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Landscape Genomics of the Flat-tailed Horned Lizard

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Landscape Genomics of the Flat-tailed Horned Lizard (*Phrynosoma mcallii*) on DoD Lands in California and Arizona



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Introduction

- Flat-tailed horned lizard (*Phrynosoma mcallii*)
- Diet specialized on ants
- Cryptic – difficult to detect
- Considered an at-risk species (Leavitt et al. 2015)
- Currently listed as a Species of Special Concern in California



Introduction



- Smallest range of any horned lizard (*Phrynosoma*) in the United States
- Occupies sandy or hardpan low desert habitats in the Colorado Desert
 - California, Arizona, northwest Mexico including DoD lands:
 - NAF El Centro
 - MCAS Yuma (BMGR)

Introduction



Introduction



Introduction

- From 1993 to 2011, *P. mcallii* was scrutinized for protection under the federal Endangered Species Act, but in a series of court cases, the U.S. Fish & Wildlife Service (USFWS) withdrew the proposed 1993 rule **four times**.
- In 1997, multiple state and federal agencies with populations of *P. mcallii* on their lands signed a voluntary **Interagency Conservation Agreement (ICA)** to implement the **Flat-tailed Horned Lizard Rangelwide Management Strategy (FTHL RMS)**
- One of the major goals of the ICA is to “encourage and support research to promote conservation of [*P. mcallii*] and desert ecosystems” (FTHL RMS 8), specifically to “***determine genetic variation among populations and effects of barriers***” (FTHL RMS 8.6)

Introduction

- In 2011, the USFWS decided against listing *P. mcallii* because *“the effects to the species associated with the implied meaning of fragmentation... are not likely to constitute a substantial threat to the species now or within the foreseeable future”* ...
- ... and because ***“the conservation efforts implemented by signatories of the [ICA] and associated [FTHL RMS] reduce the impact of existing threats in the United States and promote actions that benefit the Flat-tailed Horned Lizard throughout its range, including Mexico”*** (76 FR 14210; 14252-53)

Introduction

- In June 2014 the Center for Biological Diversity submitted a petition to list *P. mcallii* as endangered throughout their range in California under the California Endangered Species Act
- In October 2014, the California Department of Fish & Wildlife decided that the petition had merit and in February of 2015 proceeded with the mandatory one-year scientific review
- **In March 2017, the Department decided not to list this species, but will re-examine its status again in five years**
- **This study was undertaken in an effort to better understand the genetic diversity found in the FTHL, and also to use that information to support land use and management decisions**

Research Questions

- Which populations of *P. mcallii* have the highest genetic diversity?
 - How do these levels compare to other benchmark species?
- How many populations are there?
 - How are they related to each other?
- What are the natural barriers to migration (gene flow)?
- What are the management implications?

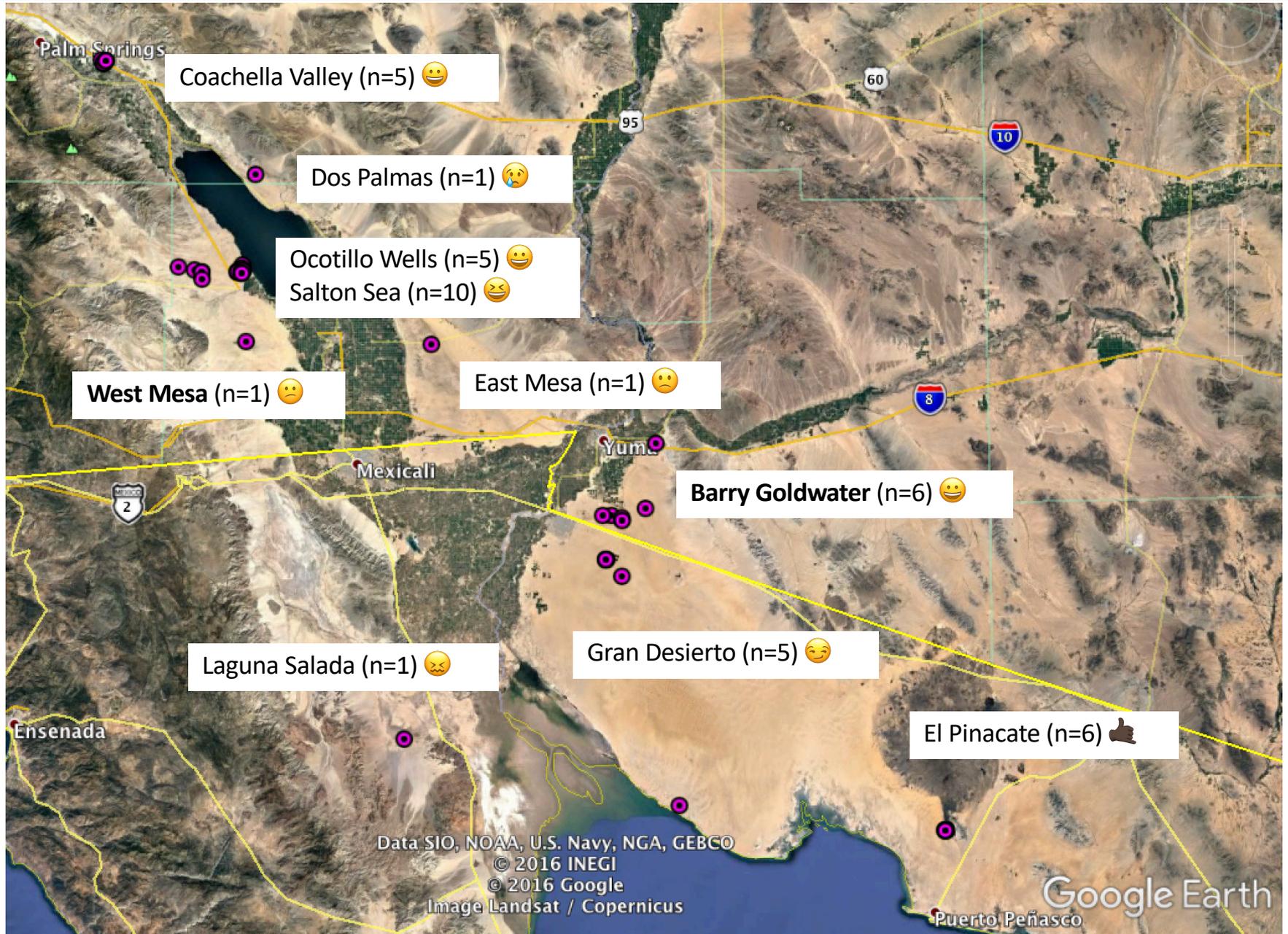


Methods: NGS Data Collection



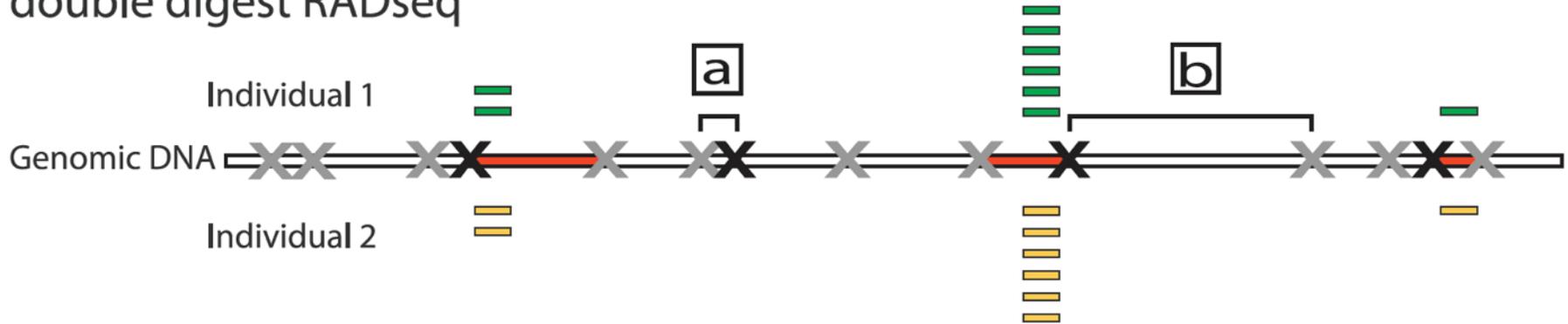
- Extracted gDNA from >100 tissue samples of *P. mcallii*
 - Including those used in Mulcahy et al. (2006) and DoD installation sites
- Final data set: 41 individuals with high molecular weight DNA

Methods: Sampling Localities



Methods: Genomic Data Collection

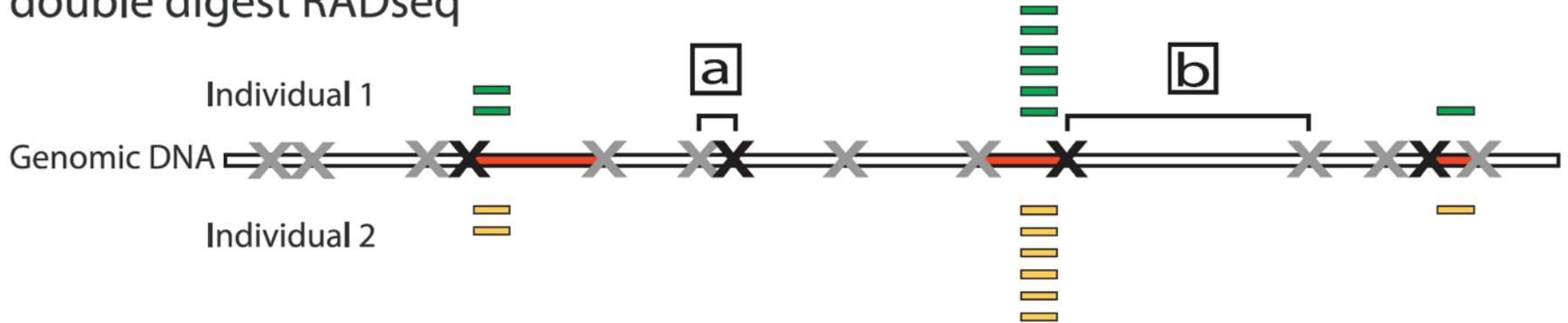
double digest RADseq



- Previous genetic research on FTHL used mtDNA
 - Mulcahy et al. 2006
 - Maternally inherited, single marker
- Double digest Restriction Associated DNA sequencing (Peterson et al. 2012)
- Can efficiently assay hundreds to thousands of genetic markers *across the genome*

Methods: Genomic Data Collection

double digest RADseq



- The final libraries were sequenced (150 bp single-end reads) on a NextSeq (Illumina) at the Institute of Integrative Genome Biology (UC Riverside).

Methods: Bioinformatics

- pyRAD v3.0.6 (Eaton, 2014) was used for raw data processing.
- Percent heterozygosity to measure genetic diversity
 - and compare to other co-distributed lizard species



Methods: Bioinformatics



- Admixture v1.23 (Alexander et al. 2009) and Discriminant Analysis of Principal Components (DAPC) (Jombart et al. 2010)
 - Used to estimate the number of populations, assign individuals to those populations, and calculate the fixation index (F_{ST}), which ranges from 0 to 1.
 - The cross-validation error (CVE) and Bayesian Information Criterion (BIC) was used to select the optimal number of populations (K), testing values ranging from 2 to 10.

Methods: Bioinformatics

- A phylogeny (evolutionary tree) was estimated with RAxML v8.2.4 (Stamatakis 2014)
 - To determine the evolutionary relationships among individuals
 - Maximum likelihood model



Methods: Bioinformatics

- EEMS (Estimated Effective Migration Surfaces; Petkova et al. 2016)
 - To gain insight into migration routes and barriers
 - The genetic variance is decomposed into **within-population** effective diversity and **among-population** effective migration

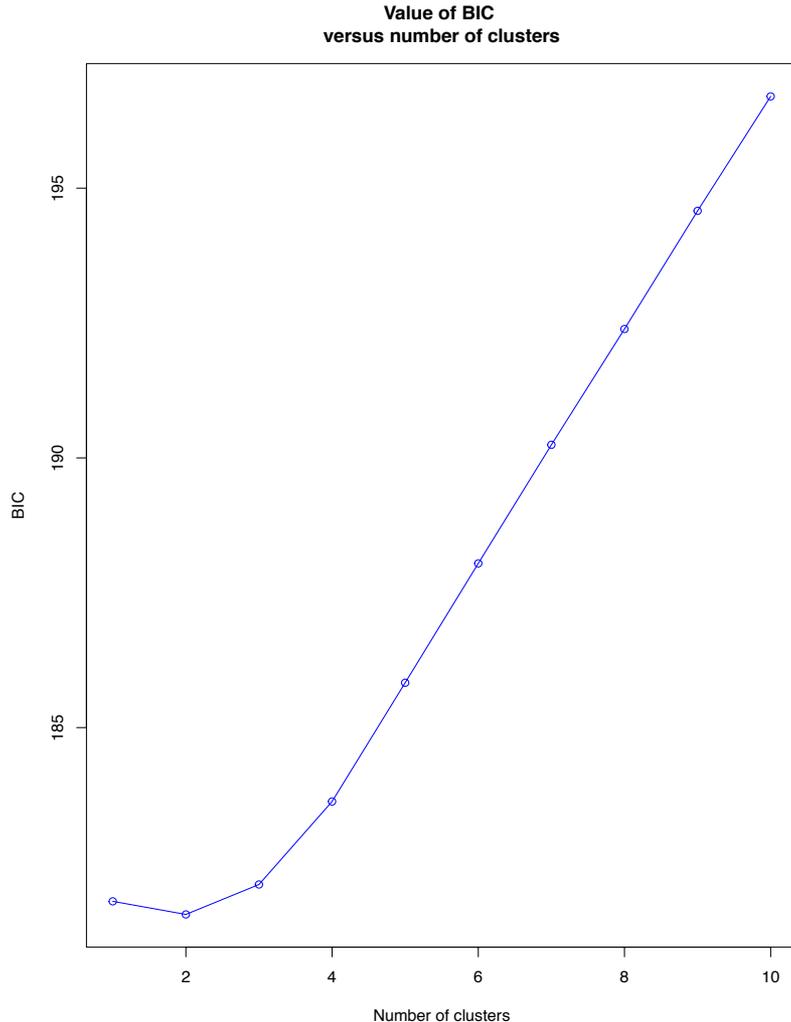


Results: Summary

- We obtained a total of 76.03 million reads (mean of 1.81 million per lizard)
 - 75% (57.14 million) passed pyRAD's initial quality filter.
- These were assembled into an average of 111,642 loci per individual.
- On average, we examined 627,732 nucleotide sites per lizard, of which 2,602 were polymorphic on average.



Results: How many populations?



K	CVE	BIC
1	0.438	181.78
2	0.446	181.54
3	0.450	182.09
4	0.484	183.63
5	0.556	185.83
6	0.601	188.06
7	0.636	190.26
8	0.712	192.53
9	0.772	194.64
10	0.793	196.74

42 individuals
851 unlinked SNPS present
in 40+ individuals

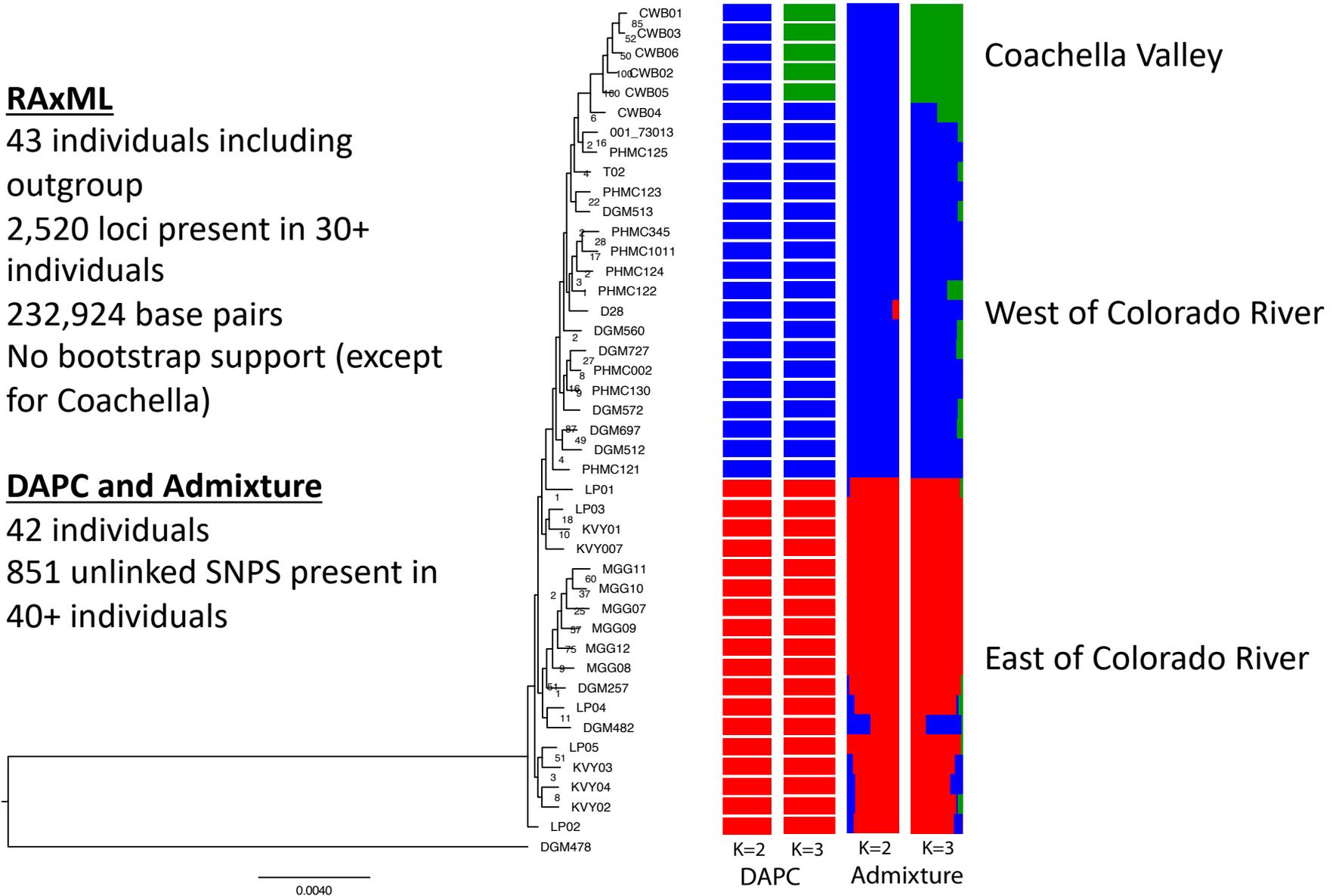
Results: Phylogeny and Population Structure

RAXML

43 individuals including
outgroup
2,520 loci present in 30+
individuals
232,924 base pairs
No bootstrap support (except
for Coachella)

DAPC and Admixture

42 individuals
851 unlinked SNPS present in
40+ individuals



Results: F_{ST}

FTHL (K=2)

	Western
Eastern	0.115

FTHL (K=3)

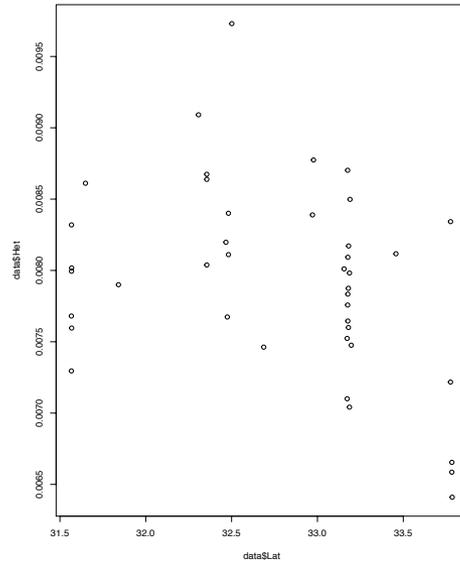
	Eastern	Western
Western	0.11	
Coachella	0.259	0.221

Uma

	inornata	notata
notata	0.297	
cowlesi	0.328	0.222

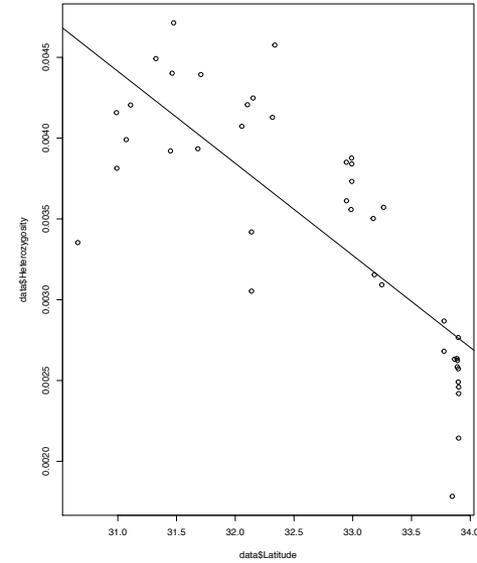
Results: Heterozygosity

FTHL



Adjusted R-squared: 0.095
F-statistic: 5.209 on 1 and 39 DF
p-value: 0.02801

Uma

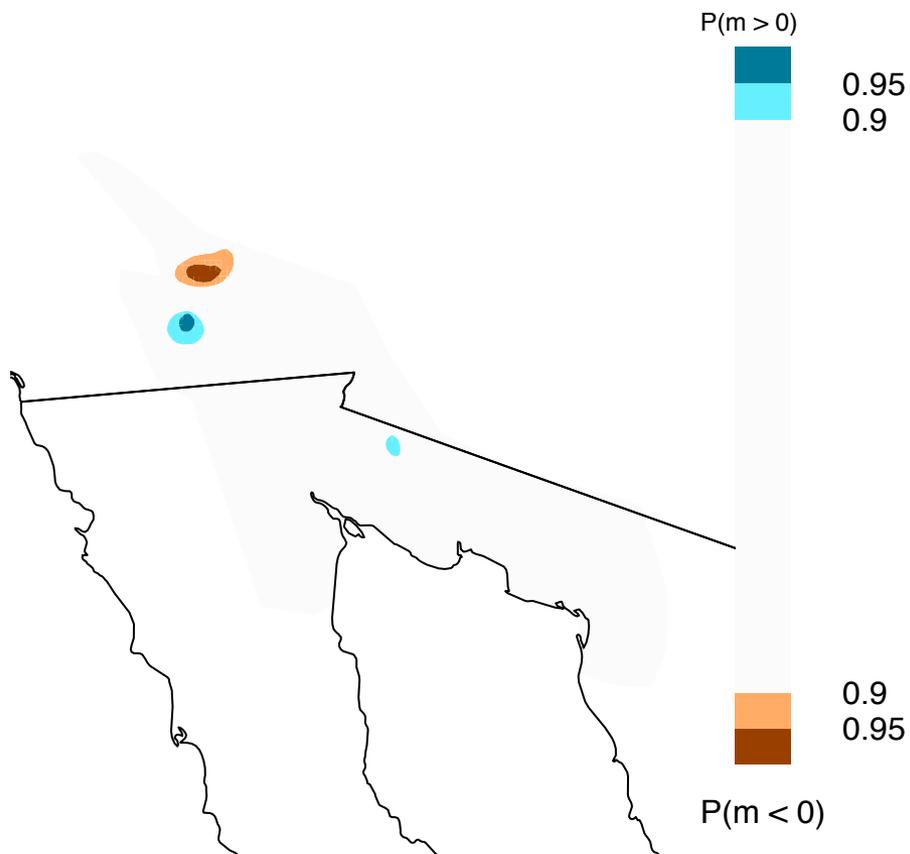


Adjusted R-squared: 0.623
F-statistic: 67.03 on 1 and 39 DF
p-value: 5.34e-10

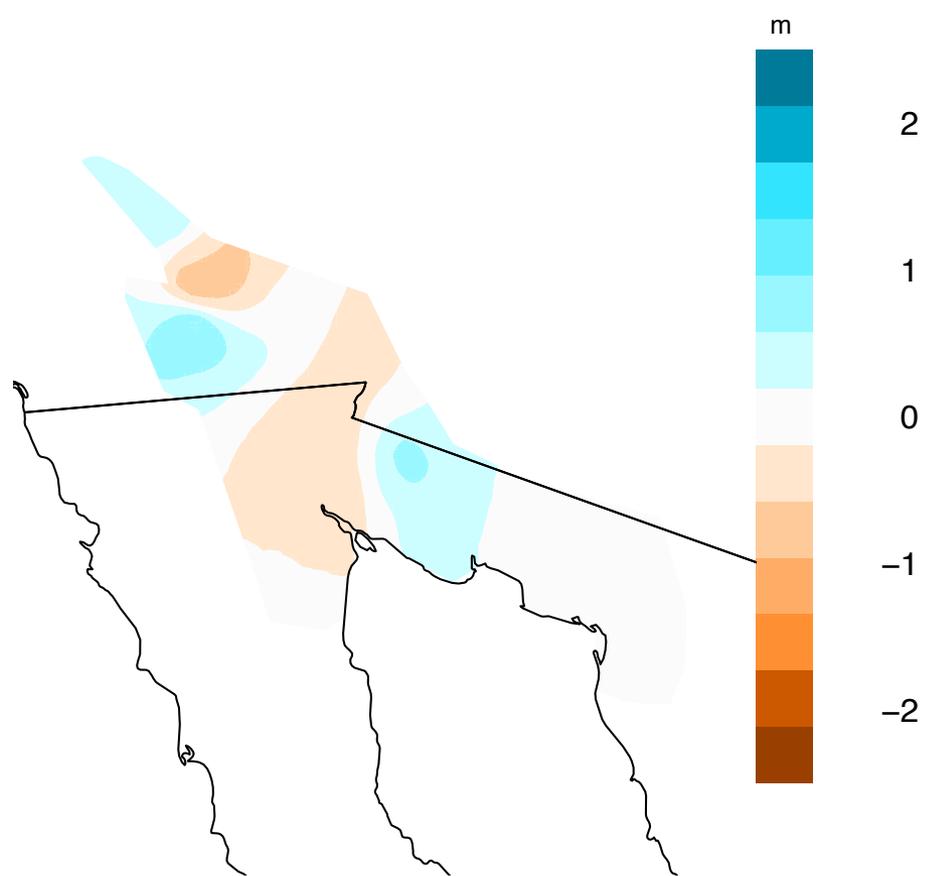
	Min	Max
<i>P. mcallii</i>	0.0064	0.0097
<i>Uma</i>	0.0010	0.0047
<i>Callisaurus</i>	0.0013	0.0051
<i>Petrosaurus</i>	0.0005	0.0034
<i>Urosaurus</i>	0.0009	0.0062
<i>Sceloporus</i>	0.0009	0.0071

Results: Estimated Effective Migration Surfaces

Posterior probabilities $P(m > 0 | \text{diffs})$ and $P(m < 0 | \text{diffs})$

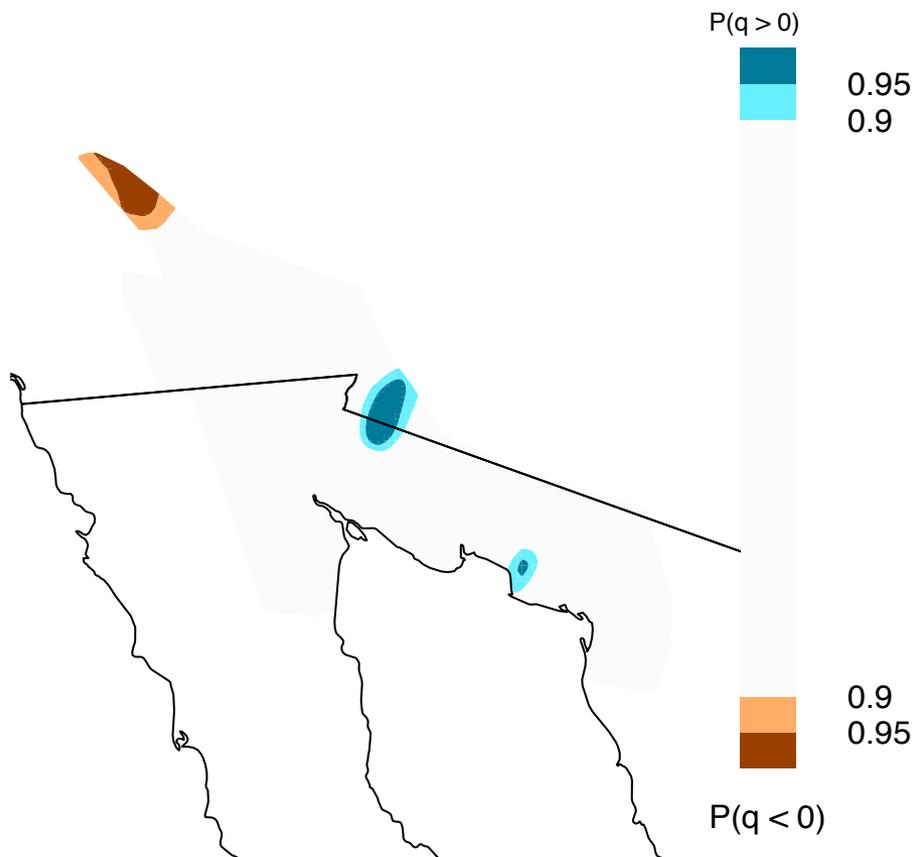


Posterior mean migration rates m (on the log10 scale)

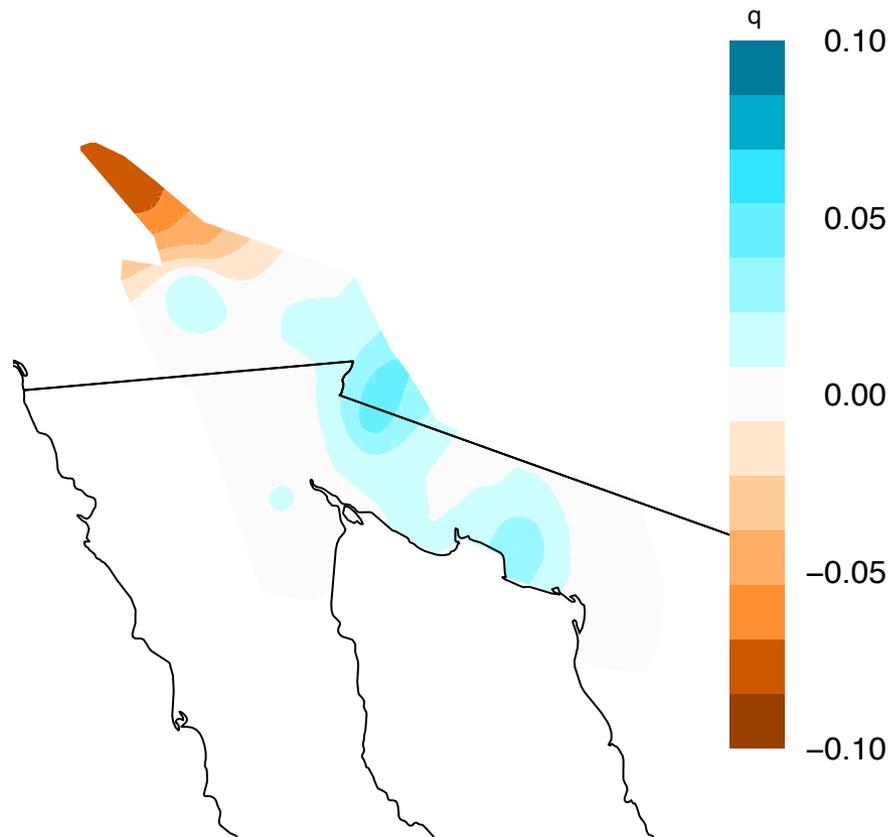


Results: Estimated Effective Migration Surfaces

Posterior probabilities $P(q > 0 | \text{diffs})$ and $P(q < 0 | \text{diffs})$



Posterior mean diversity rates q (on the log10 scale)



Conclusions

- Which populations of *P. mcallii* have the highest genetic diversity?
 - **EEMS results showed decreased local effective diversity in the Coachella Valley and increased diversity in Barry M. Goldwater Marine Corps Air Station**
- How do these levels compare to other benchmark species?
 - **Surprisingly, heterozygosity levels were higher than many common desert lizards with much larger ranges.**



Conclusions

- How many populations are there?
 - **One or two populations are best supported by our models.**
- How are they related to each other?
 - **Northwestern populations are descended from southeastern ones.**
 - **Consistent with the estimated effective diversity being highest in the Barry M. Goldwater Marine Corps Air Station in southwestern Arizona and lowest in the Coachella Valley of southern California**

Conclusions

- What are the natural barriers to migration (gene flow)?
 - **Colorado River, Salton Sea**
 - **The U.S. Mexico border is not a natural barrier to gene flow**
 - **Monitoring of populations on either side especially Barry M. Goldwater Marine Corps Air Station is recommended**



Conclusions

- What are the management implications?
 - **Still an open ended question – more data analyses in progress**
 - **Estimates of genetic diversity, heterozygosity provide a benchmark for future studies to compare to**
 - **Lab protocols provide resource for more detailed studies being conducted by other researchers**
 - **Unexpectedly high heterozygosity needs to be examined further**
 - **Confirmed importance of Colorado River as a natural barrier**
 - **Different management units on either side**
 - **The FTHL ICC continues monitoring and research on this species in concert with DoD Legacy goals**

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