

The Implications of Isolation:

A Next-Generation Sonoran Desert Researchers 6&6 Artist/Scientist Collaboration

Study ORPI-00151, Permit ORPI-2018-SCI-0010

Final Project Report: 21 March 2018

Prepared by Taylor Edwards, University of Arizona Genetics Core

Abstract

The Sonoran Desert's heterogeneous landscape is an excellent location to examine species with small, disjunct populations as well as the modern influence of increasing habitat fragmentation due to anthropogenic landscape change. Common chuckwalla, *Sauromalus ater* are saxicolous (rock dwelling) lizards that occur in disjunct, rocky environments throughout the Sonoran and Mojave deserts. Chuckwallas have limited mobility (they cannot travel as great of distances) and have short generation times, so genetic changes in a population may accumulate relatively quickly. Within the genus *Sauromalus* there are several different species, some even having evolved on islands in the Sea of Cortez and on the Baja peninsula as a result of isolating events over geologic time. We investigate the relationships among several, isolated populations of chuckwallas in northwestern Sonora, Mexico and southwestern Arizona, USA to better understand their relationship and connectivity in the surrounding landscape. We include previously published sequence data in our analysis as well as DNA samples obtained from scat. These data were used to inform a collaborative project as part of the Next Generation Sonoran Desert Researchers (N-Gen) 6&6 Arts and Science Initiative.

Introduction

Assessing the impact of habitat fragmentation is a central theme in conservation biology. Anthropogenic landscape change affects habitat connectivity and species' ability to maintain natural patterns of gene flow. As habitat fragments become smaller and smaller so to do species populations. General principles of population genetics dictate that small populations are affected by increased genetic drift and inbreeding, which decrease genetic variation and heterozygosity and make the population more vulnerable to extinction; called an extinction vortex.

Many species have populations that are naturally disjunct and these same principles apply, although the time frame of geologic or environmental change may be dramatically different than when humans manipulate the landscape. Consider, however, a species that arrives on an island; here, the few individuals that are now isolated from their mainland population face many challenges to exist. While the vast majority of these events likely lead to death, over evolutionary time some individuals do survive and establish new populations despite the detriments of inbreeding and drift. The Galapagos Islands are the classic example of small population persistence.

The Sonoran Desert's heterogeneous landscape is an excellent location to examine species with small, disjunct populations as well as the modern influence of increasing habitat fragmentation due to anthropogenic landscape change. An example of this can be observed in Desert tortoise (*Gopherus morafkai*). Tortoises in the Sonoran Desert generally inhabit rocky foothills characteristic of Arizona Upland Sonoran desertscrub. Although foothill populations appear to be isolated by low desert valleys, radiotelemetry and genetic data show that tortoises are capable of making long-distance movements between populations. The most likely scenario for the desert tortoise is that gene flow occurs not at a regular rate, but with varying frequencies over time related to environmental fluctuations (Morafka 1994). For example, there may be increased potential for successful emigration in years of high rainfall.

Another Sonoran Desert reptile that shares a similar distribution and potentially a similar evolutionary history with the desert tortoise is the common chuckwalla, *Sauromalus ater*. Chuckwallas are saxicolous (rock dwelling) lizards that occur in disjunct, rocky environments throughout the Sonoran and Mojave deserts. Chuckwallas are different, however, in that they are less mobile (they cannot travel as great of distances) and they have shorter generation times (so genetic changes in a population may accumulate more quickly). Within the genus *Sauromalus* there are several different species, some even having evolved on islands in the Sea of Cortez and on the Baja peninsula as a result of isolating events over geologic time. We investigate the relationships among several, isolated populations of chuckwallas in northwestern Sonora, Mexico and southwestern Arizona, USA to better understand their relationship and connectivity in the surrounding landscape.

Methods & Results

Sample collection

We performed visual encounter surveys for Chuckwallas (*Sauromalus ater*) at Punta La Cholla, Sonora Mexico on 11 and 12 March 2016 and at Organ Pipe National Monument (ORPI), Arizona, USA 11 May 2018. We observed live animals and sign (tracks in front of burrows and scat) at several different locations in Mexico but only observed scat at ORPI. Because of the difficulty in catching animals and the stress caused to the animal by handling and drawing blood, we collected scat samples for DNA analysis. We selected scat samples that were as fresh as possible to reduce the amount of degradation to the DNA caused by heat and UV light. We considered darker samples to be fresher because older scats tend to bleach in the sun.

DNA Isolation

To isolate Chuckwalla DNA from the scat, we targeted the epithelial cells on the outside of the scat sample which consists of cells that were shed from the intestinal lining of the animal. The scat itself is composed primarily of plant material (indigestible cellulose / fiber) and contains primarily DNA of the food items. We instead wanted to target the DNA of the study animal. We collected these cells by following the swabbing protocol of Rutledge et al. (2009). We gently

swabbed the surface of the scat with a buccal swab (Whatman OmniSwab, WB100035; Whatman Inc., USA) dampened with lysis buffer. We then stored the swab in ~650 µl of the same buffer and returned them to the University of Genetics Core for processing.

We isolated total DNA from 14 buccal swabs (collected from 10 separate scats at Punta La Cholla and 4 scats at ORPI). We performed overnight lysis with proteinase K at 55°C, followed by robotic extraction using a QIAGEN BioSprint 96 robotic magnetic-particle purification system (Qiagen, Valencia, California USA) and Aline Biosciences Buccal Swab gDNA Kit (Aline Biosciences, Woburn, Massachusetts USA). We quantified recovered DNA using a BioTEK Synergy HT (BioTEK, Vermont, USA). We successfully isolated DNA from all 14 swabs, yielding an average of 500 ng of DNA per sample (87.5–892.5), however, this is total DNA and therefore contains not only chuckwalla DNA, but also that from the plant food and bacteria.

DNA Amplification

We selected and designed primers that target just the Chuckwalla DNA in our samples and that could be used to generate DNA sequences for our analysis. Primers are used in Polymerase Chain Reaction (PCR) to target a specific locus (or location) of the DNA and make copies so that the specific DNA regions of interest can be differentiated from other DNA in the sample. Because genetic studies have previously been performed on Chuckwallas, information on DNA sequences and amplifying primers were available in the scientific literature so that we could reproduce the methods of these previous studies. We used the published primers pairs from two of these studies which amplify between 855 and 980 b.p. (basepairs; a measure of DNA sequence length) of DNA (Table 1; Kocher et al. 1989, Corl et al. 2010). Because we suspected that our DNA samples might be degraded from exposure to heat and light, resulting in only shorter fragments of DNA available for amplification, we also designed our own primer pairs from sequences available online in the GenBank database (Table 1: GenBank Accession #AF020234; Petren and Case 1997). All PCR amplification targeted the Cytochrome B (Cytb) region of the mitochondrial genome. Mitochondrial DNA (mtDNA) is widely used in phylogenetic studies and Cytb is a standard barcoding locus. MtDNA is maternally inherited and males do not pass on their mtDNA to offspring, so this marker only details female genealogies. Thus mtDNA data only address the genealogy of females.

Polymerase Chain Reaction is a process of precisely heating and cooling a chemical reaction and it is necessary to optimize each reaction for the specific combination of reagents and temperatures to ensure that the primers are targeting only the region of interest and are not producing additional sequences that may interfere with our ability to ‘read’ the sequence of interest. We optimized PCR for each primer pair using all 10 Mexican DNA samples by performing a touchdown PCR from 62-52 °C, stepping down 0.5 °C per cycle, and assessing 8 different concentrations of MgCl₂ ranging from 1.0-4.5 mM. We then ran an agarose gel on the PCR product to assess if the target amplicon was recovered during the reaction. We were able to

identify which reactions were successful by which conditions produced a visible band on the gel (PCR product).

We identified PCR conditions that worked successfully for all primer pairs; including the ones that generate longer DNA sequences. We also noted that not all of our DNA samples had viable DNA and thus failed under all conditions (Table 2). We next selected the 5 samples that were viable and ran the optimized PCR for the 2 longer sequences (855 and 980 b.p.). We next purified these PCR products for sequencing using a Rapid PCR Purification System Kit (Marligen Bioscience, Inc.). Before submitting samples for Sanger sequencing, we assessed their quantity and quality using a NanoDrop1000 spectrophotometer (ThermoFisher Scientific, Inc.). We selected 2 of these purified samples for the cytb 855 b.p. locus for sequencing.

For the ORPI samples that were obtained later, we ran the same working PCR conditions for the cytb 855 b.p. locus but did not generate any viable product. We then reran the assays for the shorter, Cytb_338 b.p. locus and were successful obtaining the targeted amplicon. The ORPI scat samples were much older by appearance (signs of bleaching from UV exposure) and were potentially from the previous season. We suspect that degradation occurred with these samples.

We submitted PCR products to the University of Arizona Genetics Core for DNA sequencing on a 3730XL DNA Analyzer (Applied Biosystems, Carlsbad, CA). We aligned sequences to a reference sequence using CLC DNA Workbench ver. 5.7.1 (CLC Bio, Aarhus, Denmark). The reference sequence (GenBank accession no. AF020233) was published by Petren and Case in 1997 for study titled *A Phylogenetic analysis of body size evolution and biogeography in chuckwalla (Sauromalus) and other iguanines*. We choose this study and sequence because it had the most available data for comparison to our sample. For each of our samples, we sequenced both strands of the amplified DNA (forward and reverse) and using the CLC software, we were able to match the Mexican sequences to the entire 855 b.p. length of the reference sequence and identify positions in the DNA that were similar and different. The 2 samples we sequenced were not different from each other. Two of the 4 ORPI samples generated viable sequence and aligned to the shorter target region.

In addition to the sequences we obtained, we used sequence data available on GenBank generated in Petren and Case (1997) for populations sampled near Caborca and Sonoyta Mexico, as well as sequences from Arizona and California. We used DnaSP (v.5.10.01; Librado & Rozas, 2009) to estimate nucleotide diversity, polymorphism and other descriptive statistics among maternal lineages. Comparing just the 855 b.p. sequences, nucleotide divergence (Jukes Cantor) between N. Mexico and Punta La Cholla was 0.92% with 3 fixed differences unique to Punta La Cholla. In contrast, between California and northwestern Mexico (including Punta La Cholla), Nucleotide divergence was 4.277%. Comparing differences just between the closest localities with sequence data to Punta La Cholla, we observed differentiation consistent with historic geographic isolation among sites (Table 3).

Although it would be desirable to have a precise estimate the amount of time that chuckwallas at Punta La Cholla and the surrounding area have been genetically isolated from regional populations (such as Sonoyta and Caborca), it is not accurate to do so using genetic data without geological or palaeontological references, such as by using fossil evidence (Benton and Ayala 2003, Reisz and Müller 2004). Unfortunately, the fossil record for iguanid lizards in western Mexico is very limited (Davy et al. 2011). As a comparison, however, Davy et al. (2011) studied spiny-tailed iguanas (*Ctenosaura spp.*) in this region and within *C. macrolopha* (located on the mainland in southern Sonora), they generated similar estimates of divergence (average pairwise genetic divergence - percentage uncorrected p-distances; 0.1 ± 0.0) to what we observed among northwestern, Mexican *Sauromalus* populations (Table 3). They estimate the time to most recent common ancestor for this node of their tree to be 1.66 Ma (95% HPD; 3.41–0.36). Assuming similar generation time, population size and dispersal ability, we might project that populations of *Sauromalus* in northwestern Mexico have undergone divergence over a similar timeframe and that individuals at Punta La Cholla and surrounding areas have been isolated at least since the Last Glacial Maximum, ~25,000 BCE. During this time, the Central Gulf Coast within the Sonoran Desert likely maintained more xeric vegetation while surrounding areas were converted to woodlands during cooling trends (Anderson & Van Devender, 1995), allowing for chuckwalla populations to expand and facilitate gene flow among disjunct populations. In contrast, one core area of desert throughout most of the Quaternary may have occurred in the Gran Desierto and other surrounding, very arid parts of the Lower Colorado River Valley of the Sonoran Desert (Van Devender et al. 1987) and chuckwallas may instead have used these areas as refugia during these glacial periods and then expanded their populations during the interglacials.

Discussion

Chuckwallas located in the studied populations are isolated and have been for a long time. Our data suggests that the Punta La Cholla and ORPI chuckwallas are closely related to other regional populations (Caborca and Sonoyta) but we cannot accurately estimate an accurate date of divergence. It is likely on the order of several thousands of years. Chuckwallas likely do not maintain gene flow across these landscapes under current conditions. Interestingly, despite being closer in geographic distance between ORPI and Sonoyta, these data suggested ORPI exhibits greater divergence from the other populations. However, these data are limited by sample size and only represent a very small portion of the mtDNA genome.

We used these data to help tell the story of this small, isolated population of chuckwallas and their home in at Punta La Cholla as part of the Next Generation Sonoran Desert Researchers (N-Gen) 6&6 Arts and Science Initiative. In a series of artists' books, we shared a narrative about our experience searching for these elusive creatures, and the increasing impact of human development that is compounding their isolation. Along the walls of the installation, a sequence of the unique sequence of La Cholla chuckwalla DNA was engraved upon sheets of mica;

expressing the close tie between the population's evolutionary history and the geologic constraints of their habitat.

Acknowledgements

We thank Hanna Strauss, Larry Jones and the Tucson Herpetological Society for their assistance in obtaining scat samples from ORPI.

Literature Cited

- Anderson, R.S. & Van Devender, T.R. (1995) Vegetation history and paleoclimates of the coastal lowlands of Sonora, Mexico - pollen records from packrat middens. *Journal of Arid Environments*, 30, 295–306.
- Benton, M.J. & Ayala, F.J. (2003) Dating the tree of life. *Science*, 300, 1698–1700.
- Corl A, Davis AR, Kuchta SR, Comendant T, Sinervo B (2010) Alternative mating strategies and the evolution of sexual size dimorphism in the side-blotched lizard, *Uta stansburiana*: a population-level comparative analysis. *Evolution* 64: 79–96.
- Davy, C.M., F.R. Méndez de la Cruz, A. Lathrop and R.W. Murphy (2011) Seri Indian traditional knowledge and molecular biology agree: no express train for island-hopping spiny-tailed iguanas in the Sea of Cortés. *Journal of Biogeography* 38:272–284.
- Jukes, T.H. & Cantor, C.R. (1969) Evolution of protein molecules. *Mammalian protein metabolism* (ed. by H.N. Munro), pp. 21–132. Academic Press, New York.
- Kocher, T.D., Thomas, W.K., Meyer, A., Edwards, S.V., Paabo, S., Villablanca, F.X. & Wilson, A.C. (1989) Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. *Proceedings of the National Academy of Sciences USA*, 86, 6196–6200.
- Librado, P. & Rozas, J. (2009) DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. *Bioinformatics*, 25, 1451–1452.

Morafka DJ (1994) Neonates: Missing links in the life histories of North American tortoises. In:

Biology of North American Tortoises (eds. Bury RB, Germano DJ), pp. 161–173. Fish and Wildlife Research no. 13. U.S. Department of the Interior National Biological Survey, Washington, DC, USA.

Petren, K. & T.J. Case. 1997. A Phylogenetic Analysis of Body Size Evolution and

Biogeography in Chuckwallas (*Sauromalus*) and Other Iguanines. *Evolution*, 51:206–219.

Reisz, R.R. & Müller, J. (2004) Molecular timescales and the fossil record: a paleontological perspective. *Trends in Genetics*, 20, 237–241.

Rutledge L., J. J. Holloway, B. R. Patterson, and B. N. White. 2009. A improved field method to obtain DNA for individual identification from wolf scat. *Journal of Wildlife Management* 73:1430-1435.

Van Devender, T.R., Thompson, R.S. & Betancourt, J.L. (1987) Vegetation history in the Southwest: the nature and timing of the late Wisconsin-Holocene transition. *North America and adjacent oceans during the last deglaciation* (ed. by W.F. Ruddiman and J.H.E. Wright), pp. 323–352. Geological Society of America, Washington, DC, USA.

Table 1. Primers designed from AF020234 submitted by Petren,K. and Case,T.J. (Sonoyta, Mexico).

Locus	PrimerName	Fwd Primer	Rev Primer	b.p.	Source
cytb	SAAT_Cytb_338f	GGCCTCTACTACGGCTCCTA	GAGTTGAGTCCGGTTGGGTT	338	Designed from AF020234
cytb	SAAT_Cytb_352f	AGCCTTCTCATCAGTTGCCC	GAATCGAGTTAGGGTCGCGT	352	Designed from AF020234
cytb	B1L / B6H	CCATCCAACATCTCAGCATGATGAAA	GTCTTCAGTTTTTGGTTTACAAGAC	980	Kocher et al. (1989)
cytb	SAAT_Cytb_855f	CCACCGTTGTTATTCAACTAC	GGTTTACAAGACCAATGCTTT	855	Corl et al. (2010)

Table 2. Results of PCR optimization assessing 4 different primer pairs and 8 different concentrations of MgCl₂ ranging from 1.0-4.5 mM (labeled as A-H). Each optimization reaction was run on each of 10 *Sauromalus* DNA samples isolated from scat.

Samples	Primers			
	<u>SAAT_Cytb_338</u>	<u>SAAT_Cytb_352</u>	<u>SAAT_Cytb_855</u>	<u>B1L / B6H</u>
SAAT_01A	<i>failed</i>	<i>failed</i>	<i>failed</i>	<i>failed</i>
SAAT_01B	<i>failed</i>	<i>failed</i>	<i>failed</i>	<i>failed</i>
SAAT_01C	<i>failed</i>	<i>failed</i>	<i>failed</i>	<i>failed</i>
SAAT_02A	A-H	A-H	A-H	E,F,G,H
SAAT_02B	A-H	A-H	A-H	H
SAAT_02C	A-H	A-H	A-H	E,F,G,H
SAAT_02L1	<i>failed</i>	<i>failed</i>	<i>failed</i>	<i>failed</i>
SAAT_02L2	A-H	A-H	A-H	G
SAAT_02L3	<i>failed</i>	<i>failed</i>	<i>failed</i>	<i>failed</i>
SAAT_02L4	E,F,G,H	G,H	E,F,G,H	<i>failed</i>

Table 3. Differentiation among *S. ater* mitochondrial Cytochrome B sequences from three sample locations in Northwestern Sonora, Mexico; Sonoyta (n = 2), Caborca (n = 2) and Punta La Cholla (n = 2) and Organ Pipe National Monument (n = 1). Sequence divergence (Dxy) estimates above the diagonal (Jukes and Cantor 1969) and number of fixed nucleotide differences between 327 b.p. total sequence below diagonal.

POP	Sonoyta	Caborca	LaCholla	ORPI
Sonoyta		0.00612	0.00612	0.01223
Caborca	2		0.00612	0.01223
LaCholla	2	2		0.01223
ORPI	4	4	4	

Supplementary Material

327 b.p. DNA Sequence obtained from *Sauromalus ater* scat collected from Organ Pipe National Monument:

>ORPI

CGGCTCCTACTTATTCAAAGAACTTGAAACATCGGAGTAGTACTACTTCTGCTAGT
AATGGCCACAGCATTCGTAGGCTATGTCCTACCCTGAGGCCAAATATCCTTCTGAGG
GGCCACAGTCATCACCAACCTACTCTCCGCCATCCCATACGTAGGAGCCACCCTAGT
AGAATGAATCTGAGGGGGGTTCTCAGTAGATAACGCCACCCTAACTCGATTCTTCAC
ATTTCACTTCCTACTGCCCTTCATAATCATCGGACTAACCATGATACACCTACTCTTC
CTTCACGAAACAGGATCAAACAACCCAACCGGACTCAACTC