

DISCORDANCE BETWEEN MITOCHONDRIAL AND NUCLEAR CONTACT ZONES  
WITHIN ANTELOPE GROUND SQUIRRELS (*AMMOSPERMOPHILUS*)

An Undergraduate Research Thesis

Presented to  
The Department of Evolution, Ecology, and Organismal Biology  
In Partial Fulfillment of the Requirements for Graduation with Research Distinction in Zoology  
The Ohio State University

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May 2020

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Biology

## Abstract

A common biogeographic pattern found in many co-distributed species along the Baja California peninsula is the genetic divergence in the Vizcaíno Desert. This separation is hypothesized to have been caused by a mid-peninsular seaway that formed during the late Miocene-middle Pleistocene and later dried, allowing contact again between formerly isolated populations. Previous phylogeographic studies on the antelope ground squirrel (*Ammospermophilus leucurus*) show a mitochondrial DNA break through the middle of the peninsula. We investigated whether (1) the mitochondrial pattern of divergence and secondary contact between the northern and southern *Ammospermophilus* clades are consistent with results from genome-wide nuclear data and (2) whether genetic admixture is occurring. One hundred thirty-three samples were collected spanning from the northwest US south into the Baja California peninsula and pooled using ddRADseq protocol. Our nuclear DNA analyses show a 335 km divergence between the two contact zones and low levels of admixture. Several individuals belonging to the southern clade have a northern mitochondrial haplotype, suggesting introgression. This introgression and lack of admixture suggests that there may have been ancestral hybridization between the now reproductively isolated populations.

## **Introduction**

Discordance in biogeographic patterns between nuclear DNA (nuDNA) and mitochondrial DNA (mtDNA) in animal hybrid zones can reveal important underlying evolutionary processes, such as incomplete lineage sorting, differential introgression, sex-biased dispersal, behavioral dominance in mating opportunities, and hybrid zone movement (Toews & Brelsford 2012). mtDNA is fundamentally different from nuDNA in two main aspects. First, mtDNA is maternally inherited rather than biparentally inherited like nuDNA and thus gender biases in dispersal or dominance of one sex over the other in mating opportunities can produce discordant biogeographic patterns between mtDNA and nuDNA. Secondly, mtDNA is haploid, which has one-fourth the population size of nuDNA, and thus ancestral polymorphisms are lost more rapidly over evolutionary time compared to nuDNA, which leads to quicker fixation of genetic differences between species (Toews & Brelsford 2012). The slower fixation rate of genetic variation in nuDNA leads to greater incomplete lineage sorting. This should leave less obvious biogeographic patterns because shared genetic variation between species should be as likely to occur away from the contact zone as it does in the contact zone. Introgressive hybridization is likely the cause in situations where shared genetic variation between species are clustered in and around the contact zone, but not farther away in the parental ranges. (Toews & Brelsford 2012). In order to completely reveal the most relevant evolutionary processes involved in biogeographic patterns, it is essential to study a variety of genetic markers. As availability and advancements in genetic sequencing technology become more prevalent, more complete understandings of biogeographic patterns of mito-nuclear discordance will be realized (Edwards & Bensch 2009; Toews & Brelsford 2012).

The Baja California peninsula has been an important region for studying secondary contact zones for a wide range of taxa. The location of the most well studied contact zone in Baja California occurs in the mid-peninsular region called the Vizcaíno Desert (Mantooth *et al.* 2013). It has been hypothesized that a seaway, recognized as the Vizcaíno Seaway, formed through this desert during the late Miocene-middle Pleistocene (Riddle *et al.* 2000). This seaway would have thus split the peninsula into two regions, including one region in the north connected to the North America continent and an insular region in the south. It has been proposed that this aquatic barrier later disappeared when sea levels dropped, which then reconnected these two regions of the peninsula. A clustering of secondary contact zones among several vertebrate systems, including mammals, reptiles, avians, and amphibians, have been documented in this region (Riddle *et al.* 2000; Leaché *et al.* 2007), thus lending support to the proposal that this was facilitated by an environmental change in the mid-peninsular region. Leaché *et al.* (2007) hypothesizes that a combination of volcanic events from the late Miocene to Holocene and marine incursions in the Gulf of California formed physical barriers to divide north and south populations. One limitation to this proposal has been the fact that this previous genetic research has largely focused on only a single or limited number of genetic markers, with a strong bias towards the use of mtDNA. Thus, we are uncertain whether biogeographic discordance in mtDNA and nuDNA is an important aspect to the secondary contact of animals in Baja California.

The antelope ground squirrel (*Ammospermophilus*) is a common rodent in the desert environments of western North America. This genus ranges from the Great Basin deserts in eastern Oregon and western Utah all the way down south through Sonoran and Chihuahuan deserts of Texas and northern Mexico, including Baja California (Mantooth *et al.* 2013).

*Ammospermophilus* is dated to have originated in the mid-Miocene (11.5 Mya) in southern California and subsequently diversified into five distinct species: *A. nelsoni*, *A. leucurus*, *A. harrisii*, *A. insularis*, and *A. interpres* (Mantooth *et al.* 2013). Previous research has shown a mtDNA break in the formerly described species of *Ammospermophilus leucurus* to occur in the mid-peninsular region (Vizcaíno Desert) of Baja California (Whorley *et al.* 2004; Whorley 2006; Mantooth 2013). The taxonomy of *A. leucurus* was subsequently revised based on the mtDNA studies with the mtDNA clade in the southern portion of the Baja California peninsula described now as *A. insularis* (Mantooth *et al.* 2013). Further studies with nuDNA are needed to verify whether these revised species relationships and species boundaries are warranted.

The goal of our research was to evaluate potential biogeographic patterns of mito-nuclear discordance in *Ammospermophilus* lineages in the Baja California peninsula. We had three main objectives. First, we investigated biogeographic patterns of divergence and secondary contact between the northern and southern lineages of *Ammospermophilus* based on nuDNA variation generated from a double digest RADsequencing (ddRADseq) approach. Next, we examined mito-nuclear discordance by comparing biogeographic patterns of secondary contact between lineages based on ddRADseq with lineages based on mtDNA (Whorley 2006). Lastly, we investigated whether genetic admixture is occurring in the zone of secondary contact.

## **Methods**

### *Sample Collection, DNA Extraction, ddRADseq Library Preparation, and Sequencing*

We analyzed 133 *Ammospermophilus* samples from Baja California and Baja California Sur, Mexico and from California, Nevada, and Utah in the United States (Figure 1). Frozen tissue samples were obtained from the following museums: Centro de Investigaciones Biológicas

(CIB); Museum of Southwestern Biology, University of New Mexico (MSB); New Mexico Museum of Natural History (NMMNH); and Burke Museum, University of Washington (UWBM).

DNA was extracted from the tissue samples using a Qiagen DNeasy kit and quantified using a High Sensitivity DNA assay on a Qubit Fluorometer. DNA was digested and size selected according to the ddRADseq protocol described by Peterson *et al.* (2012), with some modifications. Restriction enzymes MspI and SbfI were used for the double digest, with MspI being the common cutter and SbfI the rare cutter. DNA fragments were ligated with barcoded Illumina adapters, the samples were then pooled by eight samples, size-selected for fragments in the range of 300–400 base pairs (bp) using the BluePippin size selector, ligated with Illumina multiplexing indices, and final pools were assessed for quality using Agilent TapeStation electrophoresis. All pools were combined and sequenced for 50-bp single-end reads on a single lane on an Illumina HiSeq4000 at the Vincent J. Coates Genomics Sequencing Laboratory at UC Berkeley, supported by NIH S10 OD018174 Instrumentation Grant.

### *Bioinformatics and Data Analysis*

Raw Illumina data were first demultiplexed using the unique combination of barcodes and indices, and filtered using iPyrad 0.7.8 (Eaton 2014), according to the default filters with several modifications. Alterations were made in the params file at lines 9, 10, 14, 21, 22, and 23. At line 9, a maximum of 4 low-quality base pairs was allotted per read to minimize sequence uncertainty. Lines 11 and 12 were set to a minimum depth of 6, to set the lower bound of the read depth at which statistical and majority base calls were made, respectively. Line 14 was set at 0.90, which is the percent sequence similarity required for sequences to be collapsed into a single

cluster; this decreases the chances of falsely calling similar sequences the same sequence. We ran iPyrad with two different settings for line 21, the minimum number of samples that must have data at a given locus for it to be retained in the final data set. For the first iPyrad run we set this at 90% (121 out of 133 samples) and for the second run we set this at 10% (13 of 133 samples). Lines 22 and 23 were both assigned values 4 to control the maximum number of SNP's and indels allowed in a locus, respectively.

### *Population Assignment Analysis*

The number of genetic population groupings was estimated using a Bayesian clustering method in STRUCTURE 2.3.4 (Pritchard *et al.* 2000) with both datasets from iPyrad representing 10% and 90% minimum number of samples. The population assignment was analyzed using  $K = 2$  and with a 10,000 burnin period followed by 100,000 reps. Following Vähä & Primmer (2006), we categorized individuals into three clusters, using a range of q-values between 0.90–1.00 as a pure northern lineage (*A. leucurus*), between 0.00 and 0.10 as a pure southern lineage (*A. insularis*), and between 0.11–0.89 for admixed individuals.

### *Mito-Nuclear Biogeographic Discordance*

To investigate biogeographic discordance in genetic breaks between mtDNA and nuDNA, we compared the geographic location of the nuDNA (ddRADseq) break with mtDNA data from 77 samples from Whorley (2006) using Google Earth.

## Results

### *Numbers of ddRADseq Loci from iPyrad*

The two iPyrad runs with different settings for minimum numbers of missing samples had over one order of magnitude difference in numbers of polymorphic ddRADseq loci. The dataset with 90% minimum number of samples had 1,774 loci and the dataset with 10% minimum number of samples had 38,598.

### *Population Assignment*

Both Structure runs for the datasets with 90% and 10% minimum number of samples produced the same assignment groupings. Of the 133 *Ammospermophilus* samples, 16 samples had q-values that assigned them to the northern lineage (*A. leucurus*), 112 samples had q-values that assigned them to the southern lineage (*A. insularis*), and 5 samples had q-values that assigned them as hybrids (Figure 2).

### *Mito-Nuclear Biogeographic Discordance*

There is an approximate 335 km difference between the locations of the biogeographic break in nuDNA (ddRADseq) and mtDNA in the Baja California peninsula (Figure 1). The nuDNA biogeographic break occurs in northeastern Baja California between approximately 30° and 31° latitudes at the environmental transition between the San Felipe Desert and the Cataviña area of the Central Desert. The location of this environmental transition coincides with the eastern-slope rain shadow of the San Pedro Mártir mountains. The northern population extends 1004.47 km from Castle Valley, Utah, just 8 km south of the Colorado River to San Felipe, Baja California. The southern population extends 907.2 km from Puertecitos, Baja California to



Migriño, Baja California Sur. The mtDNA biogeographic break occurs in the central part of the Baja California peninsula between approximately 28.7° and 27° latitudes in the Vizcaíno Desert (Whorley 2006).

Of the 16 samples that were assigned to the northern population based on ddRADseq data, only 2 had mtDNA data. Both of these northerly assigned samples had northern mtDNA haplotypes, according to Whorley (2006). Of the 112 samples assigned to the southern population based on ddRADseq data, we had 68 samples with mtDNA data. Of these 68 samples, 12 had southern mtDNA haplotypes and 56 had northern mtDNA haplotypes. Of the 5 samples that were assigned as hybrids, only 1 had mtDNA and this had a northern mtDNA haplotype.

## **Discussion**

We found the nuDNA secondary contact zone between northern and southern lineages of *Ammospermophilus* in the northeastern portion of the Baja California peninsula in the San Felipe Desert region. This contact zone is displaced approximately 335 km north of the mtDNA contact zone that is located further south along the peninsula in the Vizcaíno Desert region. We also found only a handful of admixed individuals in the contact zone, suggesting there may be some reproductive isolating mechanisms preventing rampant interbreeding or lowered hybrid fitness. However, many individuals assigned to the southern *Ammospermophilus* lineage possess a northern mtDNA haplotype, suggesting historical introgression of the northern mtDNA genome into the southern lineage. The newly discovered boundary between northern and southern lineages based on the nuDNA data brings into question the location of the species boundary between *A. leucurus* and *A. insularis*.

The location of the nuDNA contact zone occurs in the environmental transition between the San Felipe Desert and the Cataviña area of the Central Desert. Along the Baja California peninsula, there are several distinct desert areas that vary in elevation and rainfall. The northern region of the peninsula is characterized by having very dry and hot summer climate and a winter season when most of the annual precipitation falls, usually between 3-9 cm (Holmgren *et al.* 2011). The San Felipe Desert is located in this northern region, but in an even drier region because it is in the rain shadow of the San Pedro Mártir mountains. This desert is considered the most arid desert in North America (Peinado *et al.* 2006) and has, on average, only around 3 cm of rainfall the entire year (Roberts 1989). The San Felipe desert is characterized by flat, sandy terrain with a sparse array of flora, mostly bursage and creosote bushes (Roberts 1989). This area contains the southernmost populations of *A. leucurus* in North America and low frequency hybrids with *A. insularis*. Further southwest of the San Felipe desert is the Central Desert where there are higher levels of winter rainfall than the San Felipe Desert (Bullock 2003). The Central Desert region contains the northernmost populations of *A. insularis*.

The location of the mtDNA contact zone occurs in the Vizcaíno Desert region, which represents another environmental transition in the Baja California peninsula. This Vizcaíno Desert is located at the point where seasonal precipitation patterns shift from greater levels of winter precipitation in the north to greater levels of summer precipitation in the south (10-52 cm) (Holmgren *et al.* 2011). The Vizcaíno Desert contains cooler summers with slightly more rainfall than surrounding deserts (Peinado *et al.* 2005) that support short, widely spaced flora (Roberts 1989). The hypothesized, historical Vizcaíno Seaway would have intersected the Vizcaíno Desert and is thought to have formed during the middle Pliocene era (Riddle *et al.* 2000). The lowering of sea levels since then is believed to be the time when pairs of closely related species

in the northern and southern parts of the peninsula started to experience secondary contact. The biogeographic break in the mtDNA of *Ammospermophilus* follows this pattern of secondary contact in the Vizcaíno Desert (Whorley *et al.* 2004; Whorley 2006; Mantooth *et al.* 2013). Whorley *et al.* (2004) found that the northern mtDNA clade has less haplotype diversity than the southern mtDNA clade in the Baja California peninsula, which may be indicative of a rapid southward expansion of the northern populations into the peninsula from more northerly regions of the North American continent. The greater haplotype diversity in the southern clade indicates a larger population size and longer residence time in the southern peninsula. Given that the species boundary between *A. leucurus* and *A. insularis* based on the genome-wide nuDNA appears to be further north in the San Felipe Desert, it is thus possible that mtDNA from *A. leucurus* introgressed southward into *A. insularis* and has reached as far south as the Vizcaíno Desert. An alternative explanation for the lower diversity of northern mtDNA haplotypes from *A. leucurus* in these *A. insularis* individuals is that there could have been a bottleneck of mtDNA haplotypes that introgressed across the species boundary.

Another explanation for the discordance between the nuDNA and the mtDNA biogeographic breaks could be that the hybrid zone between the two species has moved northward, leaving a mtDNA wake in the Central Desert. Ground squirrels are known to have male-biased dispersal (Lawson Handley & Perrin 2007) due to the high reproductive investment in females (Belk & Smith 1991). Males do not raise their young, so they tend to disperse farther away from their natal sites. The reduced dispersal distance in females can slow the geographic spread of the maternally inherited mtDNA, which may explain the geographic discordance between the nuDNA break and the mtDNA break. Information on male-biased dispersal in *Ammospermophilus* is limited to one study in *A. nelsoni* that shows no sex-biased dispersal

(Hawbecker 1958). Another speculation for the discordance would be if *A. insularis* males are behaviorally dominant over *A. leucurus* in copulation with females. In other word, *A. insularis* may be gradually moving northward and the possible propensity of their males to prevail over *A. leucurus* males in mating opportunities with *A. leucurus* females may be causing the maternally inherited mtDNA to be left in the wake of the northward movement of *A. insularis*.

Today the Baja California peninsula is rich in biological diversity that has evolved since the late Pleistocene. The possibility of a Pleistocene refugium in the southern Baja California peninsula coincides with the hypothesized Vizcaíno Seaway (Holmgren *et al.* 2014). With the southern area of the peninsula altering into an island, the difference in climate is inevitable. The distinct climate of the southern peninsula modified the desert biogeography and therefore the type of biota it can sustain. Riddle & Hafner (2006) identified an important phylogeographic pattern, the biota displays a high level of endemism in the southern peninsula. They postulated that this is a key finding in favor of a northward range expansion of desert taxa post-seaway. Molecular phylogeographic studies of the California gnatcatcher (*Polioptila californica*) (Zink *et al.* 2000) and the senita cactus (*Lophocereus schottii*) (Nason *et al.* 2002) support the northward expansion of taxa on the peninsula. These studies bring us to hypothesize that secondary contact between the northern and southern lineages of *A. leucurus* is an event that occurred in the last 11,000 years. Over time the change to the peninsular environment may have induced ecological isolation between the clades.

## **Conclusion**

Our study highlights the importance of collecting different kinds of genetic data. Our results for the nuDNA break between *A. leucurus* and *A. insularis* did not geographically align

with previous research showing the location of the mtDNA break and brings forth many questions. The species boundary appears to be further north than what was previously believed, but whether this is due to mtDNA introgression or hybrid zone movement is not clear. More sampling from the narrow gap between the northern and southern clades is needed to more thoroughly determine the numbers and generations of hybridization and ecological and behavioral relationships of both species.

### **Acknowledgements**

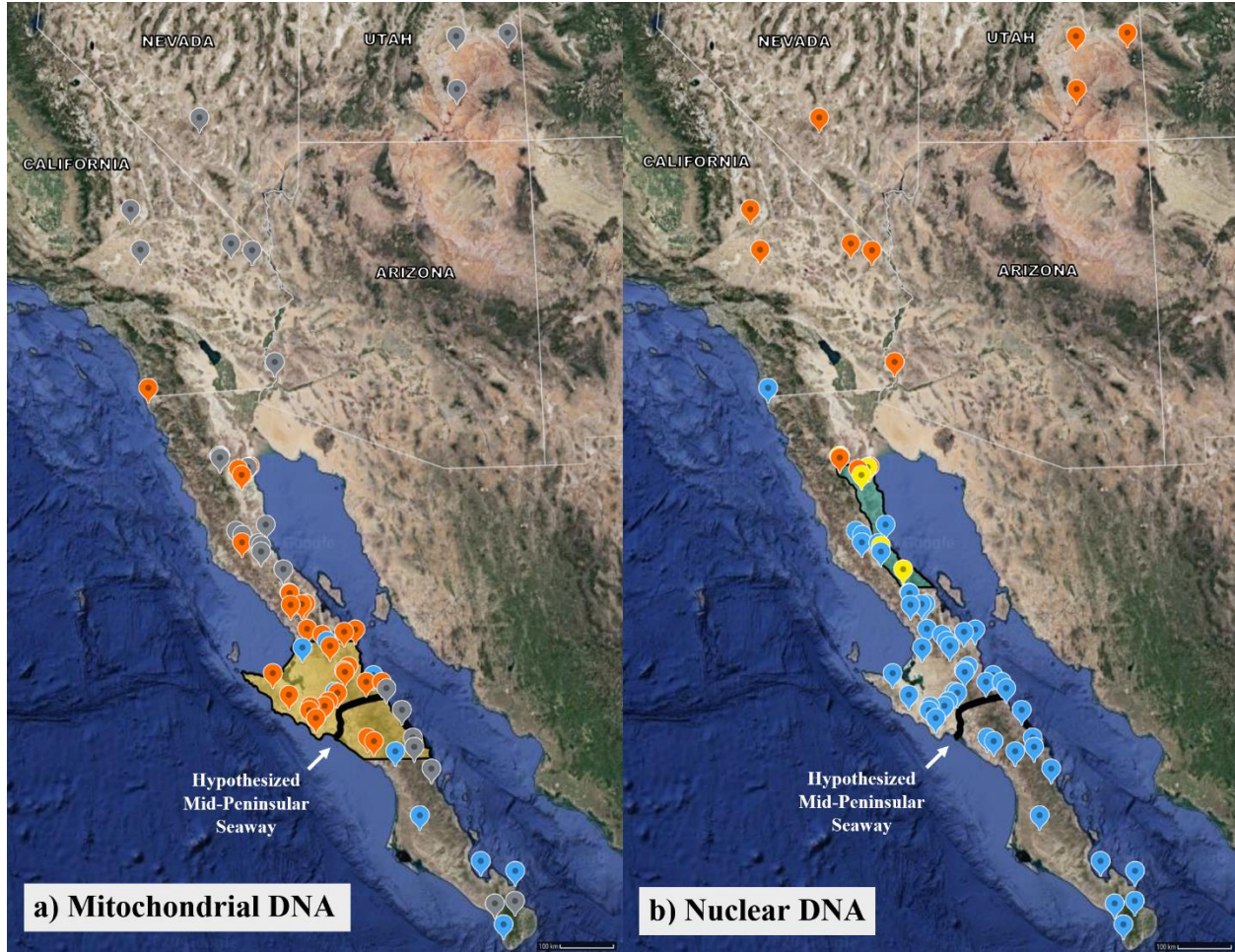
I would like to thank my project advisor, Dr. Andreas Chavez, and department representative, Dr. Ryan Norris, for their support and guidance throughout this process. I would also like to thank Sahil Patel and Nate Hofford for their assistance with laboratory work and Shay Zhao for providing writing advice. Lastly, thank you to the NSF REU program at The Ohio State University for giving me the opportunity to begin this project.

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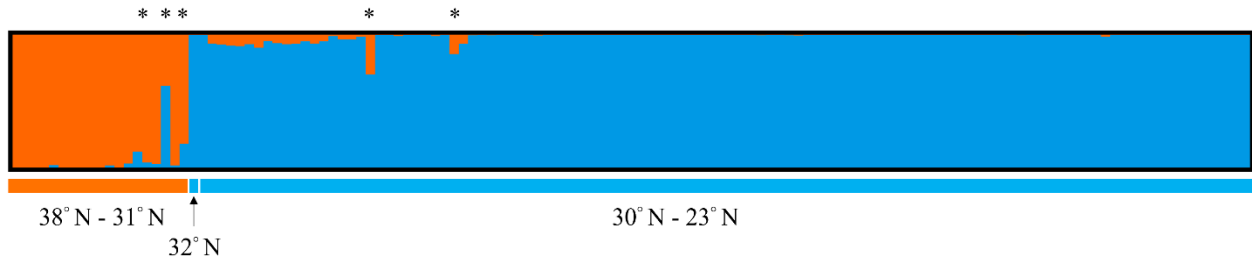
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## Figures



**Figure 1.** Maps show the location of *Ammospermophilus* samples used in this study, as well as the hypothesized mid-peninsular seaway from the Miocene. (a) The colors of each point are based on the mtDNA-clade assignment from Whorley (2006). Orange points represent samples belonging to the northern mtDNA clade (*A. leucurus*) and blue points represent samples belonging to the southern mtDNA clade (taxonomically revised as *A. insularis* by Mantooth *et al.* 2013). Gray points represent samples for which mtDNA data is unavailable. The yellow-shaded region shows the mtDNA contact zone in the Vizcaíno Desert. (b) The colors of each point are based on the nuDNA-population assignment from this study. Orange points represent pure *A. leucurus* samples, blue points represent pure *A. insularis* samples, and yellow points represent admixed samples. The green-shaded region shows the nuDNA contact zone in the San Felipe Desert. Note: we have extended the northern boundary of *A. insularis* from the mtDNA contact zone that was described in Mantooth *et al.* (2013) to the nuDNA contact zone that was discovered in this study.





**Figure 2.** Structure plot for  $K = 2$  using dataset with 90% of samples having data at a given locus. Orange color represents *A. leucurus* ancestry and blue color represents *A. insularis* ancestry. The range of latitudes for each species are shown below the plot. Asterisks show samples found to be admixed.