamics may differ from these core sections. Changes to genetic diversity and effective population size will take longer to emerge in populations than declines in abundance measured via demographic and occupancy surveys, but genetic diversity metrics can be informative of processes that population census data are not sensitive to, like selection, inbreeding, or introgression.

Effective Population Size

Effective population size estimates for each plot reveal generally large effective populations around each demographic sampling plot, apart from a small population for WMMA1. The unbounded upper confidence intervals for WMMA1 and EMMA1 can likely be attributed to limited samples (WMMA1 $n = 13$, EMMA1 $n = 39$) and low levels of linkage disequilibrium, which we see across all populations. Large effective population size surrounding the sampled individuals may also contribute to the UCI for EMMA1. Improved N_e estimation

within EMMA could be accomplished with broader sampling across EMMA by including lizards captured during occupancy surveys.

Current recommendations for populations to maintain evolutionary potential are for N_e to exceed 1,000 (Frankham et al., 2014). Although all sampling plots except EMMA1 estimate $N_e < 100$, although these sampling plots do not represent discrete, isolated populations. N_e estimation across the western Imperial Valley better represents processes at the metapopulation scale relevant to MA-focused conservation action. Western Imperial Valley N_e estimates ($N_e = 1,048$ (LCI 827, UCI 1,404)) include West Mesa and Yuha Basin management areas. These estimates fall within the northwest population post-bottleneck range of 391-5885 individuals described in Gottscho et al. (2024). At this scale (~50 km), 1,048 individuals is relatively small compared to the scale of sampling over which Payne et al. (2025) estimated roughly 800 individuals (~20 km). Populations across this sampling area, however, are not homogeneous and broader sampling that includes gaps in our sampling areas may yield more accurate estimates of effective population size. Since this estimate includes YBMA and WMMA samples, both MAs may be below the recommended 1,000 individuals, but further sampling would be necessary to make firmer conclusions here given the shallow population structure across this area (see Chapter 1).

Heterozygosity-fitness correlations

Our results do not show any significant correlation between scaled body mass index and heterozygosity. Our data show little contemporary signal of inbreeding, so it follows that we do not observe fitness effects caused by small population size. Individual body condition does not

differ by plot. Lizards on WMMA1 show greater variance in body condition, but this is due to two males with large SVLs (80+ mm) and relatively small mass (15.8 g, 17 g) in addition to small sample size relative to the other plots ($n = 11$). In an endangered rattlesnake (*Sistrurus catenatus*) with small contemporary population sizes, HFCs were detected only in a single population ($N_e = 180$), indicating that differences in fitness were better explained by ecological factors than genetic ones (Gibbs & Chiucchi, 2012). Since hundreds (or thousands) of generations have passed since the estimated range-wide *P. mcallii* bottleneck (Gottscho et al., 2024), any potential effects of inbreeding would have been resolved in the recovered populations. While differences in site-level fitness for *P. mcallii* in the Imperial Valley were not observed, populations in the Coachella Valley, which have experienced more recent significant reductions in population size, may exhibit signals of inbreeding and resultant HFCs.

Overall Synthesis

Our goal in this chapter was to use data collected on long-term FTHL ICC monitoring plots to estimate population genetic baselines for metrics critical to the long-term conservation of species. Using the data presented here and in Chapters 1 and 2, we outline practical steps forward for implementation of genetic monitoring into ongoing management of the *P. mcallii*, which may serve as a model by which other monitoring programs focused on terrestrial reptiles could integrate temporal genetic monitoring for conservation and management.

Genetic Monitoring

Based on the work detailed in this and previous chapters we echo the recommendations of Payne et al. (2025) and Gottscho et al. (2024) for incorporation of genetic monitoring into the existing monitoring framework. Temporal genetic monitoring has proven valuable in detecting management-relevant change to natural populations in a number of other systems (Gajdárová et al., 2023; Stark et al., 2025). Changes in genetic differentiation between subpopulations of Florida scrub lizards (*Sceloporus woodi*) were detected in six generations using microsatellite markers, providing managers with valuable insight into the impacts of prescribed burns and anthropogenic-maintained corridors (Orton et al., 2020). Conservation planners have incorporated these methods into actionable frameworks. Zimmerman et al. (2023) created a genetic warning system for greater sage-grouse (*Centrocercus urophasianus*) to identify management units with problematic genetic diversity and detail a roadmap for management and conservation actions in future assessments. *P. mcallii* population monitoring is ongoing and observed population genetic clusters align closely with existing management areas, so genetic sampling can be simply and effectively integrated into ongoing efforts. Our highest recommendation would be to sample tissues comprehensively each year to maximize the value of periodic genomic assessment. The geographic coverage of occupancy monitoring and the detailed demographic data from demography monitoring provide two unique aspects for temporal genetic monitoring.

Metrics of neutral genetic diversity (e.g. π , H_O , $\Theta_{\text{Watterson}}$, A_R) measured through time can inform the ICC of changes to within-population diversity that may indicate MA-specific patterns of decline or improvement. The power of genetic monitoring to detect demographic decline is

dependent on the severity and type of decline (Hoban et al., 2014). Instant decline can be reliably detected within a generation or two, but exponential decline (expected due to fragmentation, climate change) can take up to 10 generations to detect, so these tools are limited in their ability to serve as early warning signals of more gradual decline (Hoban et al., 2014). Additionally, connectivity and population structure or differentiation metrics (F_{ST} , PCA) can elucidate between-population patterns of change through time. To monitor adaptive potential partial redundancy analysis (pRDA) could be used to estimate the proportion of genetic diversity explained by environmental factors (Capblancq & Forester, 2021; Fischer et al., 2026).

Effective population size monitoring is another critical component of temporal genetic monitoring (Fischer et al., 2026; Hoban et al., 2021). To maintain adaptive potential, each MA should seek to maintain an effective population size over 1,000 individuals (Frankham et al., 2014). MA declines of N_e below 500 individuals should trigger appropriate investigative research and management action. Declines in N_e on demographic monitoring plots may also contribute to this trigger, at roughly the same 30% trigger point as the current monitoring program (*Flat-Tailed Horned Lizard Rangewide Management Strategy, 2003 Revision, 2003*).

We recommend re-assessment of genetic diversity and effective population size at least every ten years at the MA/RA scale using samples collected from all monitoring plots. If genetic data is collected annually (once/generation) then this decadal reassessment will have strong power to detect change during initial loss of diversity before genetic erosion occurs, and management action may be taken to curb continuing loss of diversity. If instant declines are

detected using existing demographic modelling, then this should spur an immediate assessment of available genetic data.

In the event of decline, targeted studies to elucidate the causes should be undertaken. Demographic survey data is a natural complement to this process and provides a substantial starting point for such an evaluation (Wood et al., 2020). Additionally, genetic data from occupancy monitoring plots have the power to detect changes in genetic diversity closer to range edge and habitat boundaries that demographic sampling may not capture. Including genetic trigger points would provide the ICC with additional information by which to take conservation action. For example, F_{ST} between YBMA and WMMA (WMS) ($F_{ST} = 0.008$) is very low, indicating historic and contemporary connectivity. EMMA and YBMA ($F_{ST} = 0.023$) similarly show recent connectivity, but there is a marked difference in differentiation compared to YBMA and WMMA given roughly 100 years of anthropogenic separation across the Imperial Valley. If YBMA and WMMA or WMMA and OWRA were to approach F_{ST} values between 0.015-0.02 this may indicate critical loss of gene flow between management units and be cause for management intervention. Management actions may include targeted research to determine the cause of a local population decline, differential land management strategies to mitigate observed impacts, or implementation of captive breeding or conservation translocations where appropriate.

Other Genomic Considerations

1) Assembly of a high-quality *P. mcallii* reference genome

One of the biggest challenges in temporal genetic monitoring is the variety of markers and sequencing types that limit comparability of data over time (Bertola et al., 2024). High-quality reference genome assembly can resolve many of these issues, and open the door to powerful whole-genome sequencing (WGS) tools (Brandies et al., 2019). WGS generates comprehensive genome coverage, can be used to identify genes under selection, allows for future reanalysis of data, and can be a resource for researchers interested in a diverse array of ecological and evolutionary questions (Bourgeois & Warren, 2021; Lu et al., 2025). Ekblom et al. (2018) assembled the wolverine (*Gulo gulo*) genome and used this data to generate genome-wide diversity, identify population differentiation, and create a reduced SNP panel to make use of low-quality DNA collected from scat. Genetic monitoring using low-quality DNA samples from scat was also made possible through microsatellite markers identified in a high-quality assembly of the Tasmanian devil (*Sarcophilus harrisii*) genome (Grueber et al., 2020).

2) Long-term storage of DNA samples

Because WGS sequencing and analysis is becoming cheaper and easier, genomic monitoring should also prioritize long-term tissue and DNA storage (Chen & Mastromonaco, 2025; Leon-Quinto et al., 2009; Magurran et al., 2010). Long-term storage will preserve specimens for periodic reassessments of *P. mcallii* genetic diversity. In the future, individual and population analysis could make use of entire genomes; thus, having a decades-long repository of tissue samples would be an invaluable resource for tracking evolution and changes to genetic

diversity during rapid environmental change (Strand et al., 2020). This could be accomplished through continued partnership with academic institutions, or regional governmental departments which possess the requisite expertise and tissue storage capacity (e.g. San Diego State University, University of Arizona, College of the Desert, USGS San Diego Field Station). Remaining samples from this dissertation will be housed at San Diego State University Biodiversity Collection for further use by the FTHL ICC or graduate students in the SDSU Ecology or Evolutionary Biology program.

Academic partnership could also include training for FTHL ICC field crews in sample collection and refinement. At the present, blood samples produce the highest quality DNA for genomic library construction and sequencing. As various molecular technologies improve in their ability to make use of smaller amounts of DNA, the relatively invasive tail-nicking procedure may be transitioned to less invasive methods such as buccal or cloacal swabs. In addition to these contributions in genomic monitoring efforts, academic partners can acquire external grants to cover the costs of sample processing, sequencing, and analysis, or make use of ICC funds earmarked for research that forwards goals set forth by the RMS.

3) Complement current research goals

The commitment of the MAs and RA to annual monitoring has generated nearly two decades of data, which is critical to modeling long-term population persistence through population viability analysis (PVA). Sensitivity analysis within the range-wide PVA revealed that slight variations in input parameters (e.g. number of broods per year, age of first offspring, mean clutch size) drastically alters the rate of change in populations, often leading to modeled

declines (Grimsley-Padron et al., 2019). Realistic estimates of these inputs can be incredibly difficult to ascertain, but genomic tools may improve some of these estimates.

Genomic data can be incorporated into PVAs with input parameters like population-level allele frequencies, kinship coefficients, and effective population sizes (Lacy, 1993). For European tree frog (*Hyla arborea*) PVA, Auffarth et al. (2017) used microsatellite allele frequencies and lethal equivalent values to estimate inbreeding effects, and found inbreeding to affect population survival through a reduction in first-year survival. Genetics-informed PVAs have also been used to validate management units (Olsen et al., 2014), and predict the efficacy of genetic rescue efforts in lowland Leadbeater's possums (*Gymnobelideus leadbeateri*) (Zilko et al., 2021).

In addition to the above demonstrations of genetic PVA inputs, our baseline HFC analysis and pedigree construction could be expanded to potentially improve PVA input parameters for each MA. For example, if a small survey team were to revisit demographic sampling plots in October to locate and collect genetic samples from late-season neonates and adult females, confirmed instances of double clutching from females would improve proportional estimates of double clutching events for PVA input, and may link body condition to fecundity, allowing for improved estimation of this parameter by year and by MA.

4) Address climate change impacts

Temporal monitoring of genetic erosion, spatiotemporal population structure, adaptation, and hybridization is critical to conservation under climate change (Jensen & Leigh, 2022). Effects of climate change on *P. mcallii* are predicted to be acute with ecophysiological modelling projecting major changes in habitat suitability across its range (Lara-Reséndiz et al.,

2026). *P. mcallii* habitat specialization is more likely to cause local extirpation than range expansion near range edges. Range contractions and increase in the number of hours of thermal restriction will put additional stress on populations, even in core sections (Lara-Reséndiz et al., 2026). As climate change alters the thermal landscape, genes associated with physiological tolerance to drought and temperature extremes may change population-level physiology or morphology, as observed in other desert lizards like *Liolaemus fuscus* (Araya-Donoso et al., 2022). Assuming a reference genome is constructed (see point 1 above), future studies should focus on adaptive potential in genes and complexes strongly correlated with important environmental variables.

5) Research beyond core range

Ongoing monitoring within MAs primarily samples core range segments. Given the future potential for range contraction, genetic monitoring of range edges, often outside MA boundaries, will increase early detection of range contractions so that management action can be taken to preserve the genetic diversity of edge populations. Populations in the Coachella Valley where severe fragmentation and recent population declines have shaped unique, decline-and-recovery driven population genetic dynamics are likely to be important in understanding how core segments may change under climate change scenarios and potential stochastic population declines.

6) Hybrid zone dynamics

Where *P. mcallii* habitat edges meet the mountain ranges that bound its distribution, population genetic dynamics are also shaped by hybridization with sympatric species like *P. platyrhinos* and *P. goodei* (Gottscho et al., 2024; Mulcahy et al., 2006). Hybridization with these species complicates conservation for several reasons. Changes in fitness due to hybrid vigor or outbreeding depression can yield different outcomes for hybrid and parental populations, including increase in adaptive potential or reduction in fitness (Allendorf et al., 2022; Rieseberg et al., 2007). Additionally, behavioral syndromes that differ between parental lineages can influence fitness in hybrids and affect hybrid zone dynamics (Johnson et al., 2015; Maag et al., 2025). The genomics underpinning the natural hybrids zones of *P. mcallii* x *P. platyrhinos* and *P. mcallii* x *P. goodei* may reveal information relevant to *P. mcallii* conservation, as demonstrated in lizards and turtles (Paris et al., 2024; Scott et al., 2019).

Conclusion

The FTHL ICC operates an unparalleled reptile monitoring program which is well situated to adopt a temporal genetic monitoring plan. The most challenging financial and logistical component of long-term monitoring is the boots-on-the-ground effort required to capture animals. Once in-hand, the collection of genetic samples requires little additional time and expense. Long-term sampling and storage of genetic tissue builds additional adaptive capacity and could highlight *P. mcallii* as an influential model for other existing wildlife monitoring programs.

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