PHYLOGENETIC SYSTEMATICS, POPULATION GENETICS, AND THE EVOLUTION OF COLOR PATTERN POLYMORPHISM AND CORALSNAKE MIMICRY IN THE SNAKE GENUS *SONORA*

by

CHRISTIAN L. COX

Presented to the Faculty of the Graduate School of

The University of Texas at Arlington in Partial Fulfillment

of the Requirements

for the Degree of

DOCTOR OF PHILOSOPHY

THE UNIVERSITY OF TEXAS AT ARLINGTON

August 2012

ACKNOWLEDGEMENTS

This project would not have been possible without the collaboration of Alison R. Davis Rabosky (University of Michigan). Early in my dissertation, we elected to collaborate rather than compete and have combined our efforts in the field, lab museum and during writing. I am so pleased that we could work together on this project for the last several years, and through collaboration the final results are far better that I could have attained working only by myself.

I would like to thank my advisors (Paul T. Chippindale and Jonathan A. Campbell) and committee (Paul T. Chippindale, Jonathan A. Campbell, James V. Robinson, Pawel Michalak, Ellen J. Pritham and Eric N. Smith) for advice, guidance, funding, and the opportunity to work independently. I would also like to thank Oscar Flores-Villela for being an unofficial sixth committee member and for his help and collaboration on this project.

My colleagues that I traveled with during fieldwork in Mexico (Thomas G. Eimermacher, Brian E. Fontenot, Jesse M. Meik, Jacobo Reyes-Velasco, Sarah Schaack, Coleman M. Sheehy, Jeffrey W. Streicher, Ruben U. Tovar) contributed to this research with many helpful discussions during lengthy van rides. Todd A. Castoe, Michael L. Logan, Robert A. Makowsky, John L. Morse, Michael Sandel, Walter E. Schargel, Michael P. Venarsky and Charles M. Watson were always willing to

converse about research ideas or help with analyses. Corey E. Roelke participated in more fieldwork than just about anybody else, and was willing to take the time to collect during many independent forays and this effort was greatly appreciated.

I thank University of Texas-Arlington Amphibian and Reptile Diversity Research Center, University of Kansas Natural History Museum and Biological Research Center, Sternberg Museum of Natural History at Fort Hays State University; Texas A & M Texas Cooperative Wildlife Collection, University of Texas Natural History Collection, University of California Museum of Vertebrate Zoology, Texas Herpetological Society, Kansas Herpetological Society, East Texas Herpetological Society, and the American Museum of Natural History for tissue loans, specimen loans and financial and logistical assistance. I owe a great deal of gratitude to Joel F. Stringer for his invaluable assistance with laboratory work. I thank Thomas G. Eimermacher, Brian E. Fontenot, Jesse M. Meik, Jacobo Reyes-Velasco, Coleman M. Sheehy, Jeffrey W. Streicher, Ruben U. Tovar, Michael L. Logan, Robert A. Makowsky, John L. Morse, Corey E. Roelke, Michael Sandel, Walter E. Schargel, Michael P. Venarsky Charles M. Watson, Joel F. Stringer, Daniel Krull, Miriam Flores-Benabib, Chris Harrison, Joseph T. Collins, James R. Dixon, Mitch F. Mulks, Sarah Schaack, Paulino Ponce-Campos, Angela Fornell, M. Ben Anders, Toby J. Hibbits, Troy D. Hibbits, Joe Farah, Brandon Pope, Travis J. LaDuc, Matt J. Ingrasci, Teddy Angarita-Sierra, Matthew A. Moseley, Paul N. Pasichnyk, Utpal Smart, Chris A. Hamilton, and any UTA Herpetology students that contributed time and effort to my research.

I thank my family (especially my parents) for enduring my muddy clothes, a parade of strange animals and fostering my interests in biology. Finally, I thank Charlotte Bakke for her love and support as I complete my research and prepare for the next step in my life and career.

ABSTRACT

PHYLOGENETIC SYSTEMATICS, POPULATION GENETICS, AND THE EVOLUTION OF COLOR PATTERN POLYMORPHISM AND CORALSNAKE MIMICRY IN THE SNAKE GENUS *SONORA*

Christian L. Cox, PhD

The University of Texas at Arlington, 2012

Supervising Professors: Paul T. Chippindale and Jonathan A. Campbell

Mimicry is a widespread biological phenomenon and is simply defined as the resemblance of one organism to another for some sort of protective purpose. Specifically, Batesian mimicry occurs when a harmless species imitates a noxious or harmful species. Neotropical coralsnakes and their colubrid snake mimics are a very diverse example of Batesian mimicry and coralsnake mimicry has been posited as a major factor underlying the diversity of Neotropical colubrids. One type of phenotypic diversity that is widespread among Neotropical coralsnake mimics is geographic variation in color and color pattern polymorphism. Color pattern polymorphism is the presence of multiple color pattern types within the same population for the same species. Although common within coralsnake mimics and within other Batesian

mimicry complexes, the evolutionary conditions underlying the evolution of color pattern polymorphism in the context of mimicry is not well understood. My dissertation research focuses on the evolutionary dynamics of color pattern polymorphism within a group of Neotropical coralsnake mimics in the genus *Sonora*. This genus contains four species that are distributed from the Balsas region of Mexico to central North America and all possess striking color pattern polymorphism. The three exclusively Mexican species are found entirely in sympatry with coralsnake species and have multiple coralsnake-mimicking morphs. In contrast, the North American *S. semiannulata* has the largest geographic range (including large areas of allopatry with coralsnakes) and has only one mimetic morph (and 3 other morphs). I use a combination of extensive museum sampling, phylogenetic systematics, population genomics, and candidate gene approaches to assess relationships among population and species, determine the selection dynamics on color pattern, and assess the genetic origin of color pattern polymorphism. This research reveals the phylogenetic distribution of coralsnake mimicry and color pattern polymorphism, geographic and temporal variation in selection on color pattern, and suggests future avenues of research for assessing the genetic determinants of color pattern.

TABLE OF CONTENTS

LIST OF ILLUSTRATIONS

LIST OF TABLES

CHAPTER 1

MIMICRY AND COLOR PATTERN POLYMORPHISM

Mimicry is a widespread biological phenomenon and is simply defined as the imitation of one organism by another for some sort of protective purpose (Brodie 1993). This definition is often narrowed to exclude forms of crypsis, whereby an animal is protected by resembling an inanimate biological structure such as a leaf or tree bark (Brodie and Brodie 2004). Although many forms of mimicry have been postulated and described, most cases of mimicry can be characterized as either Batesian or Mullerian mimicry (Brodie and Brodie 2004). Mullerian mimicry occurs when two noxious, toxic, or dangerous species resemble one another, thus exploiting predator response to a common signal that the prey may be distasteful or dangerous (Müller 1879). Batesian mimicry occurs when a harmless species (mimic) resembles a noxious, toxic or dangerous species (model), thus dishonestly convincing the predator that an otherwise suitable prey is distasteful or dangerous (Bates 1862). Mimetic systems may be more likely to evolve if the signal is clear, and so most examples in the literature of both Batesian and Mullerian mimicry involve conspicuous or bright coloration (e.g., Brodie 1993; Mallet and Joron 1999). However, cryptic mimicry has been documented, and conspicuousness to the predators driving the mimicry system may not coincide with human perception of conspicuousness (Brodie and Brodie 2004; Wuster et al. 2004).

Nonetheless, it is the diversity of conspicuous and beautiful color patterns in nature that have served as catalysts for much mimicry research.

Diversity in color pattern within mimicry complexes can be quite extensive, with some Batesian and Mullerian mimicry systems containing both geographically variable coloration and color pattern polymorphism within populations (Joron and Mallet 1998; Mallet and Joron 1999). Within the context of a mimicry complex, polymorphic species may have multiple mimetic color patterns within a single population (Brown and Benson 1974; Mallet and Joron 1999; Ceccarelli and Crozier 2007). Alternately, populations of polymorphic species may contain both mimetic and non-mimetic color patterns (Barrett 1976; Turner 1978; Nijhout 2003). These color pattern polymorphisms within a population present an interesting dilemma in evolutionary biology. Because random processes will tend to fix neutral alleles over time, the persistence of polymorphisms within populations must be explained by either adaptive (e.g. negative frequency dependent selection, heterosis, adaptive divergence with subsequent gene flow, or various forms of sexual selection) or stochastic (e.g. neutral divergence with subsequent gene flow) processes (Gray and McKinnon 2006). Adaptive divergence with subsequent gene flow and balancing selection have been invoked to explain color pattern polymorphisms in populations of frogs (Hoffman et al. 2006), lizards (Rosenblum 2006), and water snakes (King 1993; King and Lawson 1995). Within mimicry systems, color pattern polymorphism has been explained by shifting balance, coevoutionary chases, sex-limited mimetic dynamics, the genetic architecture of mimetic color, and frequency dependence (Joron and Mallet 1998;

Mallet and Joron 1999; Brodie and Brodie 2004). Specifically for Batesian mimicry systems, support for any particular mechanism maintaining color pattern polymorphism is mixed. Hence, research clarifying the mechanisms generating and maintain color pattern polymorphism in natural systems would be useful.

One of the most diverse examples of Batesian mimicry is found in Neotropical coralsnakes and their colubrid mimics (Savage and Slowinski 1992; Brodie and Brodie 2004). Coralsnakes are dangerously venomous snakes in the family Elapidae (closely related to cobras, kraits, and mambas) with 74 species of three different genera that are distributed from the North American southeast and southwest to central Argentina in South America (Campbell and Lamar 2004b). These snakes are usually bicolored and tricolored banded (Fig 1.1), with a combination of contrasting red, black, and yellow bands (Campbell and Lamar 2004b). Their harmless coralsnake mimics are conspicuously colored in a fashion similar to coralsnakes (Fig 1.1) and found in at least 39 genera in the family Colubridae and have a geographic distribution that broadly overlaps with coralsnakes (Joron and Mallet 1998; Mallet and Joron 1999). Avian predation is thought to be an important selective pressure driving mimicry (Pfennig et al. 2001; Brodie and Brodie 2004), with snakes forming an important part of the diet for many birds (Bryant 1916; DuVal et al. 2006; Sherbrooke and Westphal 2006; Specht et al. 2008; Miller et al. 2010), and some bird species displaying innate avoidance of coralsnake color patterns (Smith 1975; Smith 1977). Although historically the subject of much debate (Wickler 1968; Greene and Pyburn 1973), the mimicry of coralsnakes by colubrid species has been studied by comparing the geographic pattern of co-

occurrence of matching pattern types (Greene and McDiarmid 1981) and clay model based studies (Brodie 1993). This research has not only provided evidence for coralsnake mimicry, but also documents the evolutionary dynamics of mimicry (Brodie and Janzen 1995; Brodie and Moore 1995; Pfennig et al. 2001; Brodie and Brodie 2004), the role of density, allopatry and predator cognition on mimetic color pattern (Harper and Pfennig 2007; Kikuchi and Pfennig 2010b; Kikuchi and Pfennig 2010a; Pfennig and Mullen 2010), and the role of gene flow in impacting mimicry (Harper and Pfennig 2008).

Like many other mimicry systems, color pattern polymorphism is present in some coralsnake species and some coralsnake mimics. One of the most striking examples of a coralsnake mimic with color pattern polymorphism is in the genus *Sonora* (Greene 1997; Ernst and Ernst 2003; Campbell and Lamar 2004b). This genus contains four species that are distributed from Mexico to central North America (Echternacht 1973; Cox et al. 2012). All members of the genus are considered coralsnake mimics, have color pattern polymorphism and possess 2-4 discrete color morphs (Savage and Slowinski 1992; Brodie and Brodie 2004; Cox et al. 2012). Three of the species are found exclusively in Mexico and are entirely in sympatry with more than one species of coralsnake, and all of these species have multiple coralsnake-mimicking morphs (Echternacht 1973; Cox et al. 2012). In contrast, *S. semiannulata* has the largest geographic range (including large areas of allopatry with coralsnakes), and only one of the four morphs is clearly mimetic (Ernst and Ernst 2003). This species possesses the greatest variation in color pattern in the genus, with four different dorsal

color patterns; uniform, red-striped, darkly cross-banded, or both cross-banded and red-striped (Fig 1.2). Beyond this discrete polymorphism, *S. semiannulata* also has great variation in both ground color and the shape, size, and number of dorsal stripes and bands (Fig 1.2). Two other colubrid genera (*Chilomeniscus* and *Chionactis*) are hypothesized close relatives of Sonora and are also mimetic with color pattern polymorphism (Ernst and Ernst 2003). It is my contention that this group serves as an ideal natural system with which to explore the evolutionary determinants of color pattern polymorphism in a mimicry complex.

For my dissertation research, I have combined phylogenetic systematics, population genomics, candidate loci approaches and sequence analysis to assess the evolutionary and selection dynamics of color pattern polymorphism in a group of coralsnake mimics. For my second chapter, I used supermatrix and coalescent approaches to assess phylogenetic relationships among *Sonora*, focusing on the exclusively Mexican species. I then used Bayesian species delimitation to amend the taxonomy of this group and discuss the phylogenetic distribution of coralsnake mimicry and color pattern. This chapter is in collaboration with Alison R. Davis Rabosky, Jacobo-Reyes Velasco, Paulino Ponce-Campos, Eric N. Smith and Jonathan A. Campbell. In the third chapter, I used AFLPs to study how landscape influences patterns of neutral genetic variation across their geographic range. This research is in collaboration with Alison R. Davis Rabosky and Paul T. Chippindale. For my fourth chapter, I combined estimates of population differentiation with color pattern frequencies to determine whether selection is acting on color pattern. I supported these

results with museum data on temporal and size-class shifts in morph frequency within populations. This chapter is in collaboration with Alison. R. Davis Rabosky. In the fifth chapter, I used the candidate loci approach and sequenced a color pattern gene (Mc1R) for all *Chilomeniscus*, *Chionactis* and *Sonora* species and morphs, and then used association tests to infer the role of this gene in controlling color pattern polymorphism. I also used tests for selection to determine the selection dynamics on this color pattern gene. This chapter is in collaboration with Alison Davis-Rabosky and Paul T. Chippindale. Finally, in the sixth chapter I summarized the results of my research and discusssed future and ongoing research within this system. This research furthers our understanding of the selection and evolutionary dynamics influencing color pattern polymorphism in mimicry complexes.

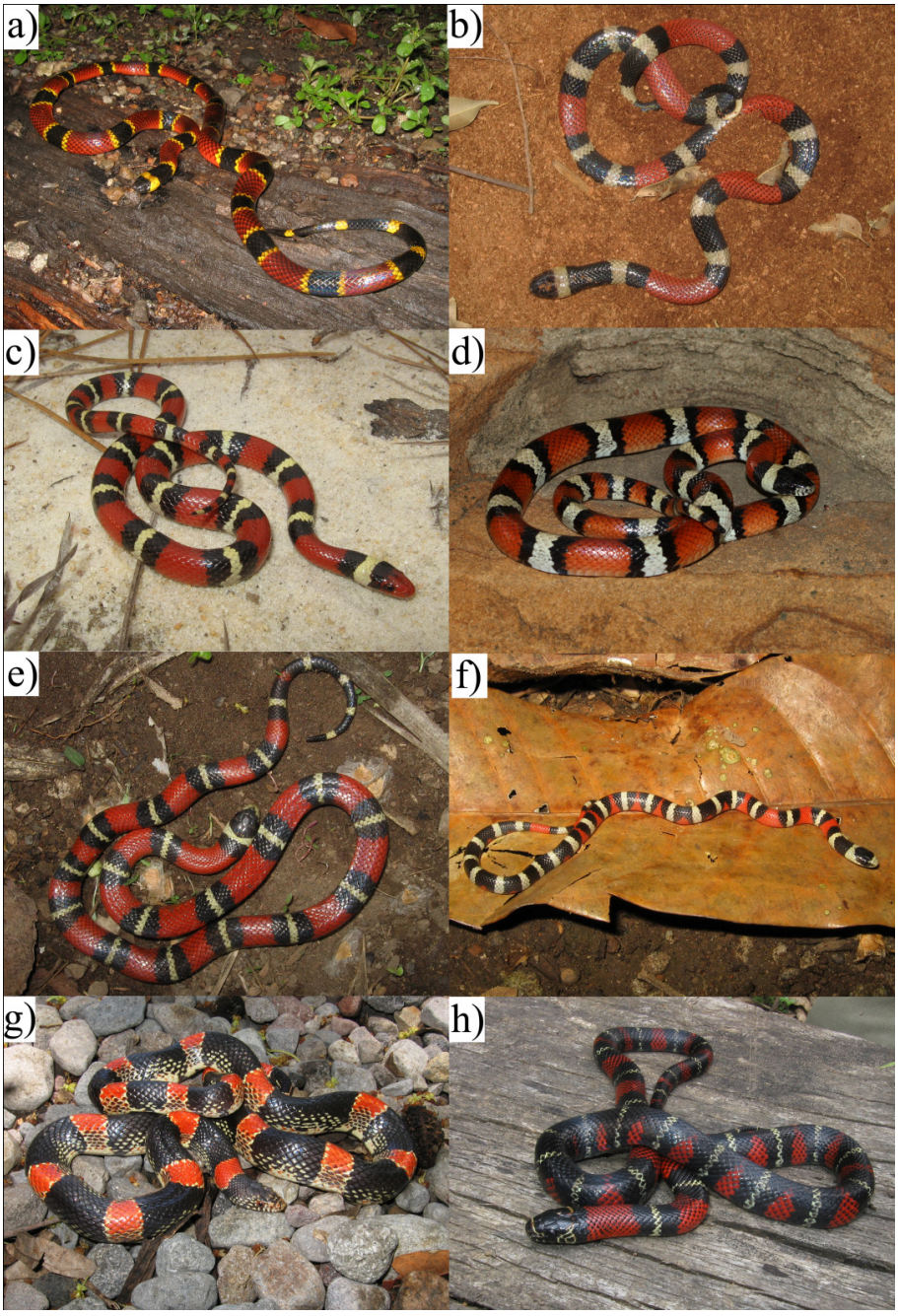

Figure 1.1. Examples of coralsnake species and coralsnake mimics. a) *Micrurus browni*, b) *Micrurus laticollaris*, c) *Lampropeltis elapsoides*, d) *L. triangulum subsp.*, e) *L. t. nelsoni*, f) *Sonora mutabilis*, g) *Rhinocheilus lecontei*, h) *L. t. subsp*. Photos by Christian L. Cox.

Figure 1.2. Variation in ground color, band and stripe morphology, and number of bands for *Sonora semiannulata*. Photos by Alison R. Davis Rabosky and Christian L. Cox.

CHAPTER 2

MOLECULAR SYSTEMATICS OF THE GENUS *SONORA* (SQUAMATA: COLUBRIDAE) IN CENTRAL AND WESTERN MEXICO

2.1 Abstract

Mexico possesses high levels of endemic biodiversity, especially for squamate reptiles. However, the evolutionary relationships among many reptiles in this region are not well known. The closely related genera of *Sonora* Baird and Girard 1853 and *Procinura* Cope 1879 are coralsnake mimics found from the central and western United States to southwestern Mexico and Baja California. Although species delimitation in this group has historically relied upon color pattern and other morphological characters, many populations of these species display color pattern polymorphism, which may confound taxonomy. We use molecular phylogenetics to assess the evolutionary relationships and delimit species within *Sonora*, focusing on the phylogenetic position of *Procinura* and the validity of *S. mutabilis* and *aequalis*. We sequenced two mitochondrial (*ND4* and *cytb*) and two nuclear (*c-mos* and *RAG-1*) genes for the single species of *Procinura* and each of the four species of *Sonora*. We analyzed these sequences using maximum parsimony, maximum likelihood, and Bayesian phylogenetic analyses on separately concatenated mitochondrial and nuclear datasets. Additionally, we used Bayesian coalescent methods to build a species tree (Bayesian species tree analysis) and delimit species boundaries (Bayesian species delimitation). All methods indicated that

Procinura is deeply nested within *Sonora*, and most individual species are well supported. However, we found that one taxon (*S. aequalis*) is paraphyletic with regard to another (*S. mutabilis*). We recommend that the genus *Procinura* be synonymized with *Sonora* and that *S. aequalis* be synonymized with *S. mutabilis.* Additionally, the phylogenetic patterns that we document are broadly congruent with a Miocene or Pliocene divergence between *S. michoacanensis* and *S. mutabilis* along the Trans-Mexican Volcanic Belt. Finally, our data are consistent with the early evolution of coralsnake mimicry and color pattern polymorphism within the genus *Sonora*.

2.2 Introduction

The country of Mexico is an extremely diverse region (Mittermeier et al. 2005), especially for squamate reptiles (Flores-Villela and Canseco-Márquez 2004). High endemism and species richness of this country has been explained by its complex landscape, geology, tropical latitude and ecological diversity(Peterson et al. 1993; Ramamoorthy et al. 1993; Flores-Villela and Gerez 1994). Despite this diversity (or perhaps because of it), genetic relationships of many squamate species in Mexico are unknown and their taxonomy is unstable. Contributing to this taxonomic uncertainty for squamate reptiles is variable and polymorphic color pattern, which can cause taxonomists to either assign multiple species designations within single polymorphic species or to lump geographically widespread species under a single "polymorphic" species. This leads to the potential for cryptic biodiversity and thus the systematics of such species complexes are a matter of high taxonomic priority.

The genus *Sonora* Baird and Girard 1853 is one lineage of snakes that is relatively poorly known and displays striking color pattern polymorphism. Members of *Sonora* are small, arthropod-consuming, semifossorial snakes that are found in the central and western United States to southwestern Mexico and Baja California (Figure 2.1; Stickel 1943; Ernst and Ernst 2003). These snakes are normally placed in the colubrid tribe Sonorini with the genera *Chilomensiscus, Chionactis, Conopsis, Ficimia, Gyalopion, Pseudoficimia, Stennorrhina*, and *Sympholis* (Dowling 1975; Dowling and Duellman 1978), although some authors include *Tantilla* and *Geagras*, and by extension *Tantillita* and *Scolecophis* (Savitzky 1983; Greene 1997). However, some authors have questioned the traditional Sonorini based upon molecular and morphological data (Holm 2008; Goynechea 2009).

There are five species that have recently been included in the genus *Sonora* (Echternacht 1973; Ernst and Ernst 2003; Ponce-Campos et al. 2004). *Sonora semiannulata* Baird and Girard 1853 is found in the central and western United States and northern Mexico. *Procinura aemula* Cope 1879 was until recently (Lemos-Espinal et al. 2004a; Lemos-Espinal et al. 2004b; Lemos-Espinal et al. 2004c) included in the genus *Sonora* (Bogert and Oliver 1945; Zweifel and Norris 1955; Nickerson and Heringhi 1966) and is found in western Mexico in the states of Chihuahua, Sonora and Sinaloa (Figure 2.2). *Sonora mutabilis* Stickel 1943 and *S. aequalis* Smith and Taylor 1945 are found mostly sympatrically in the foothills of the Sierra Madre Occidental in Jalisco, Nayarit, Aguascalientes, southern Zacatecas and extreme southern Sinaloa (Fig. 2.2). *Sonora michoacanensis* Duges in Cope (1885) is currently known from the Balsas

basin of Michoacan, Guerrero, Morelos, Puebla and Colima and the coastal regions of Colima and Guerrero (Figure 2.2). Notably, all species possess color pattern polymorphism, with uniform, striped, banded, bicolor and tricolor morphs known for the different species (Figure 2.1). Herein, we focus on the exclusively Mexican species of *P. aemula, S. mutabilis, S. michoacanensis*, and *S. aequalis*.

Taxonomic confusion has reigned in the exclusively Mexican species of *Sonora* and *Procinura*. While the validity of the species *P. aemula* is not generally questioned, this species was recently placed in the monotypic genus *Procinura* on the basis of its unusual caudal morphology, a "file-like" tail (Lemos-Espinal et al. 2004a; Lemos-Espinal et al. 2004b; Lemos-Espinal et al. 2004c). However, a phylogenetic analysis was not undertaken at the time of the genus re-elevation, and so the reciprocal monophyly of *Procinura* and *Sonora* is not established. The three species of *Sonora* (*S. aequalis, S. michoacanensis, S. mutabilis*) from southern and western Mexico have been at various times considered a single species with up to two subspecies of *S. michoacanensis michoacanensis* and *S. m. Mutabilis* (Stickel 1943; Echternacht 1973) or up to three species including *S. erythrura*, *S. mutabilis*, and *S. michoacanensis* (Taylor 1937; Smith and Taylor 1945) Most recently, Ponce-Campos et al. (2004) elevated *S. michoacanensis michoacanensis* and *S. m. mutabilis* to full species based on color pattern, and resurrected the name *S. aequalis* for bicolor ground snakes formerly included under *S. mutabilis*.

One reason for the unstable taxonomy of Mexican *Sonora* is their extreme color pattern polymorphism (Figure 2.1). *Procinura aemula* is considered a coralsnake

mimic (Echternacht 1973; Campbell and Lamar 2004b)and possesses morphs that are uniform red or tricolor, monadal or triadal with a varying number of triads (Nickerson and Heringhi 1966) . According to current taxonomy, *S. mutabilis* is tricolored and *S. aequalis* is bicolored(Ponce-Campos et al. 2004), with both considered coralsnake mimics (Echternacht 1973; Campbell and Lamar 2004b). Finally, *S. michoacanensis* is also considered a coralsnake mimic (Echternacht 1973; Campbell and Lamar 2004b) and possesses uniform red and tricolor morphs (some of the bands on tricolored animals may appear as white dots with a black centre). These three species are currently distinguished based solely on color pattern; *S. mutabilis* is tricolored, *S. aequalis* is bicolored, and *S. michoacanensis* can be distinguished from *S. aequalis* and *S. mutabilis* by the absence of banding on its tail. Given that color pattern polymorphism is documented within all members of the genera *Sonora* and *Procinura* and is a well-known characteristic of mimicry complexes(Echternacht 1973; Mallet and Joron 1999; Brodie and Brodie 2004), taxonomy based solely on color pattern in coralsnake mimics may be deceptive.

With current taxonomy based on color pattern, a revision of the genera *Sonora* and *Procinura* based upon more appropriate characters is necessary. Morphological characters such as scale counts and color pattern have traditionally been used in snake systematics, but may suffer from problems of homoplasy and environmentally induced variation (e.g. Burbrink et al. 2000; Devitt et al. 2008) especially because many snake genera such as *Sonora* are morphologically conservative. We use a molecular approach to evaluate the phylogenetic relationships of the genera *Sonora* and *Procinura*.

Our goals are to use both mitochondrial and nuclear loci to: 1) determine the number of distinct genetic lineages of the genera *Sonora* and *Procinura* in western Mexico, 2) determine the phylogenetic relationships among the different species of the genera *Sonora* and *Procinura*, and 3) assess the match between current taxonomy and molecular phylogeny of the genera *Sonora* and *Procinura*. Based upon the results of this analysis, we make taxonomic recommendations for this group and discuss morphology in the context of this taxonomy.

2.3 Materials and Methods

2.3.1 Taxonomic sampling

We obtained at least one tissue for *P. aemula* and *S. aequalis, S. michoacanensis, S. mutabilis,* and *S. semiannulata* during fieldwork (2001–2009) and/or from museum collections (Figure 2.2; Table 2.1). We also obtained one sequence for *P. aemula* from an unpublished dissertation (Holm 2008). Specimens and photos were deposited in the University of Texas at Arlington Amphibian and Reptile Diversity Research Centre and Digital Collection (UTA ARDRC and UTA ARDRC DC) and the Museo de Zoología, Facultad de Ciencias (MZFC). We chose to use a hierarchical outgroup scheme to test the monophyly of the ingroup, using *Coluber constrictor,* a closely related member of the subfamily (Colubrinae) containing *Sonora* and *Procinura* (Pyron et al. 2011) and *Agkistrodon contortrix*, a member of the family Viperidae.

2.3.2 Molecular methods

Muscle, liver and skin tissue was taken from freshly killed specimens and stored in 95% ethanol or tissue lysis buffer at -80° C. Genomic DNA was extracted from tissues using

the DNAeasy Blood and Tissue Kit (Qiagen) using standard protocol. We chose to amplify two separate mitochondrial loci, a partial fragment (639 bp) of cytochrome b (*cyt-b*) and a fragment (777 bp) containing part of NADH dehydrogenase subunit 4 (*ND4*) including complete RNA^{His} and complete and partial $tRNA^{Ser(AGY)}$ (Table 2.2) using primers modified from previous studies (Arevalo *et al.*, 1994; Harvey *et al.*, 2000). We also amplified two nuclear genes, a partial fragment (997 bp) of the recombination activating gene 1 (*RAG-1*) and a fragment (546 bp) of the oocyte maturation factor (*c-mos*; Table 2.2). *Cyt-b* and *ND4* were both amplified using polymerase chain reaction (PCR) under the following thermocycling protocol: Initial denaturation at 94°C for 3 min, then 35 cycles of denaturation for 30 sec at 94°C, annealing for 45 sec at 55°C, and extension for 90 sec at 72°C, followed by a final extension at 72°C for 10 min. *RAG-1* and *cmos* were amplified using the same PCR protocol as the mitochondrial genes, except that the annealing temperature was 58°C. Successful amplification was determined by gel electrophoresis of the PCR product along a 1% agarose gel, and PCR products were prepared for the sequencing reaction by using the ExoSAP-IT kit (United States Biochemical). We used the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems Inc.) following the manufacturer's protocol. The sequenced products were precipitated using an ethanol/sodium acetate method and rehydrated in HPLC purified formamide (Hi-Di). The sample was then analyzed either on a ABI PRISM 3100xl Genetic Analyzer in the Genomics Core Facility at the University of Texas-Arlington or on a ABI 3730 Genetic Analyzer at the Museum of Vertebrate Zoology at the University of California, Berkeley. Sequences were edited

and assembled using Sequencher (Genes Code Corps., Inc.). Individual sequences were exported to MEGA (Tamura et al. 2011), aligned in MEGA using the CLUSTAL algorithm (Larkin et al. 2007) with default parameters and manually adjusted if necessary.

2.3.3 Concatenated sequence analysis

We assembled concatenated mitochondrial (*cyt-b*, *ND4*, and tRNAs) and nuclear (*cmos* and *RAG-1*) datasets for maximum parsimony (MP), maximum likelihood (ML) and Bayesian analyses. Phylogenetic analysis using the MP criterion was implemented for separately concatenated mitochondrial and nuclear datasets in MEGA (Tamura et al. 2011) with nodal support assessed by 1000 bootstrap replicates. For maximum likelihood and Bayesian phylogenetic analysis we used four separate partitioning schemes. Both mitochondrial and nuclear datasets were 1) unpartitioned, 2) partitioned by gene or gene region, 3) partitioned by gene region and two codon partitions for protein encoding genes (the first two codon positions partitioned separately from the last codon position) and 4) partitioned by gene and three codon partitions (one for each codon position). The best-fitting model of molecular evolution for each gene was determined using MEGA (Tamura et al. 2011), with models ranked by Bayes factors. Maximum likelihood phylogenetic reconstruction was implemented in RaxML (Stamatakis 2006) with 100 independent searches using the GTRGAMMA (GTR+G) model. Nodal support for the best scoring ML tree was bootstrap proportions from 1000 pseudoreplicates. Bayesian phylogenetic reconstruction was completed in MrBayes v 3.1 (Huelsenbeck and Ronquist 2001). The HKY+G model of evolution

was used for both nuclear and mitochondrial datasets. Excepting a variable rate prior, we used the default parameters in MrBayes (Huelsenbeck and Ronquist 2001). Markov-chain Monte-Carlo searches were run for 1,000,000 generations sampling trees every 100 generations with 4 chains (3 heated chains and one cold chain). We considered that the Bayesian searches had converged when the average standard deviation of split frequencies declined to below 0.01 and by examining log-likelihood versus generation plots. Additionally, we used the online program AWTY (Wilgenbusch et al. 2004) to confirm that our analyses reached stationarity. When the runs were completed, we discarded the first 25% of trees as burnin. Bayesian posterior probabilities were used to assess nodal support in the Bayesian analysis. Trees from all analyses were visualized and manipulated using FigTree v1.3.1 (Rambaut 2007).

2.3.4 Species tree analysis and Bayesian species delimitation

We conducted a species tree analysis to provide a guide tree for species delimitation analyses. Although species-tree coalescent methodology is most appropriate when applied to datasets with multiple individuals for each species, the focus of these analyses is the genetic distinctness of *S. aequalis* and *S. mutabilis* for which we have multiple samples. We used the program *BEAST (Heled and Drummond 2010) in the BEAST software package (Drummond and Rambaut 2007) to estimate a species tree from our four separate loci (*ND4*+tRNA's, *cyt-b*, *c-mos* and *RAG-1*). For the species tree we initially assigned taxa to *P. aemula, S. aequalis, S. michoacanensis, S. mutabilis* and *S. semiannulata*. We generated species trees with unpartitioned data and the first two codon positions partitioned separately from the last, with separate models of

molecular evolution for each gene (*cmos*= HKY, *cyt-b*=HKY+G, *ND4*=HKY+I, *RAG-1*=HKY+G) determined by model selection using the Bayesian Information criterion in MEGA (Tamura et al. 2011). The approximately 125 bp of tRNAs in *ND4* was trimmed prior to analysis. We considered the default priors in *BEAST (Heled and Drummond 2010) to be appropriate for our analysis, although for each partitioning scheme we varied the tree prior (Yule process or birth-death process). We used searches of 10 million generations (with trees sampled every 1000 generations) for two independent runs, and burned in 50% of runs. Data were combined using LogCombiner. Nodal support for the resulting species tree was posterior probabilities and was mapped onto the tree using TreeAnnotator.

We used the species tree from the species tree analysis as a guide tree for Bayesian species delimitation (focused on *S. aequalis* and *S. mutabilis*). We used the program BPP v2.1 (Yang and Rannala 2010), which uses reverse jump Markov-Chain Monte Carlo (rjMCMC) to infer the posterior probabilities of a fully resolved guide tree and each partially or completely collapsed version of the guide tree, but see Leache and Fujita (2010)and Yang and Rannala (2010) for details. For our guide tree, we used the species tree generated by *BEAST (all partitioning schemes and prior sets yielded the same topology). Initially, we varied the fine-tuning parameter and starting seeds, and conducted analyses for 100,000-500,000 generations to ensure homogeneity of results. Final analyses were conducted for 100,000 generations, sampled every 10 and burned in the first 50% of trees. The fine-tuning parameters and algorithms for rjMCMC mixing were set to give consistent results and were similar to those in Leache and Fujita (2010)

with all speciation models given equal priors. Additionally, we used the same three prior sets as in Leache and Fujita (2010) for ancestral population size (θ) and root age (τ). We set both θ and τ to a gamma distribution, initially with 1) G (α, β) ~ G (1, 10) for both θ and τ. Two other prior combinations were also used, 2) G (2,2000) for both θ and τ and 3) θ ~ G (1,10) and τ ~ G (2,2000). Acceptance proportions for each parameter were within the recommended range (0.3–0.7) for Bayesian species delimitation (Yang and Rannala 2010). Support for species was assessed as Bayesian speciation probabilities for each node, which is different from Bayesian posterior probability nodal support which indicates the probability a clade is true and presumably monophyletic (Huelsenbeck et al. 2002) in that it indicates a probability ("Bayesian speciation probability, BSP") that a node is fully resolved or fully bifurcated.

2.3.5 Morphological analysis

We collated morphological data from Echternacht (1973) including data originally from Stickel (1943) for one *S. aequalis,* 18 *S. michoacanensis,* and eight *S. mutabilis* and measured the same traits on eight additional specimens (Table 2.3). We also collected additional color pattern data for species diagnosis information from museum specimens that were mentioned but not illustrated in Echternacht (1973) or Stickel (1943). Length measurements were taken to the nearest mm using digital callipers, and Jacobo Reyes-Velasco conducted all morphological measurements. We also studied the hemipenial morphology of three specimens of *S. mutabilis,* and compare it to that of *S. michoacanensis*. We followed the standard procedures to prepare hemipenes as

suggested by Myers and Cadle (2003) and Zaher and Prudente (2003). Morphological definitions are based on Dowling and Savage (1960).

2.4 Results

2.4.1 Concatenated analyses

Bayesian, maximum likelihood, and maximum parsimony phylogenetic analyses all yielded similar topologies for both nuclear and mitochondrial datasets. Similarly, all gene and codon partitioning schemes yielded similar topologies in both Bayesian and maximum likelihood analyses with both datasets. Because we prefer to present an optimal tree, we elected to include the best maximum likelihood tree for both mitochondrial and nuclear datasets (partitioned by gene and first two codon positions partitioned separately from the third) with nodal support assessed as Bayesian posterior probabilities (BPP), maximum likelihood bootstrap proportions and maximum parsimony bootstrap proportions (Figure 2.3). Phylogenetic trees from both the mitochondrial and nuclear datasets recover *Sonora+Procinura* as a monophyletic group (BPP=1.0), with maximum uncorrected pairwise sequence divergence of 18% and 0.8% for the mitochondrial and nuclear dataset, respectively. The mitochondrial dataset (Figure 2.3) recovers a southern clade (S. *mutabilis, S. aequalis* and *S. michoacanensis*) and a northern clade (*S. semiannulata* and *P. aemula*) separated by 15.5% mitochondrial uncorrected sequence divergence (BPP=1.0). In contrast, *S. michoacanensis* is recovered as sister to the *S. semiannulata/ P. aemula* clade (BPP=0.71) in the phylogenetic tree based on nuclear loci (Figure 2.3). Both

mitochondrial and nuclear datasets find *Procinura* nested within *Sonora* (BPPs=1.0 and 0.99), sister to *S. semiannulata* (Figure 2.3). Additionally, both nuclear and mitochondrial phylogenetic trees indicate that *S. aequalis* is paraphyletic to *S. mutabilis* (Figure 2.3) and recover *S. michoacanensis* as being quite divergent (12.5% in the mitochondrial data) from *S. mutabilis* and *S. aequalis* (Figure 2.3). The mitochondrial phylogenetic tree displays limited geographic structuring within clades, with *S. aequalis* and *S. mutabilis* clustering by locality (not taxonomy, Figure 2.3).

2.4.2 Species tree and Bayesian species delimitation analyses

Tree prior and codon partitioning combinations for the species tree analyses resulted in very similar topologies, so we present the partitioned dataset using a Yule process tree prior with nodal support of Bayesian posterior probabilities. The coalescent analysis largely agreed with the concatenated dataset analyses (Figure 2.4). In agreement with the mitochondrial dataset, a southern clade (S. *mutabilis, S. aequalis* and *S. michoacanensis*) and a northern clade (*S. semiannulata* and *P. aemula*) are well supported (Figure 2.4; BPP=1.0). *Procinura* is deeply nested within *Sonora*, sister to *S. semiannulata*. *Sonora aequalis* and *S. mutabilis* are recovered as a monophyletic group (but with almost no sequence divergence; BPP=1.0) and are sister to *S. michoacanensis* (Figure 2.4: BPP=1.0).

Bayesian species delimitation returned similar results for each prior set, and was mostly congruent with the other analyses (Figure 2.4). Generally, this analysis supported a topology that was resolved at all nodes except the *aequalis/mutabilis* node (Figure 2.4). The *P. aemula/S. semiannulata* node had mixed support (based upon prior

set), perhaps as the result of limited sampling for these two species (Figure 2.4). Nonetheless, these analyses demonstrate that *P. aemula* is nested within the currently recognized species of *Sonora*.

2.4.3 Morphological analysis

Hemipenial and meristic scale characters were mostly overlapping between *S. aequalis*, *S. michoacanensis*, and *S. mutabilis* (Table 2.3). *Sonora aequalis* possessed overlapping but somewhat higher number of temporal scales than *S. michoacanensis* or *S. mutabilis*. The only consistent morphological difference between *S. michoacanensis* and *S. mutabilis/aequalis* is the complete banding on the tail of *S. mutabilis/aequalis* and the lack of banding on the tail of *S. michoacanensis* (Table 2.3).

2.4.4 Species diagnoses

Below we provide species accounts for *S. aemula*, *S. michoacanensis* and *S. mutabilis*. We refrain from presenting a species account for *S. semiannulata* due to our limited sampling from this geographically widespread species.

Sonora aemula (Cope, 1879)

Procinura aemula Cope (1879) Holotype: Academy of Natural Sciences in Philadelphia (ANSP) 11614 (Bogert and Oliver 1945). Type Locality: "Batopilas, Chihuahua" (Cope 1879).

Scolecophis aemulus—Amaral (1929)

Sonora aemula—Bogert and Oliver 1945

Sonora aemula—Zweifel and Norris 1955

Procinura aemula—Lemos-Espinal *et al*., (2004a)

Diagnosis: This species can be distinguished from both *S. michoacanensis* and *S. mutabilis* by the presence of distinctly raised tubercular scales or caudal spines (Figure 2.5) creating a "file-like" tail (Bogert and Oliver 1945).

Variation: This species is extremely variable in color pattern, ranging from a uniformly red to banded tricolored pattern (Bogert and Oliver 1945; Zweifel and Norris 1955; Nickerson and Heringhi 1966). In tricolored animals, the number and arrangement of triads can vary greatly (Bogert and Oliver 1945; Zweifel and Norris 1955; Nickerson and Heringhi 1966). A more detailed description of meristic characters and a hemipenial description are found in Bogert and Oliver (1945).

Distribution: This species is found on the Pacific versant of the Mexican states of Chihuahua, Sonora and Sinaloa (Figure 2.2).

Sonora michoacanensis Duges *in* (Cope 1885)

Contia michoacanensis Duges in Cope (1885). Holotype: Neotype British Museum of Natural History (BMNH), now the Natural History Museum, London (NHMUK) 1903.3.21, now 1946.1.14.65. The original holotype from the Museo Alfredo Dugès was lost (Stickel 1943); a specimen collected in Michoacan with no additional locality information was designated as neotype by Stickel (1943).Type locality: None given in Duges in Cope (1885). Neotype locality is given as "Michoacán" (Stickel 1943). Restricted to "Apatzingan, Michoacán" by Smith and Taylor (1950).

Elapomorphus michoacanensis—Cope (1895)

Homalocranium michoacanense—Gunther (1895)

Chionactis michoacanensis—Cope (1896)

Scolecophis michoacanensis—Boulenger (1896)

Sonora erythura—Taylor (1937)Holotype: University of Illinois Museum of Natural History (UIMNH) 25063. Type Locality: "16 km S of Taxco, Guerrero".

Sonora michoacanensis michoacanensis—Stickel 1943

Sonora michoacanensis—Ponce-Campos *et al*., 2004

Diagnosis: This species can be distinguished from *S. mutabilis* based on the almost invariable absence of banding on the tail, and from *S. aemula* based on the absence of a file-like tail (Figure 2.5). We note that one specimen from the University of Michigan Museum of Zoology (UMMZ 109904) has a single narrow band on the tail.

Variation: This species is extremely variable in color pattern, ranging from uniform red to banded tricolored pattern (Echternacht, 1973). In tricolored animals, the number of bands and shape of bands varies greatly (Echternacht, 1973). In some individuals, the black and yellow bands appear as black-bordered yellow spots (Figure 2.1). Morphological measurements and meristic characters are mostly overlapping between *S. mutabilis* and *S. michoacanensis* (Table 2.3). The hemipenis is depicted in Cope (Cope, 1895, Plate XXIX, Figure 6).

Distribution: This species is found on the Pacific coast and Balsas basin in the Mexican states of Colima, Guerrero, Michoacan, Morelos, and Puebla (Figure 2.2).

Sonora mutabilis Stickel 1943

Sonora michoacanensis mutabilis—Stickel 1943. Holotype: The holotype is in the Field Museum of Natural History (FMNH) 105257, with paratypes FMNH 105296, NHMUK 1946.1.14.63– NHMUK 1946.1.14.64 and American Museum of Natural

History (AMNH) 19714–19716 (Stickel 1943; Echternacht 1973). Type locality: "Magdalena, Jalisco" (Stickel 1943).

Sonora aequalis—Smith and Taylor 1945. Holotype: Museum of Comparative Zoology (MCZ) 6444. Type Locality: Originally given as "Matagalpa, Nicaragua" (Stickel 1943), later concluded to be "within or somewhat to the east of the ranges of *mutabilis* and *michoacanensis*, on the southern part of the Mexican plateau or in the surrounding mountains" (Stickel 1943; Echternacht 1973).

Sonora michoacanensis mutabilis—Echternacht 1973

Sonora aequalis—Ponce-Campos *et al*., 2004

Sonora mutabilis—Ponce-Campos *et al*., 2004

Diagnosis: Both bicolored (formerly *aequalis*) and tricolored forms of this species can be distinguished from *S. michoacanensis* based on complete banding on the tail and from *S. aemula* based on the absence of a file-like tail (Figure 2.5).

Variation: *Sonora mutabilis* possesses bicolored (red and black) and tricolored (red, black, and yellow) morphs (Echternacht 1973). In tricolor morphs, the extent of black interspaces between bands may be quite variable, and bands may have red dorsal or lateral inclusions (e.g., Figure 2.1). Bands may be regular, irregular, or absent ventrally. Morphological measurements and meristic characters are mostly overlapping between *S. mutabilis* and *S. michoacanensis* (Table 2.3). The hemipenis of *S. michoacanensis* was described by Stickel (1943). His description was based on one specimen of *S. michoacanensis* and one of *S. mutabilis.* Here we describe the hemipenis of *S. mutabilis* (Figure 2.6) and compare it to that of *S. michoacanensis* (Cope 1895). The

hemipenis is slightly bilobed, differentiated, and with a simple sulcus spermaticus. The apical lobes are covered with numerous papillated calyces; the papillae are so numerous and large that the calyces are nearly indiscernible. The papillae become enlarged towards the base of the calyces and grade into spines. The calyces cover 54% of the hemipenis in a specimen from Jalisco (UTAR-53487) and 38% of the hemipenis in a specimen from Plomosas, Sinaloa (UTAR-7227), and 39% in another bicolored specimen (formerly *S. aequalis*) from Jalisco (UTA R-59761). Approximately 45-60 hooked spines cover the surface between the base and the calyces; this area represents 28% of the hemipenis of UTA R-53487, 35% of UTA R-7227, and 31% of UTA R-59761. Two large basal hooks are found in all specimens. The basal area of the hemipenis is naked and this area comprises 19% of the hemipenis for UTA R-53487, 27% for UTA R-7227, and 29% for UTA R-59761. The everted hemipenis of UTA R-53487 is 6 subcaudals long, while that of UTA R-7227 and UTA R-59761 are 7 subcaudals *in situ*. The main difference between the hemipenis of *S. mutabilis* and *S. michoacanensis* is the size of the papillae in the apical region, being very large and abundant in *S. mutabilis,* to the point of making the calyces undistinguishable, while in *S. michoacanensis* the calyces are conspicuous.

Distribution: *Sonora mutabilis* is found in the Mexican states of Aguascalientes, Jalisco, Nayarit, southern Zacatecas and extreme southern Sinaloa (Figure 2.2).

2.5 Discussion

2.5.1 Taxonomic implications

We adhere to the evolutionary species (Wiley 1978) and general lineage (de Queiroz 1998) theoretical species concepts when evaluating the taxonomy of the genera *Sonora* and *Procinura,* and implement the focal-species approach of Schargel *et al.* (Schargel et al. 2010). We consider putative geographic barriers, and consider that ecological differentiation and morphological divergence represent additional evidence that lineages are valid species (i.e., Schargel et al. 2010). Our results have implications for both generic and species-level taxonomy for the genus *Sonora*. Both nuclear and mitochondrial datasets, and combined coalescent analyses recover *P. aemula* as sister to *S. semiannulata* (the type-species of the genus *Sonora*) and nested within the other *Sonora* species, rendering *Sonora* paraphyletic (BPPs > 0.99). In fact, many previous taxonomic treatments of *P. aemula* have considered this species to be within the genus *Sonora* (Bogert and Oliver 1945; Zweifel and Norris 1955), and it was only re-elevated to the monotypic genus *Procinura* (Lemos-Espinal et al. 2004a; Lemos-Espinal et al. 2004b; Lemos-Espinal et al. 2004c) based on a single morphological autapomorphy (the file-like caudal anatomy). We propose that *P. aemula* be returned to the genus *Sonora*, which renders *Sonora* monophyletic and accurately reflects the evolutionary history of this genus.

Our molecular analyses also indicate that *S. aequalis* and *S. mutabilis* are paraphyletic with regard to one another (BSPs<0.21). Specimens group genetically based upon locality, not color pattern, and so *S. aequalis* is best considered a bicolor

morph of *S. mutabilis* and not a valid species. *Sonora mutabilis* has taxonomic priority (Stickel, 1943), so we suggest that *S. aequalis* be placed in synonymy with *S. mutabilis* and that the species diagnosis for *S. mutabilis* revert to the diagnosis by Stickel (1943), with the inclusion of a bicolor morph. In contrast, the results of this study reveal a deep genetic divergence between *S. mutabilis* and *S. michoacanensis*. This genetic divergence is reflected in discontinuity in their respective geographic distribution. We concur with previous recommendations that both *S. mutabilis* and *S. michoacanensis* should be considered separate species(Stickel 1943; Echternacht 1973; Ponce-Campos et al. 2004) and suggest the species diagnosis for *S. michoacanensis* be as in Stickel (Stickel 1943). We note that the lack of banding on the tail of *S. michoacanensis* is a reliable morphological feature that can be used to distinguish it from *S. mutabilis* (Figure 2.5, Table 2.3). While color pattern variation is probably an underlying factor in the taxonomy uncertainty in *Sonora*, it is also useful as a field character for distinguishing *S. michoacanensis* from *S. mutabilis*. Besides the consistent differences in tail banding, *S. michoacanensis* is either uniform red or tricolored, with bands or saddles that vary in size and position. In contrast, *S. mutabilis* is either bicolored or tricolored with regularly shaped bands (e.g., Figure 2.1) and has no uniformly red morph. While color pattern polymorphism is easier to interpret in the context of a molecular phylogeny, prior generations of herpetologists reached the same taxonomical conclusions as our study based on careful assessment of morphology, including color pattern(Bogert and Oliver 1945; Zweifel and Norris 1955; Echternacht 1973).

Although our study focused on Mexican *Sonora* (mostly *S. michoacanensis* and *S. mutabilis*), there is still great need for molecular and taxonomic reviews of some of the other *Sonora* species and related taxa. *S. semiannulata* was only represented by a single specimen in this study, and so we cannot comment on either the biogeography or taxonomy of this taxon. Because *S. semiannulata* is 1) morphologically distinct from other *Sonora* species, and 2) has a non-overlapping geographic range with other *Sonora* species, inclusion of additional *S. semiannulata* specimens should not change the conclusions of this study. Our study did not include the genera *Chionactis* and *Chilomeniscus*, which are hypothesized to be close relatives of Sonora (Dowling 1975; Dowling and Duellman 1978), with *Chionactis* at one time considered synonymous with *Sonora* (Stickel 1938; Stickel 1943). Multiples species and subspecies have been recognized for both of these genera (Ernst and Ernst 2003), and evaluating the taxonomy and molecular systematics of these genera was beyond the scope of this study. A complete molecular evaluation of all species and subspecies of *Chionactis*, *Chilomeniscus*, and *S. semiannulata* is needed to clarify the complex biogeographic history and taxonomic nomenclature of this group.

2.5.2 Methodological congruence

We found marked differences in rates of molecular evolution between mitochondrial and nuclear loci. Maximum pairwise divergence within *Sonora* varied by two orders of magnitude (from 0.8% uncorrected divergence for nuclear loci compared to 18% for mitochondrial loci) for nuclear (*c-mos, RAG-1*) and mitochondrial loci (*cyt-b, ND4*) commonly used in snake systematics(Burbrink et al. 2000; Townsend et al. 2004;

Noonan and Chippindale 2006; Vidal and Hedges 2009). Rate variation between nuclear and mitochondrial loci is well known (Vawter and Brown 1986; Hare 2001) and often causes incomplete lineage sorting in nuclear loci(Madison and Knowles 2006; Makowsky et al. 2010). Yet despite great differences in rates of evolution, separate mitochondrial and nuclear phylogenetic analyses supported very similar topologies (Figure 2.3; except for the phylogenetic position of *S. michoacanensis*). These results demonstrate the potential for rate heterogeneity between snake clades and between mitochondrial and nuclear genomes.

In addition to traditional analytical methods (maximum parsimony, maximum likelihood and Bayesian phylogenetic analysis), we used coalescent-based species tree analyses within a Bayesian framework and Bayesian species delimitation. Generally, each method supported the same taxonomy and evolutionary relationships among focal taxa. All methods supported the monophyly of *Sonora* + *Procinura*, the nesting of *Sonora* (formerly *Procinura*) *aemula* within the genus *Sonora*, and the distinctness of *S. michoacanensis* (BPPs>0.98). None of the methods supported the genetic distinctness of *S. mutabilis* (formerly *aequalis*) and *S. mutabilis* (BSPs<0.21). We obtained inconsistent results for one relationship (between *S. aemula* and *S. semiannulata*) with Bayesian species delimitation analysis (BSPs from 0.49-0.83), which is sensitive to prior conditions (Yang and Rannala 2010). The resolution of this node received some support with high θ and τ parameters, but was not supported with the other two prior conditions with lower θ and τ parameters. Given that the validity of *S. aemula* and *S. semiannulata* is well supported by multiple lines of evidence (Stickel 1938; Bogert and

Oliver 1945; this study), we suspect that this mixed support was due to our very limited sampling of both of these species. In fact, both species tree analyses and Bayesian species delimitation use coalescent methodology that are more appropriate for studies with greater molecular and specimen sampling (Knowles and Kubatko 2010; Leache and Fujita 2010; Yang and Rannala 2010). Nonetheless, all methodologies consistently recover key relationships among focal taxa, suggesting that coalescent methods may be somewhat robust to limited sampling (Burbrink et al. 2011; Leache and Rannala 2011), at least if focal taxa are very genetically distinct.

2.5.3 Historical biogeography

Phylogenetic relationships among Mexican *Sonora* species are generally consistent with the biogeographic patterns documented in many other Mexican vertebrates. In the south, *S. michoacanensis* and *S. mutabilis* are separated by the Trans-Mexican Volcanic Belt, which has been implicated in biogeographic breaks in other snakes(Devitt et al. 2008; Bryson et al. 2011), anurans(Mulcahy and Mendelson 2000; Greenbaum et al. 2011), fish (Mateos 2005), and many other taxa (Ferrusquia-Villafranca 2007). We note that although the uplift of the Trans-Mexican Volcanic Belt has been implicated in these biogeographic patterns, they could also arise from geographic features associated with this uplift, including the closing and aridification of the Balsas Basin (Gómez-Tuena and Carrasco-Núñez 2000; Ruiz-Martinez et al. 2000). Although we lacked appropriate data for detailed divergence analyses, our results (12.5% uncorrected mitochondrial sequence divergence between *S. mutabilis* and *S. michoacanensis*) are consistent with a Pliocene or Miocene divergence between these two species given the potential for an

accelerated rate of mitochondrial evolution in snakes (Mateos 2005; Jiang et al. 2007; Bryson et al. 2011). This temporal framework is broadly consistent with the diversification in other Mexican fauna (Mulcahy and Mendelson 2000; Mateos 2005; Devitt et al. 2008; Greenbaum et al. 2011). Highland diversification is thought to be a major driver of species richness of vertebrates in Mexico(Demastes et al. 2002; Jaeger et al. 2005; Riddle and Hafner 2006; Bryson et al. 2011). Our data may support that hypothesis within *S. mutabilis*, with the specimens from Bolaños, Jalisco forming a moderately (1.8% uncorrected sequence distance) divergent mitochondrial clade. Finally, our data are structured latitudinally, with most analyses (BPPs>0.98) supporting a southern clade (*S. mutabilis* and *S. michoacanensis*) and a northern clade *(S. aemula* and *S. semiannulata*). While greater geographic sampling is necessary for *S. aemula* and *S. semiannulata*, many other Mexican species with latitudinally structured phylogenies show evidence for northern range expansion from the southern and central highlands of Mexico into central North America(Savage 1982; Mulcahy and Mendelson 2000; Mateos 2005) and a similar pattern in *Sonora* would be unsurprising.

2.5.4 Evolution of color pattern in the genus Sonora

All Mexican *Sonora* are thought to be coralsnake mimics (Campbell and Lamar 2004b), and it is likely that red and black coloration in *S. semiannulata* has evolved in the context of mimicry given the probable Mesoamerican origin of the genus (Savage 1982). Additionally, each of the currently recognized species of *Sonora* contains populations that have color pattern polymorphism (Figure 2.1). Both *S. michoacanensis* and *S. aemula* are either uniform red or tricolored, with variation in the shape,

arrangement, and number of bands (Figure 2.1; Echternacht 1973). In contrast, *S. mutabilis* has bicolor (red/orange and black banded) or tricolor morphs. The most northern distributed member of the genus (*S. semiannulata*) displays the most extreme color pattern polymorphism, with individuals that are plain, red-striped, darkly banded, or both banded and red-striped (Ernst and Ernst 2003). The phylogenetic distribution of color pattern polymorphism in these coralsnake mimics may support the ubiquity of color pattern polymorphism in mimicry complexes (Mallet and Joron 1999; Brodie and Brodie 2004; Kunte 2009).

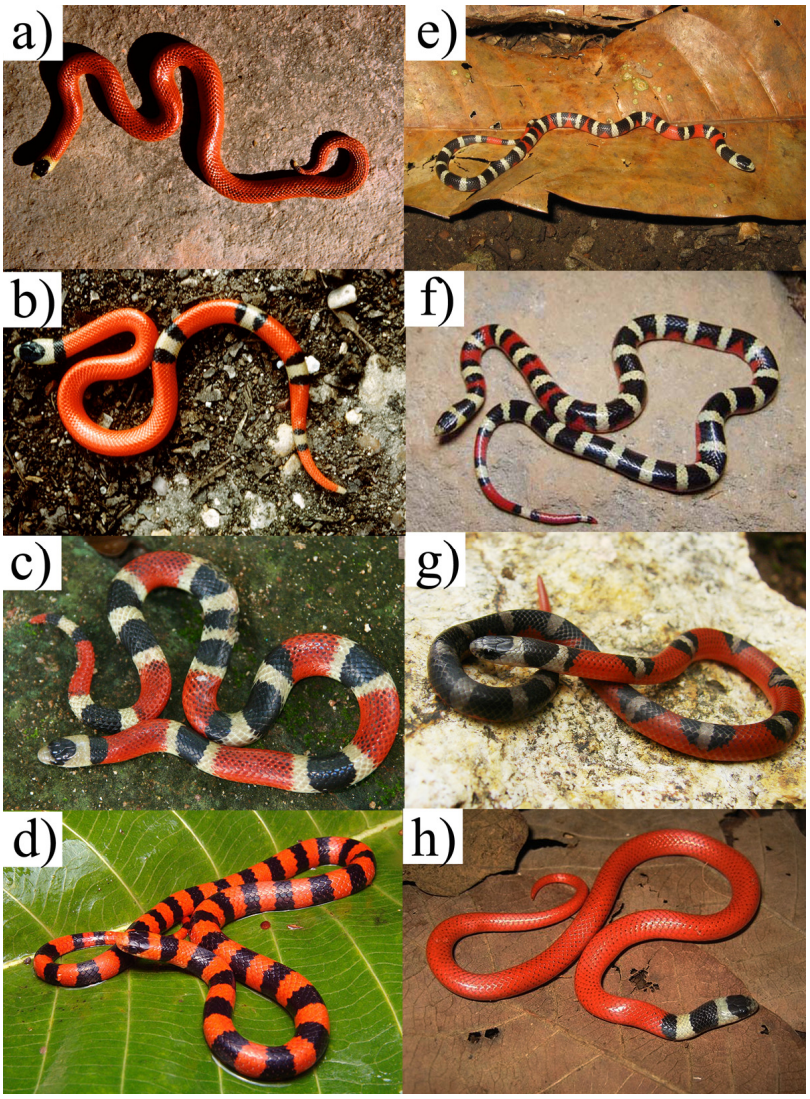

Figure 2.1. Snakes of the genus *Sonora* found exclusively in Mexico. Images deposited in the University of Texas-Arlington Digital collection (UTADC). (1) Uniform morph of *Sonora* (*Procinura*) *aemula* from near Rio Cuchojaqui, Sonora (Photo by C. M. Bogert, UTADC 7405); (2) *S. aemula* from Rio Cuchojaqui with a few bands (Photo by C. M. Bogert, UTADC 7406); (3) tricolor morph of *S. aemula* from near Alamos, Sonora (Photo by C. Rodriguez, UTADC 7407); (4) bicolor *S. mutabilis* from near Guadalajara, Jalisco (*aequalis*; Photo by C. Grunwald, UTADC 7408); (5) tricolor *S. mutabilis* from near Rio Blanco, Jalisco (Photo by C. L. Cox, UTADC 7409); (6) tricolor *S. mutabilis* from Rio Blanco, Jalisco (Photo by J.Reyes-Velasco, UTADC 7410) (7) tricolor *S. michoacanensis* from near Arcelia, Guerrero (Photo by A. Mendoza, UTADC 7411); (8) uniform morph of *S. michoacanensis* from near Tacambaro, Michoacan (Photo by O. Medina-Aguilar, UTADC 7412).

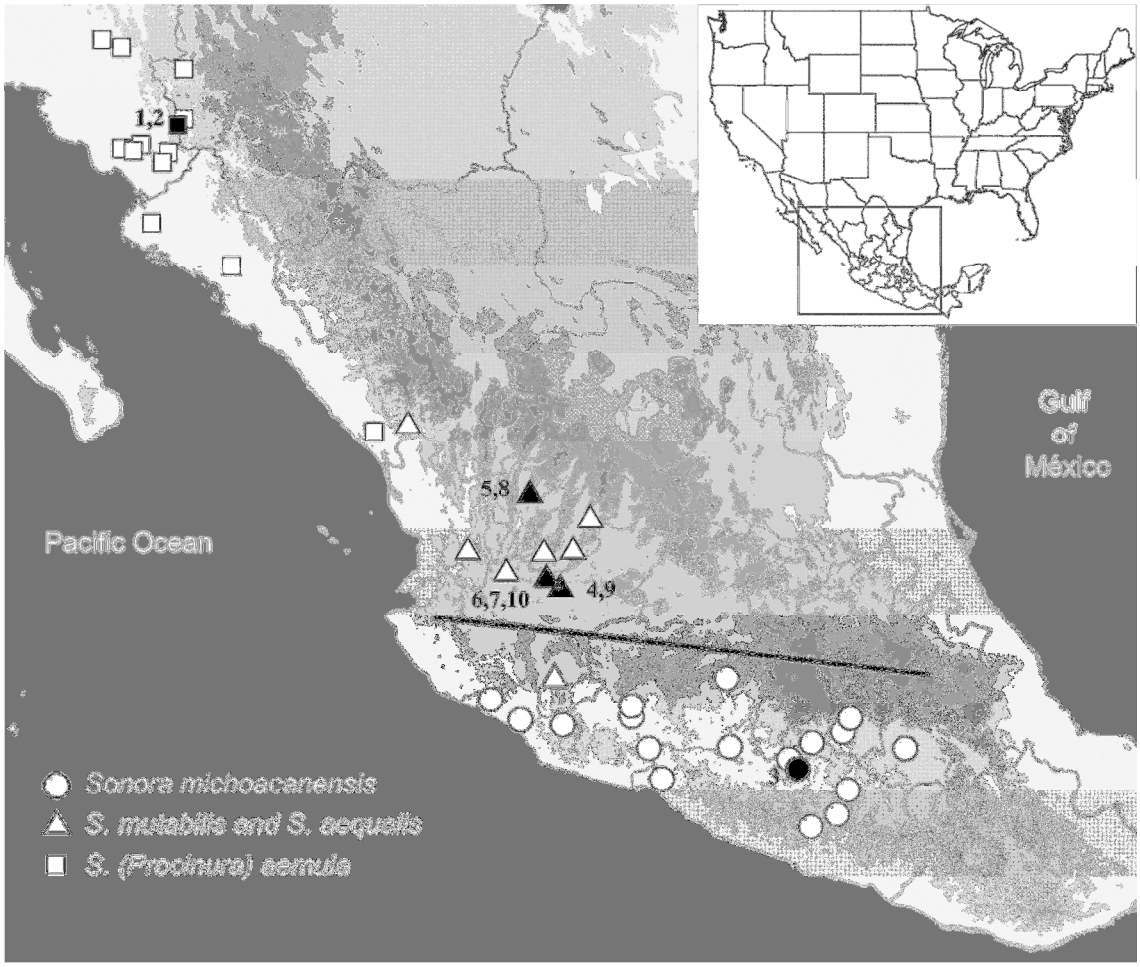

Figure 2.2. Map of specimen localities for snakes of the genus *Sonora* found exclusively in Mexico. Inset displays the geographic context of the map. Filled symbols represent localities with the tissue samples that are used in this study, and numbers next to symbols indicate localities from Table 1. Elevation is indicated on the map using shaded areas, with sea level represented by white and shaded areas in dark grey to a maximum of 5636 m. The approximate position of the Trans-Mexican Volcanic Belt is indicated with a solid line.

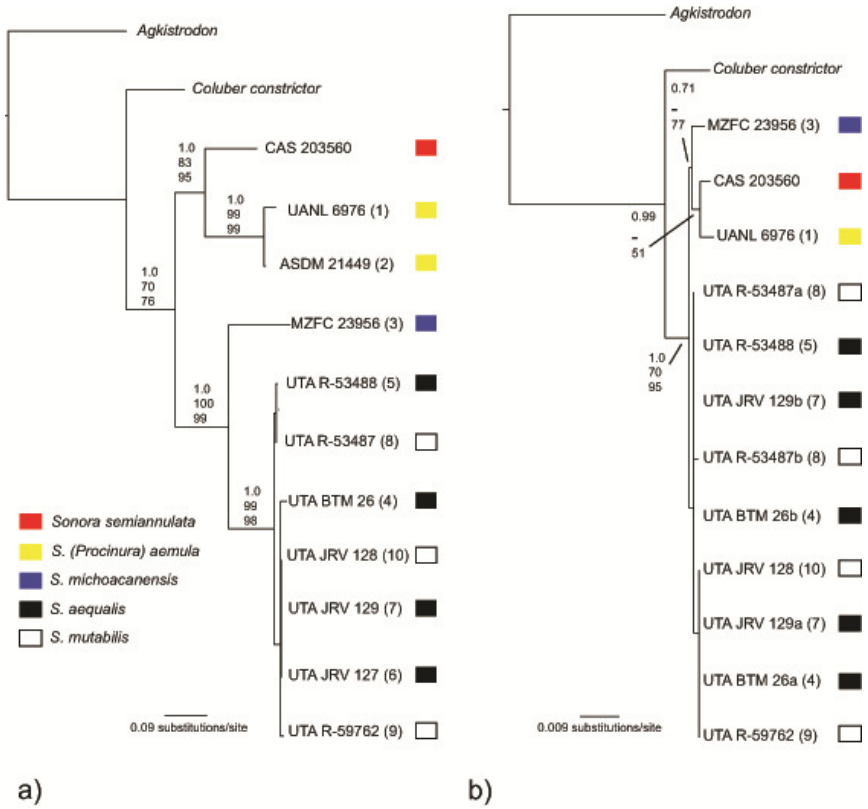

Figure 2.3. Phylogenetic relationships among *Sonora* based upon mitochondrial and nuclear data. Maximum likelihood phylogenetic tree of relationships among *Sonora* and *Procinura* species using (10) a concatenated mitochondrial dataset (*ND4* and *cyt-b*) and (11) a concatenated nuclear dataset (*c-mos* and *RAG-1*). Numbers in symbols next to specimen numbers correspond to localities in Table 1 and Figure 2.2. In the Figure 2.3a , a lower case letter after each specimen name indicates the phase for phased heterozygous individuals. Support values for nodes are Bayesian posterior probability (top), bootstrap proportions from maximum likelihood analysis (middle) and bootstrap proportions (1000 pseudoreplicates) from maximum parsimony analysis (bottom) > 50 (maximum likelihood and maximum parsimony) or 0.8 (Bayesian posterior probability). A dash (-) denotes support lower than the cutoff value for maximum likelihood or maximum parsimony. On the phylogenetic tree derived from nuclear loci, lower case letters nest to specimen numbers represent gametic phases. Note that for both datasets, *Procinura* is deeply nested within *Sonora*, and that *S. aequalis* is paraphyletic with regard to *S. mutabilis*.

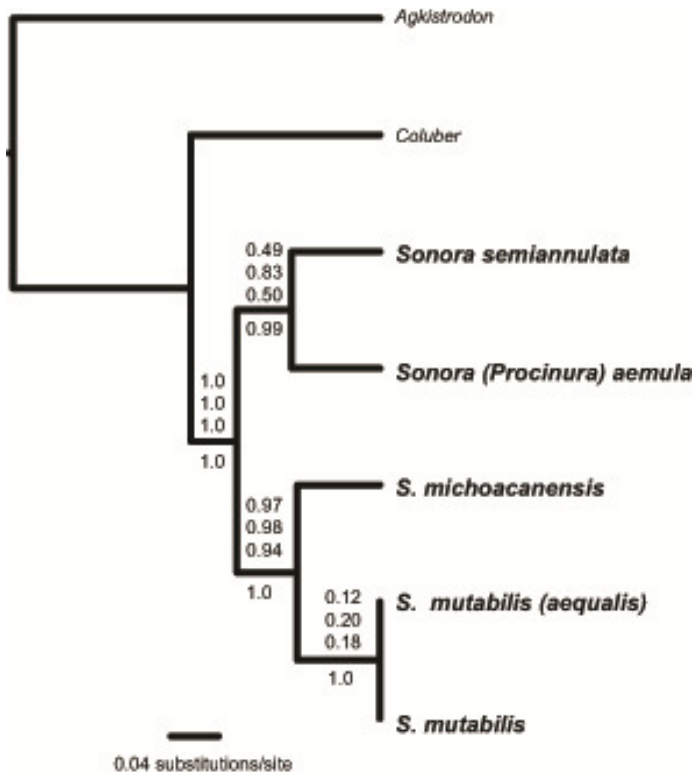

Figure 2.4. Species tree of *Sonora* and *Procinura* based upon four genes (*ND4, cyt-b, c-mos, RAG-1*). Tree is annotated with recommended taxonomic nomenclature (previous nomenclature in parentheses). Support values above the node are speciation probabilities from the Bayesian species delimitation analysis, which represents the probability that a node is fully resolved (or fully bifurcates). The top value represents the probability from prior set 1 (G [1,10] for both $\boldsymbol{\theta}$ and $\boldsymbol{\tau}$), the middle value is from prior set 2 (G [2, 2000] for both $\boldsymbol{\theta}$ and $\boldsymbol{\tau}$), and the bottom value from prior set 3 (G [1, 10] for $\boldsymbol{\theta}$ and G [2,2000] for $\boldsymbol{\tau}$). The support value below the node is the Bayesian posterior probability of that node from the species tree analysis.

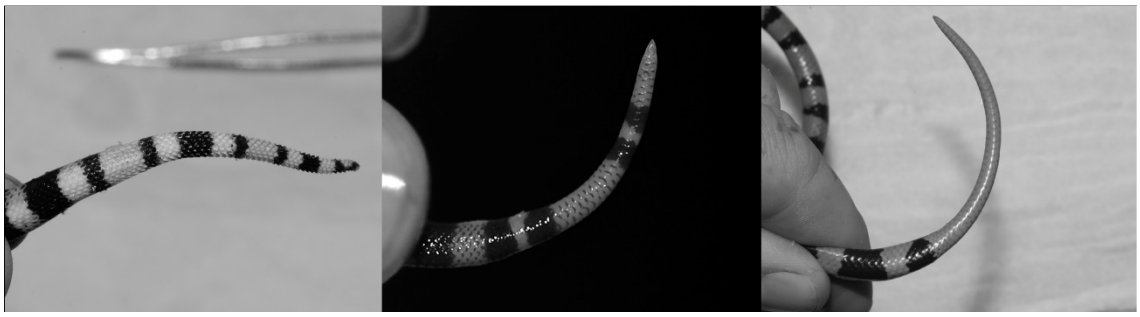

Figure 2.5. Differences in tail morphology among *Sonora*. Comparison of tail morphology for *Sonora aemula* (left, UAZ 45675, note caudal spines), *S. mutabilis* (centre, KU 23791, note banding on tail) and *S. michoacanensis* (right, MVZ 71356, note lack of banding on tail).

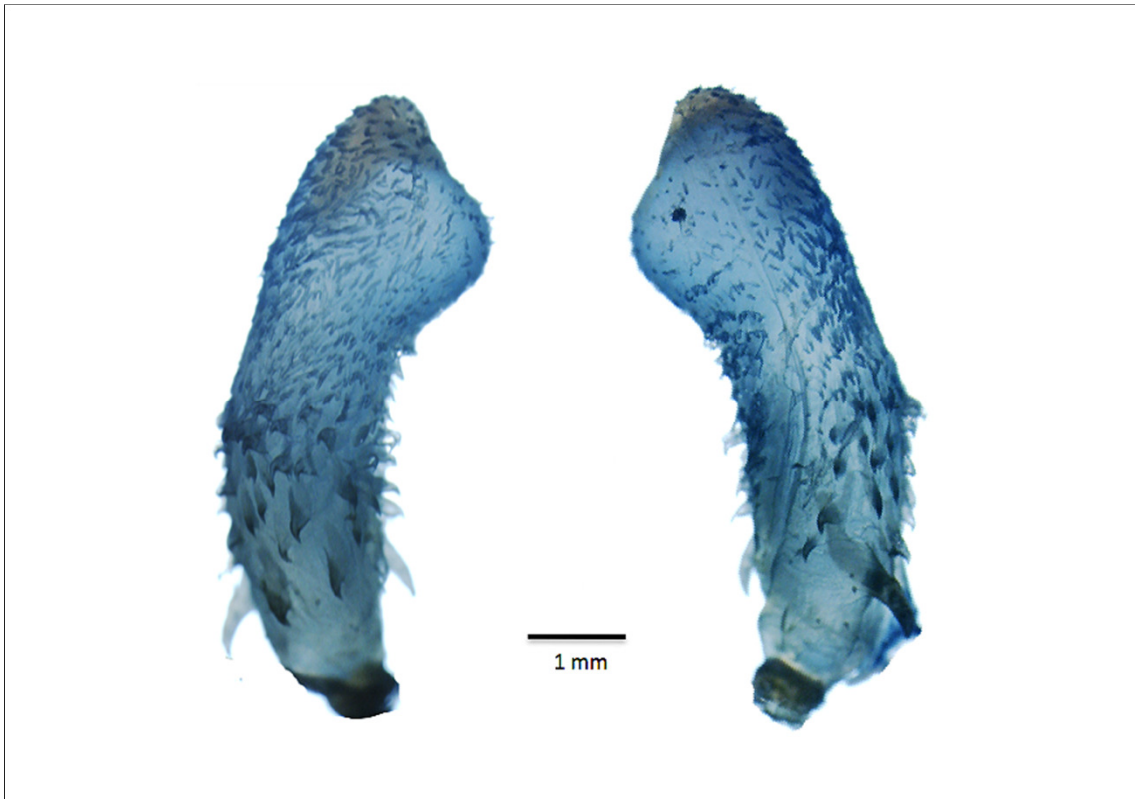

Figure 2.6. Hemipenis of *Sonora mutabili.* Specimen is UTA R-53487. Right, sulcate side, left, asulcate side.

Table 2.1. Sample information and Genbank accession numbers. Numbers correspond to localities in Figure 2.2. Voucher IDs are either museum numbers or field numbers. ASDM 211449 *cytb* sequence is published in Holm (2008). Field notes and tissues for UTA BTM and UTA JRV specimens are deposited at the UTA ARDRC. Genes for all outgroup taxa were downloaded from Genbank. Accession numbers for phased RAG-1 sequences are indicated with a and b and correspond to identifiers in Figure 2.3.

#	Voucher ID	Taxon	Country: State	Locality	Lat	Long	Elevation (m)	*cytb*	*ND4*	*c-mos*	*RAG-1*
1	UANL 6976	*Sonora (Procinura) aemula*	Mexico: Sonora	near Alamos	27.02458	-108.9397	400	JQ265959	JQ265979	JQ265952	JQ265970
2	ASDM 21449	*S. aemula*	Mexico: Sonora	near Alamos	27.02458	-108.9397	400	Holm (2008)	NA	NA	NA
	CAS 206503	*S. semiannulata*	USA: California	Inyo County near Bishop	36.24532	-117.4531	907	AF471048	JQ265981	AF471164	JQ265970
3	MZFC 23956	*S. michoacanensis*	Mexico: Guerrero	Campo Morado, Canada "El Naranjo"	18.19316	-100.1609	1072	JQ265958	JQ265980	JQ265951	JQ265969
4	UTA BTM 26	*S. mutabilis ("aequalis")*	Mexico: Jalisco	Barranca del Rio Santiago	20.79239	-103.3297	107	JQ265954	JQ265975	JQ265945	a) JQ265967; b) JQ265968
5	UTA R-53488	*S. mutabilis ("aequalis")*	Mexico: Jalisco	near Bolanos	21.87539	-103.8207	1633	JQ265953	JQ265973	JQ265947	JQ265962
6	UTA JRV 127	*S. mutabilis ("aequalis")*	Mexico: Jalisco	Huaxtla: canyon below town	20.72845	-103.6567	1450	JQ265955	JQ265976	JQ265950	NA
7	UTA JRV 129	*S. mutabilis ("aequalis")*	Mexico: Jalisco	Huaxtla: canyon below town	20.72845	-103.6567	1450	JQ265956	JQ265978	NA	a) JQ265960; b) JQ265951
8	UTA R-53487	*S. mutabilis*	Mexico: Jalisco	near Bolanos	21.87539	-103.8207	1633	NA	JQ265972	JQ265946	a) JQ265965; b) JQ265966
9	UTA R-59762	*S. mutabilis*	Mexico: Jalisco	Road to Pueblitos near Barranca del Rio Santiago	21.02544	-103.4607	1350	JQ265957	JQ265974	JQ265948	JQ265963
10	UTA JRV 128	*S. mutabilis*	Mexico: Jalisco	Huaxtla: canyon below town	20.72845	-103.6567	1450	NA	JQ265977	JQ265949	JQ265964
	CAS 212760	*Coluber constrictor*	USA: California	Mendocino National Forest	39.16058	-122.6681	597	EU180467	AY487041	AY486938	NA
	SDSU 3929	*Coluber constrictor*	-	-	-	-	-	NA	NA	NA	EU402841
	Moody338	*Agkistrodon contortix*	-	-	-	-	-	NA	AF156576	NA	NA
	LSU H0607	*Agkistrodon contortix*	-	-	-	-	-	EU483403	NA	NA	EU402833
	CAS 214406	*Agkistrodon piscivorous*	-	-	-	-	-	NA	NA	AF471096	NA

Table 2.2. Primer name and primer sequence for the amplification and sequencing of gene fragments analyzed in this study.

Primer Name	Fragment	Sequence (5'-3')
ATRCB3	*cytb*	TGA GAA GTT TTC YGG GTC RTT
GLUDG	*cytb*	TGA CTT GAA RAA CCA YCG TTG
ND4F	*ND4*	CAC CTA TGA CTA CCA AAA CCT CAT GT
LeuR	*ND4*	CAT TAC TTT TAC TTG GAT TTG CAC CA
RAG1_f1a	*RAG-1*	CAG CTG YAG CCA RTA CCA TAA AAT
RAG1_r2	*RAG-1*	CTT TCT AGC AAA ATT TCC ATT CAT
S77cmos	*c-mos*	CAT GGA CTG GGA TCA GTT ATG
S78cmos	*c-mos*	CCT TGG GTG TGA TTT TCT CAC CT

Table 2.3. Morphological measurements of *S. michoacanensis* and *S. mutabilis.* Morphological measurements from Echternacht (1973) and this study. We excluded some specimens included in Echternacht (1973) from this table because their locality is unknown. TBL= total body length. TL= tail length. Meristic counts are presented as left-right. This specimen has a single narrow band on the tail.

Catalog #	Taxon	State	Sex	TBL (mm)	TL	Temporals	Supralabials	Infralabials	Ventrals	Subcaudals	Banding on tail	Source
NHMUK 1946.1.14.65	*michoacanensis*	Michoacan	M	244	56	-	-	-	165	44	no	Echternacht 1973
FMNH 37141	*michoacanensis*	Michoacan	M	205	50	3 – 2	7 – 7	7 – 7	152	44	no	Echternacht 1973
FMNH 39128	*michoacanensis*	Michoacan	F	169	31	2 – 2	7 – 6	8 – 7	173	36	no	Echternacht 1973
FMNH 39129	*michoacanensis*	Michoacan	F	201	38	2 – 2	7 – 7	8 – 7	171	39	no	Echternacht 1973
Holotype	*michoacanensis*	Michoacan	M	160	35	3 – 3	7 – 7	6 – 6	152	37	no	Echternacht 1973
HSM RS-596	*michoacanensis*	Colima	F	220	36	2 – 3	7 – 7	7 – 7	161	32	no	Echternacht 1973
KU 23790	*michoacanensis*	Guerrero	M	237	46	3 – 3	7 – 7	7 – 7	177	41	no	Echternacht 1973
KU 23791	*michoacanensis*	Guerrero	M	275	55	2 – 2	7 – 6	7 – 6	175	42	no	Echternacht 1973
MCZ 33650	*michoacanensis*	Guerrero	F	272	58	3 – 3	7 – 7	6 – 7	175	46	no	Echternacht 1973
Museo Dugés -	*michoacanensis*	Guerrero	F	-	-	-	-	-	177	43	no	Echternacht 1973
MVZ 45123	*michoacanensis*	Guerrero	F	253	54	4 – 3	7 – 7	6 - ?	175	45	no	Echternacht 1973
MVZ 76714	*michoacanensis*	Michoacan	F	228	45	2 – 3	8 – 8	7 – 7	170	40	no	Echternacht 1973
UIMNH 25063	*michoacanensis*	Guerrero	M	110	23	3 – 2	8 – 8	7 – 7	163	46	no	Echternacht 1973
UMMZ 109904	*michoacanensis*	Michoacan	F	192	34	3 – 3	6 – 7	6 – 5	168	37	no[d]	Echternacht 1973
UMMZ 109905	*michoacanensis*	Michoacan	F	234	41	3 – 3	7 – 7	6 – 7	171	38	no	Echternacht 1973
UMMZ 109906	*michoacanensis*	Michoacan	F	120	19	3 – 3	7 – 7	6 – 6	171	33	no	Echternacht 1973
UMMZ 119457	*michoacanensis*	Michoacan	M	211	47	3 – 3	7 – 7	7 – 7	157	41	no	Echternacht 1973
UIMNH 41688	*michoacanensis*	Puebla	F	257	51	3 – 3	6 – 6	7 – 7	177	40	no	Echternacht 1973

Table 2.3 - continued

Catalog #	Taxon	State	Sex	TBL (mm)	TL	Temporals	Supralabials	Infralabials	Ventrals	Subcaudals	Banding on tail	Source
UTA R-38146	*michoacanensis*	Guerrero	F	205	38	5 – 5	7 – 7	6 – 7	171	37	no	This Study
UTA R-59760	*michoacanensis*	Colima	-	76	16	-	-	-	164	46	no	This Study
AMNH 74951	*mutabilis*	Nayarit	M	215	41	-	7 – 7	6 – 6	171	40	yes	Echternacht 1973
NHMUK 1946.1.14.63	*mutabilis*	Zacatecas	M	229	54	-	-	-	160	45	yes	Echternacht 1973
NHMUK 1946.1.14.64	*mutabilis*	Zacatecas	M	220	48	-	-	-	166	46	yes	Echternacht 1973
FMNH 105296	*mutabilis*	Jalisco	M	191	44	3 – 3	7 – 7	6 – 6	163	44	yes	Echternacht 1973
FMNH 105297	*mutabilis*	Jalisco	M	189	43	3 – 3	7 – 7	6 – 6	161	44	yes	Echternacht 1973
KU 106286	*mutabilis*	Zacatecas	F	230	45	3 – 3	5 – 7	6 – 6	178	43	yes	Echternacht 1973
MVZ 71356	*mutabilis*	Jalisco	M	99	15	3 – 3	7 – 7	6 – 6	171	34	yes	Echternacht 1973
UIMNH 18754	*mutabilis*	Jalisco	F	210	42	3 – 3	7 – 7	6 – 6	169	41	yes	Echternacht 1973
UTA R-7227	*mutabilis*	Sinaloa	M	225	52	6 – 5	7 – 7	7 – 7	174	48	yes	This Study
UTA R-53487	*mutabilis*	Jalisco	M	235	51	7 – 6	7 – 7	7 – 7	162	40	yes	This Study
UTA R-59762	*mutabilis*	Jalisco	-	-	-	3 – 3	7 – 7	7 – 7	189	41	yes	This Study
MCZ 6444	*mutabilis ("aequalis")*	-	F	225	40	3 – 3	7 – 7	6 – 6	174	38	yes	Echternacht 1973
UTA R-16169	*mutabilis ("aequalis")*	Jalisco	F	256	52	5 – 5	7 – 7	7 – 6	169	41	yes	This Study
UTA R-53488	*mutabilis ("aequalis")*	Jalisco	F	252	41	7 – 6	7 – 7	7 – 6	169	39	yes	This Study
UTA R-59761	*mutabilis ("aequalis")*	Jalisco	M	-	-	6 – 6	7 – 7	6 – 6	168	47	yes	This Study

CHAPTER 3

THE EFFECT OF LANDSCAPE ON THE POPULATION GENETIC STRUCTURE OF GROUND SNAKES (*SONORA SEMIANNULATA*) IN THE GREAT PLAINS REGION

3.1 Abstract

Genetic variability within populations and gene flow between populations are both important factors in the long-term persistence of populations and species. However, knowledge of population and landscape genetics is limited in geographic and taxonomic scope. For this study, we focus on patterns of gene flow and genetic variability within the Great Plains region. This expansive region is a continuous landscape extending from southern Canada to central Texas. Notably, this region possesses a species-rich assemblage of ecologically similar semifossorial snakes. However, the influence of ubiquitous anthropogenic disturbance in the Great Plains on gene flow and genetic variability is unknown. Additionally, the role of landscape in structuring populations is not well known. We sampled 17 populations of the ground snake (*Sonora semiannulata*) from across the Great Plains region as a proxy to understand the population and landscape genetics of small semifossorial snakes in the Great Plains. We used AFLPs and standard population genetics analyses to determine their genetic structure of populations. In addition, we used bivariate and matrix correlations to assess the role of landscape on genetic variation and gene flow. The minimal genetic structure in our dataset was not structured geographically, and gene flow and genetic variability

were relatively high within and among populations. Similarly, we were unable to detect any landscape genetic structure, suggesting that the continuous landscape of the Great Plains must not exert a strong influence on patterns of genetic differentiation among populations. Generally, our work suggests that the ground snake in the Great Plains has relatively large population sizes, despite the greatly modified habitat that they are found in. Future research should incorporate ecological data on ground snakes into landscape analyses and should expand the taxonomic scope of population genetics research in the Great Plains.

3.2 Introduction

The genetic structure of populations has important implications for the conservation and management of species (Conner and Hartl 2004). Genetic structure of populations can be evaluated by genetic variability within populations, and by gene flow and connectedness to other populations. Gene flow to other populations and genetic diversity within a population can affect the persistence of populations in time and space (Conner and Hartl 2004). Additionally, low genetic diversity within populations can compromise the long term persistence of a species (Bouzat et al. 1998; Wisely et al. 2002), while genetically divergent and isolated populations may represent important conservation management units (Conner and Hartl 2004).

Aspects of organism biology can influence the genetic structure of populations. Breeding behavior such as lekking can structure populations genetically (Johnson et al. 2003; Bouzat and Johnson 2004), while male-biased dispersal may create specific patterns of mitochondrial and nuclear genetic structure (Fontenot et al. 2011).

Additionally, low vagility may lead to highly structured populations, while high-vagility organisms may have minimally genetically structured populations (Conner and Hartl 2004). Organisms may also experience differential susceptibility to anthropogenic disturbance, which may fracture populations and decrease gene flow (Conner and Hartl 2004; Goosens et al. 2006). The landscape can also impact patterns of genetic diversity and gene flow, with rivers and mountains serving as vicariant or filter barriers to gene flow, and isolated habitats (i.e. glades in the Ozarks, moist springs in deserts) serving as fosters of genetic diversity (Conner and Hartl 2004). These landscape effects are mediated by organismal biology, with the influence of landscape on organisms taxon-specific (e.g., xeric habitats and saltwater are effective barriers for many amphibians). Despite the importance of understanding population structure and landscape effects of population genetics for conservation, many taxonomic groups and geographic regions have poorly characterized patterns of population genetic structure.

The Great Plains region of the United States and Canada contains some of the most imperiled habitats in the United State (Burke et al. 1991), with particular habitats intact in only a small fraction of their previous range (Samson and Knopf 1994; Samson et al. 2004). The Great Plains region is a large contiguous region of grasslands, riparian woodlands, and wetlands throughout central North America that extends from the southern United States to southern Canada (Samson et al. 2004). Although this continuous landscape does not possess any major geographic barriers, rivers and boundaries between habitat types may subtly influence patterns of genetic diversity and gene flow for certain organisms (Samson et al. 2004). Thus, understanding how genetic

diversity and gene flow are distributed spatially across this landscape for different organisms can contribute information about the health of this habitat.

The Great Plains region contains a species-rich guild of small semifossorial colubrids (Ernst and Ernst 2003). Although these snakes are not closely related, they are remarkably ecologically similar- primarily fossorial, spring emergers, of small body size and consumers of a diet containing mostly invertebrates (Ernst and Ernst 2003). In certain habitats, they may be tremendously abundant and even the most prevalent vertebrate by numbers and biomass (Fitch 1999). However, small squamates may especially be impacted by habitat fragmentation and construction of roads because of their generally low vagility and dispersal capability (Branch et al. 2003). Indeed, some species are rare, threatened or endangered in parts of their geographic range (Table 1). Due to their potential ecological and conservation importance in parts of their range, understanding the landscape effects on population genetic structure may be important for informing conservation and land management decisions in the Great Plains. Because a detailed analysis for each species is not practical at this time, we seek to understand the landscape genetics of this guild of snakes using a representative proxy species.

The ground snake is a small insectivorous snake that is distributed across the central and western United States (Ernst and Ernst 2003). The ground snakes within the Great Plains region form a monophyletic clade (A. R. Davis Rabosky, unpublished data), can be locally abundant and are found in a variety of habitats. Because of their wide geographic range and abundance, ground snakes represent an excellent

opportunity to study the population and landscape genetic structure of the ecological guild of small semifossorial snakes.

We used amplified fragment length polymorphism (AFLP) to assess the genetic structure of ground snakes across their range in the Great Plains. We used a variety of different analytical techniques to describe population structure, gene flow and genetic diversity within populations. Using correlation methods, we also test whether some landscape features are associated with gene flow and genetic diversity.

3.3 Material and Methods

3.3.1 Specimen collection

Snakes were collected by turning rocks in appropriate habitat across the Great Plains from 2008 to 2010. We focused on collections from 17 different localities or populations (Figure 3.1). We preserved muscle, liver, or skin tissue in lysis buffer, 95% ethanol or an RNA-preserving buffer. Although some specimens were sampled for tissues and released, most specimens were fixed in 10% formalin and fluid-preserved in 70% ethanol. Specimens were deposited in the University of Texas-Arlington Amphibian and Reptile Diversity Research Center and Sternberg Museum at Fort Hays State University.

3.3.2 Molecular methods

We assessed the genetic structure of 247 ground snakes collected from 17 different localities within the Great Plains region by using amplified fragment length polymorphisms (AFLPs) following Vos (1995). Briefly, DNA was isolated using

Quiagen© DNAeasy Blood and tissue kit using the standard protocol. DNA isolates were digested for 6 hours with EcoR1 and Mse1 at 37 ° C, and adaptors (Table 3.2) were ligated to digested DNA using T4 DNA ligase at 16° C for 12 hours. The ligated products were amplified using preselective primers (Table X, thermocycle protocol). For the next amplification, one primer was fluorescently labeled with FAM (EcoR1) with additional selective bases (Table 3.2, thermocycle protocol). These labeled selective PCR products were purified using an ethanol/ sodium acetate precipitation, and were rehydrated in HPLC purified formamide (HiDi) with a Rox 500 bp size standard. Samples were analyzed on an ABI PRISM 3100 xl Genetic Analyzer in the Genomics Core Facility at the University of Texas-Arlington. For a sizable subset of the data, we repeated this analysis to estimate repeatability and error. Resulting data was assembled and binned for possible analysis in Genemarker. We used the R-script (R Development Core Team 2008) AFLPscore to objectively score loci, which uses mismatch distributions and Bayesian error rates for a duplicated dataset to set loci inclusion and peak threshold for scoring loci to minimize error (Whitlock et al. 2008). We then removed AFLP loci that were present in less than three specimens (Yeh and Boyle 1997) and converted that dataset for further analysis using the R-script (R Development Core Team 2008) AFLPdat (Ehrich 2006). We used Bayescan (Foll and Gaggiotti 2008) and Mcheza (Antao and Beaumont 2011) to identify loci that may be under selection, and removed those loci from the dataset. After data pruning, 112 AFLP loci remained that were used in subsequent analysis.

3.3.3 Population genetic analyses

We assessed genetic structure among populations using two different approaches. First, we calculated an overall Fst (PhiPt), which increases in value with increasing population subdivision. This statistic was calculated in Genalex, and significance was assessed using 1000 bootstrap pseudoreplicates. Second, we used the program Structure to infer the likelihood of different number of populations (K). Although in preliminary analyses we experimented with different population models, for final analyses we used a population model with genetic admixture and correlated allele frequencies, and analyzed 100000 generations for a K ranging from 1 to 9. We consider the lowest value of K when likelihood values had reached a plateau to be a reasonable estimate of K.

We assessed genetic variation within each of our populations using Nei's unbiased heterozygosity and Shannon's I and determined the number of loci unique to a single population. Genetic variation within populations was compared to sample size using Pearson's and Spearman's Rank Correlation. We also calculated genetic distance between populations, using Nei's unbiased genetic distance and binary genetic distance. All of these summary statistics were calculated in Genalex.

3.3.4 Landscape genetic analyses

We tested the role of landscape factors on genetic variability within populations. Specifically, we tested for an association between latitude, longitude, and sample size and genetic variability using Spearman's Rank and Pearson's correlation. Additionally, we tested whether large rivers (largest 20 rivers in the United States based upon

discharge, drainage area, or length; Krammer 1990) separating a population from other populations (maximum and total number of rivers) was correlated with genetic variability (Figure 3.1). Finally, we tested whether populations in the periphery and center of the ground snakes geographic range and from different ecoregions (ecoregions defined as in Omernik 1987) differed in genetic variation using the non-parametric Kruskal-Wallis test. We also tested for the effect of landscape factors on genetic distance between populations using simple and partial (controlling for geographic distance) Mantel tests. We tested whether the number of ecoregions (ecoregions defined as in Omernik 1987), number of rivers (largest 20 rivers in the United States based upon discharge, drainage area, or length; Krammer 1990), vertical or horizontal distance (in km) separating populations were associated with genetic distance. Mantel tests were conducted in zt (Bonnet and van de Peer 2002), and significance was assessed using 100,000 bootstrap pseudoreplicates.

3.4 Results

3.4.1 Population genetic structure

We found that the overall Fst among populations was low but significantly different from zero, suggesting some minimal population structure. Using Bayesian cluster analyses we found that likelihood values reached a plateau of around K = 3 or 4, although likelihood values declined with increasing K (Table 3.3). We did not observe any geographic structuring of genotypes for any K value.

We found moderate within-population genetic variation that was similar among localities for both Nei's unbiased heterozygosity (Table 3.4; 0.054-0.233) and

Shannon's I (Table 3.4, 0.059-0.341). Few population possessed unique loci, with Barber, Barn and Steph containing a single unique loci and Geary with three unique loci. Within population genetic variation was significantly positively associated with increasing sample size (P=0.019) However, this relationship was driven by two populations with very low sample sizes (Sutton and San Saba; Table 3.4) and analyses excluding these populations did not recover a significant association between sample size and genetic variation. These two populations were excluded from the within-population genetic variation landscape analyses, although they were included in genetic distance analyses. Genetic distance between populations was generally low and similar between populations for both population binary genetic distance (Table 3.5; 11.00-27.083) and Nei's unbiased genetic distance (Table 3.5; 0.096-0.009).

3.4.2 Landscape genetics

We found no relationship between genetic variation within populations and any landscape feature (*P's*>0.05). There was no detectable effect of isolation by distance (*P*>0.50). Similarly, we found no significant relationship between landscape features and genetic distance (*P's*>0.05), even when controlling for geographic distance (*P's*>0.05).

3.5 Discussion

Populations of *S. semiannulata* in the Great Plains possess minimal genetic structure, with no evidence of geographically based genetic structure. We found evidence for abundant gene flow between populations, and also that genetic variability within populations was relatively high and similar among populations. Similarly, we

were unable to detect any impact of landscape on genetic variability and gene flow. In the ensuing discussion we discuss our findings and compare to previous research.

We found that genetic variability within *S. semiannulata* populations was relatively high, which suggests the population size of these prairie denizens may be quite high (Conner and Hartl 2004). This is consistent with field observations, as multiple individuals could be found with only a few hours of searching in most populations. Thus *S. semiannulata* might be insensitive to the anthropogenic disturbance that is ubiquitous in the Great Plains region (Samson et al. 2004), although we note that only one population (Tulsa) was near a major metropolitan area, and other localities were found in rural areas. Other Great Plains animal species such as prairie chickens (Bouzat et al. 1998) and black-footed ferrets (Wisely et al. 2002) have experienced precipitous population crashes in response to anthropogenic disturbance. Our study suggests that *S. semiannulata* and perhaps other semifossorial snakes in the Great Plains are less affected by anthropogenic disturbances than are some other prairie species.

Gene flow was relatively high between localities, and we were unable to detect even the simplest form of landscape genetic structure, isolation by distance. We note that while we refer to the collecting localities in this study as populations, the lack of detectable population structure probably indicates that all localities in this Great Plains region could be considered a single population. This pattern could be the result of three processes that are not mutually exclusive. First, our results could be the result of recent range expansion and low subsequent gene flow (Ibrahim et al. 1996; Ray et al. 2003;

Wegman et al. 2006). Second, the patterns we report may reflect high rates of gene flow between localities, even those at great geographic distance. Finally, these findings may suggest a very large population size within the Great Plains (Conner and Hartl 2004). To address this question, we separated our AFLP loci into derived and ancestral loci (data not shown, see Chapter 4), with population structure from derived loci reflecting recent gene flow and population structure from ancestral loci reflecting historical gene flow. We found that the F_{st}s from each dataset were statistically indistinguishable from one another, suggesting that the population genetic patterns that we report are the result of both recent and historic gene flow. In addition, the high genetic variability that we report also suggests a large population size. Hence, the patterns that we report could plausibly be the result of recent range expansion and high rates of gene flow combined with large population sizes.

We did not detect the statistical effect of any landscape factor that we tested on genetic structure. This result is consistent with our observation of high genetic variability and minimal genetic distance between localities. However, we acknowledge that the statistical approach that we used may not be sufficient to detect subtle genetic structure. A powerful technique for determining the impact of landscape on genetic structure is least-cost path analysis (Storfer et al. 2007; Wang and Shaffer 2009). Briefly, this GIS-based, spatially explicit method uses information about movement and habitat preference to parameterize a landscape cost surface, and calculate a least-cost path (Storfer et al. 2007; LaRue and Nielsen 2008; Spear et al. 2010). This least-cost path matrix can then be compared to genetic and geographic distance matrices using

Mantel tests to ascertain the influence of landscape on patterns of genetic differentiation among populations (Storfer et al. 2007; Spear et al. 2010). Unfortunately, there is no detailed behavioral or habitat use data for secretive and semifossorial *S. semiannulata*. While some authors have developed *de novo* cost surfaces by iteratively using genetic distance to develop parameters for the cost surface (Wang and Shaffer 2009), in the context of our study this approach might suffer from the philosophical problem of tautology. Future research into the behavior and habitat use of *S. semiannulata* or other semifossorial snakes from the Great Plains region could facilitate the implementation of least-cost analysis to test for subtle genetic structure in these little-studied snakes.

Our results have conservation and management implications for ground snakes and perhaps other semifossorial snakes in the Great Plains. Given a likely large population size and robust gene flow among localities, this species is probably not genetically imperiled, nor are any localities genetically isolated from others. It is possible that their small size and diet consisting largely of arthropods (Ernst and Ernst 2003) has allowed them to persist in an anthropogenically altered landscape, and even allowed them to maintain large population sizes (e.g., Fitch 1999). Although it might be reasonable to extend these conclusions to other members of the guild of small semifossorial colubrids, only population genetic studies for these other species can clarify the broad applicability of our results.

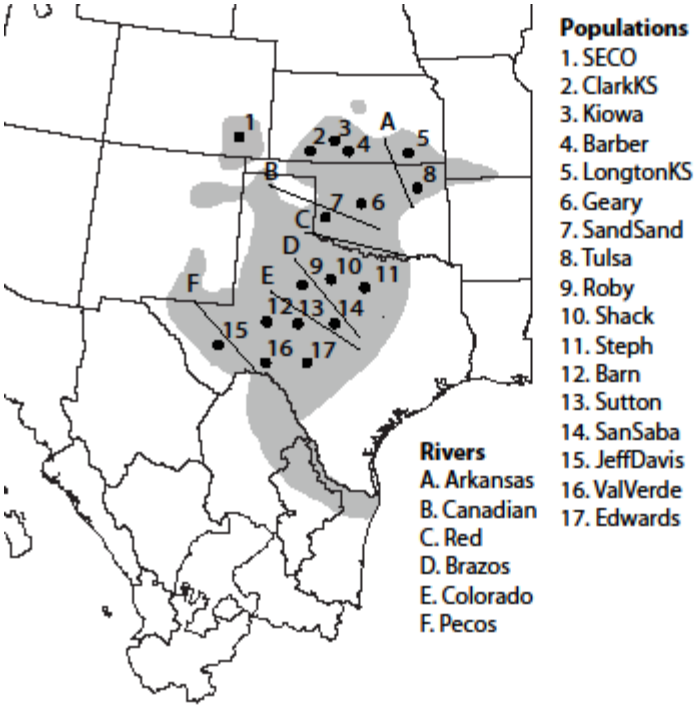

Figure 3.1. Populations used for landscape genetic analysis. Lines indicate how river barriers separated populations.

Table 3.1. Conservation status of small semifossorial snakes of the Great Plains.

Species	Conservation Status	Source
Western worm snake (*Carphophis vermis*)	Threatened and Endangered List in IA	http://www.iowadnr.gov/wildlife/files/wildinfo.html
Rough Earth Snake (*Virginia striatula*)	Species in Need of Conservation in KS	http://kdwp.state.ks.us/news/Other-Services/Threatened-and-Endangered-Species
Flathead Snake (*Tantilla gracilis*)	Not considered threatened in any state	
Plains Blackhead Snake (*Tantilla nigriceps*)	Status of NSS3 in WY (habitat is restricted)	http://gf.state.wy.us/wildlife/nongame/SpeciesList/index.asp
Prairie Ringneck Snake (*Diadophis punctatus arnyi*)	Not considered threatened in any state	
Night Snake (*Hypsiglena torquata*)	Species in Need of Conservation in KS	http://kdwp.state.ks.us/news/Other-Services/Threatened-and-Endangered-Species
Texas Blind Snake (*Leptotyphlops humilis*)	Species of special concern in CO, Threatened and Endangered list in KS	http://wildlife.state.co.us/WildlifeSpecies/SpeciesOfConcern/Reptiles/; http://kdwp.state.ks.us/news/Other-Services/Threatened-and-Endangered-Species
Ground Snake (*Sonora semiannulata*)	Only two records from AR, isolated population in CO	http://www.agfc.com/wildlife-conservation/reptiles/snakes/ground-snake.aspx

Table 3.2 Primer sequences and sources for AFLP study. Sequences for adaptors and primers based upon primers in Vos (1995) and Makowsky (2009). Selective bases are indicated with brackets.

Primer	Sequence (5'-3')
Mse1 Adaptor 1	GACGATGAGTCCTGAG
Mse1 Adaptor 2	TACTCAGGACTCAT
EcoR1 Adaptor 1	CTCGTAGACTGCGTACC
EcoR1 Adaptor 2	AATTGGTACGCAGTCTAC
Mse1 Primer 1	GATGAGTCCTGAGTAA[GC]
EcoR1 Primer 1	GACTGCGTACCAATTC[G]
Mse1 Primer 2	GATGAGTCCTGAGTAA[GCC]
EcoR1 Primer 2 (FAM)	GACTGCGTACCAATTC[GA]

Table 3.3 Likelihood values for different population clusters (K)

Populations (K)	Likelihood	Likelihood Variation
1	-7036	53.5
2	-6179.9	184.4
3	-5974.3	318
4	-5825	333.9
5	-5828	535.8
6	-5791.4	706.6
7	-5667	655.7
8	-5627.4	709.2
9	-5648.3	882.1

Table 3.4. Ecoregion identity and population genetic variability. Population codes correspond to localities in Figure 3.1 and Appendix A.

Population Code	N	Region*	Shannon's I	Nei's Unbiased Heterozygosity	Private Loci
Barber	12	SWTL	0.181	0.123	1
Barn	41	EP	0.286	0.191	1
ClarkKS	16	SWTL	0.282	0.190	0
Edwards	5	EP	0.236	0.180	0
Geary	18	CGP	0.341	0.233	3
Kiowa	21	SWTL	0.213	0.141	0
LongtonKS	9	CT	0.294	0.207	0
Roby	17	SWTL	0.271	0.182	0
SandSand	15	CGP	0.174	0.119	0
SanSaba	3	EP	0.077	0.061	0
SECO	23	SWTL	0.178	0.117	0
Shack	31	CGP	0.281	0.188	0
Steph	21	CT	0.246	0.161	1
Sutton	2	EP	0.059	0.054	0
Tulsa	4	CIP	0.233	0.178	0
ValVerde	6	CHID	0.161	0.117	0
West	3	CHID	0.145	0.112	0

Table 3.5. Genetic distance between *S. semiannulata* populations. Binary genetic distance is above the diagonal, while Nei's unbiased genetic diversity is below the diagonal. Population codes correspond to information in Appendix A.

Population	Barber	Barn	Clark KS	Edwards	Geary	Kiowa	Longton KS	Roby	Sand Sand	San Saba	SECO	Shack	Steph	Sutton	Tulsa	Val Verde	West
Barber		14.6	15.3	19.0	19.9	12.7	19.1	14.4	12.6	12.4	14.3	15.2	15.8	16.2	23.2	14.5	16.2
Barn	0.026		15.8	18.4	20.2	13.5	19.4	14.8	13.1	12.9	13.6	16.0	15.9	14.9	22.5	14.9	15.6
ClarkKS	0.031	0.009		19.9	21.1	14.5	19.9	15.9	13.7	14.4	15.6	16.0	17.2	15.6	22.5	15.7	17.3
Edwards	0.057	0.030	0.038		23.8	18.6	23.4	18.7	17.4	17.3	17.7	18.5	19.6	18.5	25.4	18.4	18.5
Geary	0.049	0.018	0.024	0.043		19.3	23.6	20.2	18.8	19.2	19.3	21.0	21.8	21.1	27.1	21.1	21.6
Kiowa	0.023	0.016	0.020	0.060	0.042		17.9	13.5	11.5	11.6	12.7	14.6	14.6	13.6	21.9	13.5	14.7
LongtonKS	0.041	0.020	0.017	0.041	0.024	0.028		19.4	17.5	17.4	18.3	20.6	20.8	19.4	24.9	20.1	20.4
Roby	0.021	0.010	0.016	0.032	0.032	0.019	0.025		12.9	12.4	14.1	15.2	16.1	14.4	22.5	15.2	15.7
SandSand	0.024	0.023	0.022	0.053	0.050	0.017	0.035	0.018		11.0	11.9	13.7	13.9	13.2	21.3	12.9	14.2
SanSaba	0.038	0.042	0.051	0.077	0.081	0.032	0.053	0.031	0.026		12.1	13.2	13.6	11.2	20.1	12.6	14.1
SECO	0.036	0.026	0.038	0.050	0.056	0.025	0.040	0.023	0.019	0.036		14.8	14.1	15.7	20.9	14.2	13.5
Shack	0.031	0.011	0.011	0.020	0.025	0.025	0.027	0.009	0.024	0.049	0.033		16.0	15.6	22.5	15.3	16.4
Steph	0.027	0.016	0.025	0.037	0.045	0.024	0.033	0.020	0.022	0.038	0.023	0.019		16.7	22.9	16.1	16.3
Sutton	0.072	0.055	0.062	0.094	0.096	0.039	0.065	0.048	0.051	0.039	0.069	0.071	0.064		21.8	15.8	15.0
Tulsa	0.058	0.038	0.039	0.052	0.062	0.050	0.036	0.036	0.051	0.054	0.044	0.039	0.034	0.076		21.8	22.1
ValVerde	0.030	0.030	0.038	0.049	0.070	0.027	0.063	0.023	0.027	0.038	0.033	0.029	0.035	0.073	0.055		15.7
West	0.050	0.029	0.047	0.052	0.066	0.028	0.048	0.022	0.043	0.054	0.035	0.036	0.035	0.048	0.043	0.036	

CHAPTER 4

DYNAMICS OF SELECTION ON COLOR PATTERN IN NATURAL POPULATIONS OF MIMETIC SNAKES

4.1 Abstract

The biological phenomenon of mimicry is an excellent example of phenotypic novelty and convergence. Within Batesian systems, some mimics possess color pattern polymorphism, or the presence of multiple discrete color pattern types within a single population. Although not uncommon, the evolutionary mechanisms that generate and maintain color pattern polymorphism in mimicry complexes are not well characterized. We examined temporal and age-class variation in color pattern morph frequency and compared patterns of population structure generated using putative neutral genetic markers (AFLPs) and color pattern to infer selection on color pattern polymorphism. We found that the frequency of color pattern morphs changed between juveniles and adults, consistent with selection. Additionally, color pattern varied temporally, with morphs exhibiting opposing frequency trends. Finally, we found minimal evidence of population structure using a neutral genetic marker, but that populations were highly subdivided with regards to color pattern. Our results suggest that color pattern is subjected to selective as opposed to neutral evolutionary forces in a coralsnake mimic. We also conclude that selection is driving geographically and temporally variable color

pattern morph frequencies. This landscape variation in the results of selection among populations is consistent with a geographic mosaic of coevolution or mimicry.

4.2 Introduction

The biological phenomenon of mimicry, which can be broadly defined as the imitation of one species by another, is an excellent example of phenotypic convergence, coevolutionary interactions, and adaptation (Mallet and Joron 1999; Brodie and Brodie 2004). Mimicry has even been postulated to be a major driver of diversity (Joron and Mallet 1998; Mallet and Joron 1999). Although most classic theoretical (Nur 1970; Charlesworth and Charlesworth 1975a; Charlesworth and Charlesworth 1975c; Charlesworth and Charlesworth 1975b; Matthews 1977) and empirical (e.g. butterflies: Bates 1862; Müller 1879; Mallet and Joron 1999; hoverflies: Waldbauer 1988; spiders: Ceccarelli and Crozier 2007) studies of mimicry have focused on invertebrates, Neotropical coralsnakes and their mimics offer a promising opportunity for evaluating generalities and differences about how mimicry systems evolve across taxa (Greene and McDiarmid 1981; Pfennig et al. 2001; Brodie and Brodie 2004). In this Batesian mimicry system, the bright warning coloration (red, black, and sometimes yellow or white bands) of venomous coralsnakes in the family Elapidae is deceitfully imitated by many genera of harmless snakes in the family Colubridae to deter potential predators (Greene and McDiarmid 1981; Brodie and Brodie 2004).

One major prediction of mimicry is that color pattern should be under the influence of strong selection. Previous research in coral snake mimicry complexes has studied selection on color pattern using plasticine or clay model studies (Brodie 1993;

Brodie and Janzen 1995; Pfennig et al. 2001; Kikuchi and Pfennig 2010b). This research has been essential to advancing the study of mimicry by 1) providing the first direct evidence that coralsnake color patterns are more avoided by predators than cryptic color patterns and clarifying the ecological agents of selection, and 2) highlighting the role of frequency-dependence, allopatry with the model, mimetic accuracy, and other factors in the evolution of color pattern in coralsnake mimics (Brodie 1993; Pfennig et al. 2001; Kikuchi and Pfennig 2010a; Kikuchi and Pfennig 2010b; Pfennig and Mullen 2010). Replica studies are especially attractive because they offer control over the types, abundance, and location of color patterns encountered by predators, but such observations are proxies for the real measurement of interest: response to selection in natural populations of free-living snakes.

A more direct approach for studying selection on color pattern is to examine landscape and temporal phenotypic variation in coralsnake mimics with color pattern polymorphism (multiple color morphs within single populations). Color pattern polymorphism is a common feature of Batesian mimicry complexes (Joron and Mallet 1998; Mallet and Joron 1999; Edmunds 2000; Nijhout 2003; Ceccarelli and Crozier 2007) and has been documented in multiple coralsnakes and their colubrid mimics (Brodie 1993; Brodie and Brodie 2004). This discrete phenotypic variation can be leveraged to study selection on color pattern in two different ways. First, morph frequencies within populations can be compared between both age classes and time points. Marked shifts in color pattern morph frequencies between juvenile and adult age classes or over time are consistent with selection on color pattern. Second, geographic

variation in color pattern morph frequency can be compared to geographic variation in neutral genetic markers, with strong discordance markers considered evidence for selection (Gillespie and Oxford 1998; Hoffman et al. 2006; Croucher et al. 2011).

We used both of these approaches to study selection on color pattern in the mimetic ground snake, *Sonora semiannulata*. Ground snakes possess striking color pattern polymorphism (Fig. 4.1) and are found throughout the central and western United States and northern Mexico (Ernst and Ernst 2003). Ground snakes have both mimetic and non-mimetic morphs, with individuals either uniform brown, gray, or tan, red-striped dorsally, darkly banded, or both banded and red-striped (the coralsnake mimic phenotype, fig. 4.1). All *Sonora* species are considered coralsnake mimics (Savage and Slowinski 1992; Greene 1997; Brodie and Brodie 2004) and possess all of the key features typical of other mimetic species, including 1) geographic ranges with broad areas of sympatry with coralsnakes, 2) highly visible red and black coloration, and 3) color pattern conservatism across the genus suggesting an evolutionary history of mimicry (Cox et al. 2012). Color polymorphism also extends throughout the genus (Echternacht 1973; Cox et al. 2012), as black and red, solid red, and even tricolor (black, red, and yellow) morphs are found in the three Mexican congeners (*S. michoacanensis*, *S. mutabilis*, and *S. aemula*).

We used the extraordinary color pattern diversity in ground snakes to characterize landscape-scale patterns of selection on color pattern. Specifically, we compared patterns of within- and among-population variation in color pattern morph frequency. We accomplished this by using three separate but complementary

approaches: 1) comparing morph frequencies among ground snake size classes to determine morph-associated survivorship within a populations, 2) modeling temporal variation in morph composition within populations to infer variation in selection over time, 3) using Bayesian genome scans with population AFLP data and the locus-comparison approach (Gillespie and Oxford 1998) to characterize geographic patterns of selection among populations on color pattern.

4.3 Material and Methods

4.3.1 Field and tissue sampling

Snakes were collected by turning rocks in appropriate habitat across their geographic range from 2008 to 2010. We preserved muscle, liver, or skin tissue in lysis buffer, 95% ethanol or buffer. Although some specimens were sampled for tissues and released, most specimens were fixed in 10% formalin and fluid-preserved in 70% ethanol. Specimens were deposited in the University of Texas-Arlington Amphibian and Reptile Diversity Research Center, the Museo Zoologica de la Facultad de Ciencias at the Universidad Nacional Autonoma de Mexico, and Sternberg Museum at Fort Hays State University.

4.3.2 Temporal and age-class variation analysis

To assess changes in color morph frequency across age classes and time, we scored the color pattern of 458 fluid preserved *S. semiannulata* specimens from **9** different institutional collections (see Appendix B). For each specimen, we measured snout-vent length (SVL) and scored the color pattern as uniform (U), red-striped (S), black-banded (B), or both striped and banded, or mimetic (M). Each individual was

also photographed, and in some cases, the photograph was re-examined to ensure correct color pattern scoring. Field collected specimens were scored while alive, except for one small subset also scored after fluid preservation for several months. In all cases, our color pattern assessments before and after preservation were identical.

To test for a change in color morph frequencies between juveniles and adults, we analyzed 203 preserved specimens collected between 1952 and 1962 from the greater Phoenix metropolitan area in Maricopa Co., AZ (see Appendix B), which was the only population for which we had a robust sample size within a small enough time window to reasonably approximate a single generation. We considered specimens juveniles if they were less than 230 mm SVL (after Kassing 1961). First, we tested whether the frequency of the four morphs varied between juveniles and adults using Fisher's exact test in R v2.14.0. To conservatively rule out potential bias due to the better preservation of black than red pigment in museum specimens, we then repeated the analysis on snakes coded only for the presence or absence of black bands (collapsing individuals scored M and B into a "banded" and S and U into an "unbanded" category).

To test for change in morph frequency over time, we first isolated all populations from our museum and field dataset which had a) more than 30 specimens over a time span of at least 40 years, and b) all four color morphs (see Appendix B). For each of these five populations, we used a multinomial regression from the 'mlogit' package in R v2.14.0 to calculate the probabilities of detecting each morph over that population's sampling interval.

4.3.3 Molecular and population sampling

We assessed the genetic structure of 261 ground snakes from across their geographic range. We focused on 225 ground snakes from 11 different populations within the Great Plains region (Appendix A; Table 1; Figure 4. 2). We focused on this area because 1) the geographic region forms a monophyletic molecular clade (and is closely related to clades from south Texas and the Western United States; A.R. Davis pers. obs.), 2) populations are often highly polymorphic for dorsal color pattern, and 3) snakes are abundant enough for population genetic sampling. We used amplified fragment length polymorphisms (AFLPs) to determine the neutral genetic structure of ground snakes, following Vos (1995) using slightly modified primers (Table 2). We objectively scored AFLPs using the R script (R Development Core Team 2008) AFLPscore (Whitlock et al. 2008), and processed AFLP loci for analysis using AFLPdat (Ehrich 2006).

4.3.4 Genome scans for selection

We used two different Bayesian-based genome scan approaches to detect selection for both color pattern and AFLP loci. First, we used Mcheza (Antao and Beaumont 2011), which implements the DFdist backend program (Beaumont and Balding 2004), to identify candidates for selection. Briefly, this approach uses hierarchical Bayesian methods with Markov Chain Monte Carlo (MCMC) to identify loci with outlier F_{st} values. We followed suggested parameter settings (Antao and Beaumont 2011) and estimated outliers using 100,000 generations and a conservative false discovery rate (FDR=0.001). Second, I used the program Bayescan (Foll and

Gaggiotti 2008) to identify candidate loci. Briefly, this program uses a Bayesian approach with reverse-jump Markov Chain Monte Carlo (rjMCMC) to identify outlier loci and differs from Mcheza (Antao and Beaumont 2011) in implementing more realistic population models that may be more robust to false positives (Foll and Gaggiotti 2008). We used suggested parameter settings (Foll and Gaggiotti 2008) and estimated outliers using 100,000 generations and a conservative false discovery rate (FDR=0.001). We analyzed our dataset in a stepwise fashion by analyzing an AFLP-only dataset and removing selected loci and then analyzing a combined AFLP (with selected loci removed) and color pattern dataset. Loci considered putatively under selection were excluded for the remainder of the analyses.

4.3.5 Population structure analysis

Selection on color pattern polymorphism may be inferred simply based upon a locus-comparison study (Gillespie and Oxford 1998; Andres et al. 2000; Hoffman et al. 2006; Abbot et al. 2008; Croucher et al. 2011). This approach compares the population parameters derived from a putatively neutral marker to that of a phenotypic marker. High differentiation of neutral markers relative to the variable phenotypic trait suggests directional selection among populations on color pattern, while low differentiation of neutral markers relative to variable phenotypic traits suggests diversifying selection among populations on color pattern (Gillespie and Oxford 1998). This approach has been used to infer selection on color pattern polymorphisms in invertebrates such as damselflies (Andres et al. 2000; Abbot et al. 2008) and spiders (Gillespie and Oxford

1998; Croucher et al. 2011), but has only rarely been applied to vertebrates (Hoffman et al. 2006).

We used the locus comparison approach to examine genetic variation within populations, genetic distance between populations, and levels of population segregation for both neutral genetic markers (AFLPs) and color pattern. We assessed AFLP variation in four ways: principle coordinates analysis (PCO), Shannon's information index (I), Nei's unbiased genetic distance (h), and fixation index (F_{ST}). For analyses of color pattern, we treated banding and striping as separate dominant loci, similar to AFLP loci (i.e. Croucher et al. 2011). Although the inheritance of color pattern has not been tested specifically in ground snakes, pigmentation synthesis pathways are known to be highly conserved across vertebrates (Bagnara et al. 1979; Kondo and Shirota 2009; Kanehisa et al. 2012). As in amphibians and fish, black (melanins) and red (pteridines) pigments in snakes are synthesized inside cellular organelles within two different chromatophores (melanophores and erythrophores, respectively) from different dermal tissue layers (Bagnara 1983). Common garden breeding experiments (Bechtel and Bechtel 1962; Bechtel 1978; Bechtel and Bechtel 1978) have shown that red and black coloration in other colubrid snakes is genetically controlled, likely by separate unlinked loci with simple Mendelian inheritance, justifying our decision to code black and red coloration as separate dominant loci. We excluded AFLP loci that were statistically associated with color pattern loci and that were identified as candidates for selection from the genome scan analyses for a final dataset of 106 AFLP loci.

To assess neutral genetic variation, we visualized population subdivision by performing a principle coordinates analysis (PCO) using the packages 'ape' and 'vegan' in R v2.14.0. We analyzed both the dataset restricted to the 11 populations within the Great Plains clade and the larger dataset which included 3 populations in the Western US clade (data not shown). We then generated Shannon's I and Nei's unbiased genetic variation statistics in Genalex (Peakall and Smouse 2006) to compare variation within populations for color pattern and AFLPs. We used Pearson's R (significance determined Bonferroni probabilities) and Spearman's Rank correlation (significance determined by bootstrapping) to assess the relationship between color pattern and AFLP variation (untransformed and log-transformed for Pearson's R correlation) within populations using the program Systat (Systat Software, Inc.). We then used simple and partial (with geographic distance as a covariate) Mantel tests, implemented in zt (Bonnet and van de Peer 2002), to assess the relationship between color pattern and AFLP variation between populations. For comparisons of genetic variation within populations and genetic distance between populations, we interpret a positive correlation to be consistent with no selection, and no correlation or a negative correlation to be consistent with selection.

We also compared patterns of population segregation between AFLP and color pattern loci. We used Genalex (Peakall and Smouse 2006)to calculate θ [$F_{st,}$ denoted as ϕ_{pt} in Genalex (Peakall and Smouse 2006)] for both types of loci, and generated 95% confidence intervals by bootstrapping our dataset with 9999 replicates. Because current genetic structure is a combination of ancestral as well as current gene flow, we

separated our AFLP dataset to parse out these patterns. We identified loci that were variable in all ground snake clades as basal loci (n=3) that would indicate more historical population structure. Conversely, loci that were variable only in the Great Plains clade were considered derived loci (*n*=44) that reflect more recent patterns of populations structure. These separate datasets were used to determine the relative importance of recent and historic gene flow in generating the current population structure in ground snakes. Estimates of θ were then calculated for both the derived (*n*=3) and basal datasets (*n*=44) and bootstrapped 99% confidence intervals as previously described. In all cases, we consider $\theta_{color} = \theta_{AFLP}$ indicative of no selection, $\theta_{color} \leq \theta_{AFLP}$ to be indicative of balancing selection, and $\theta_{color} \geq \theta_{AFLP}$ indicative of selection for local adaptation among populations (Gillespie and Oxford 1998; Abbot et al. 2008).

4.4 Results

4.4.1 Temporal and age-class variation in color pattern

We found a dramatic decrease in the frequency of the uniform morph in adults compared to juveniles (FET, P<0.05), and a corresponding increase in the frequency of mimetic and striped adults (Figure 4.3) in the Phoenix populations. When considering only bands, we found a significant increase (FET, P<0.05) in the frequency of individuals with bands and a corresponding decrease of individuals without bands in adults compared to juveniles (Figure 4.3). We found that the probability of detection of each morph varied over time in four of five populations that we considered (Figure

4.3). For these populations, detection probabilities of different morphs displayed opposing trends within the same population (Figure 4.3).

4.4.2 Genome scans for selection

We found that 15 AFLP loci were identified as putatively under selection, and these were removed from the analysis. Both Bayescan and Mcheza identified banding and striping loci as significant outliers (both P's<0.001), while neither program identified the neutral AFLP loci as significant outliers (Figure 4.2).

4.4.3 Population structure analyses

We found that the genetic structure of populations was inconsistent with geography, with most individuals from all populations occupying a similar location in genotypic space (Figure 4.2). We found no significant correlation (*P'*s from 0.10 to 0.19) between color pattern and AFLP variation within populations (Shannon's I and Nei's Unbiased heterozygosity) using parametric (Pearson's R) and non-parametric correlation (Spearman's Rank) for any measure of population variation (Shannon's I and Nei's unbiased genetic distance on transformed and untransformed data). Notably, although not significant, all correlations were negative (*R'*s from -0.60 to -0.43). Similarly, we found no significant relationship (*P'*s from 0.20 to 0.51) between color pattern and AFLP distance between populations (binary genetic distance and Nei's unbiased genetic distance) using simple (*r'*s from -0.06 to 0.13) and partial (controlling for geographic distance, *r'*s from -0.06 to 0.18) Mantel tests. We found that ϕ_{pt} for AFLP loci (0.034) was significantly (*P*<0.001) lower (Figure 4.2) than for color pattern (0.49). Conversely, separating AFLP loci into derived and basal loci (Figure 4.2)

resulted in ϕ_{pt}'s that were statistically similar (P>0.5) to the entire AFLP dataset (derived and basal ϕ_{pt}'s = 0.041 and 0.038, respectively), but significantly different from the color pattern ϕ_{pt} (P<0.001).

4.5 Discussion

The two main conclusions of our study are that 1) selection is acting upon color pattern within populations of ground snakes, and 2) selection is driving landscape-scale variation in morph frequencies among populations of ground snakes. Within populations, we found clear evidence that morph frequencies vary between juvenile and adult age classes and oscillate over time within single populations, which are hallmarks of the selective process. Among populations, we found large differences in phenotypic frequencies of color morphs, even between neighboring populations, despite minimal genetic population structure across all of the Great Plains. This striking discrepancy between phenotypic and genetic markers strongly suggests that selection, as opposed to neutral forces such as gene flow and drift, promotes color pattern variation among populations (i.e. Jorgensen et al. 2006; Abbot et al. 2008). Additionally, we found that the temporal dynamics of color pattern varied across populations (Fig. 4.3), which could also contribute to color pattern variation among populations. Although locus comparison results alone could suggest the presence of selection, the additional museum-based lines of evidence supports the conclusion that selection is important for both within-population color pattern polymorphism and among-population variation in morph frequency for ground snakes.

Both the shift in morph frequency between age classes in the Phoenix population and the mismatch between the F_{st} for AFLP loci and color pattern that we found are quite dramatic. Indeed, the long-term persistence of color patterns experiencing very low survivorship in Phoenix may superficially seem counterintuitive, and we offer two explanations for this pattern. First, frequency-dependent selection may cause a reversal in the direction of selection, and we note this apparent reversal as the uniform morph increases in frequency over time in the Phoenix population (Figure 4.3). Second, even in the presence of strong selection, the uniform morph may persist in the population through recessive null alleles carried by any of the other three color morphs. In our population genetic data, although we found that the F_{st} for color pattern was much greater than that for AFLPs (0.54 compared to 0.034), this is close to the range of values (0.008 to 0.437 for color pattern, 0.01 to 0.14 for neutral markers) obtained in similar studies (Gillespie and Oxford 1998; Andres et al. 2000; Hoffman et al. 2006; Jorgensen et al. 2006; Abbot et al. 2008; Croucher et al. 2011). Thus, we consider our finding of strong selection using multiple types of data to simply underscore the strength of the patterns that we report.

There are two different (although not mutually exclusive) selective mechanisms that could underlie the discordance between genetic and phenotypic markers, the age-class shifts in morph frequencies and temporal variation in morph frequency: frequency-dependence and environmental variation. Frequency-dependent selection occurs when the fitness of one phenotype is dependent on the frequency of other phenotypes in the population (Joron and Mallet 1998; Gray and McKinnon 2006). This

type of selection is often characterized by temporal and geographic variation in the strength and direction of selection because the dynamics of colonization, population size, and gene flow will influence the tempo and mode of frequency-dependent cycles within each local population (Joron and Mallet 1998; Gray and McKinnon 2006). Beyond frequency-dependence, geographic variation in the biotic (e.g., predator assemblages) and abiotic (e.g., access to refugia) environment could alter selection on color pattern among populations. Our findings of temporal variation in the frequency of morphs, with different morphs exhibiting opposing trends in temporal frequency, as well as the uncoupling of neutral genetic and color pattern population structure is consistent with frequency-dependent selection within populations driving color pattern variation between populations.

Our results have two important implications for understanding the evolution of mimicry systems. First, both frequency-dependence and environmental variation can be thought of under the umbrella concept of a geographic mosaic of coevolution (Thompson 1994; Thompson 2005), which unites within- and between-population dynamics across a landscape. This pattern of geographic and temporal variation in phenotype controlled by selection could be produced by a complex interaction between frequency-dependent selection, range expansion and contraction, geographic variation in selection and variation in population size (Brodie et al. 2002; Thompson 2005; Pfennig and Mullen 2010). Our results provide an empirical example of coralsnake mimicry that fits the theoretical expectations of a geographic mosaic. Because color pattern polymorphism is widespread throughout coralsnake (Savage and Slowinski

1992; Brodie and Brodie 2004) and other mimicry complexes (Clarke et al. 1968; Joron and Mallet 1998; Mallet and Joron 1999), studies that explicitly consider temporal and geographic variation in the evolutionary dynamics of mimicry should be considered critical to understanding the evolutionary dynamics and mechanisms underlying mimicry.

Finally, our study empirically links research in coralsnakes and classic invertebrate systems to conceptually unify our understanding of mimicry. Because studies of invertebrate mimics have also found high levels of spatial and temporal variation in selection regimes (Waldbauer 1988; Joron and Mallet 1998; Mallet and Joron 1999; Gilbert 2003), this complexity may be a fundamental outcome of mimetic interactions across taxa. Robust geographic sampling is often more challenging in vertebrate systems, but comparisons across taxa are the best way to assess generalities in the patterns and processes driving mimicry. Despite the deceptively simple logic behind how mimicry systems operate, our results highlight the inherent complexity in the selective forces driving the mimetic phenotype.

Figure 4.1. Different morphs of the ground snake (*S. semiannulata*). Clockwise from bottom left; Striped morph (S) with red dorsal stripe (bottom left), Banded morph (B) with black crossbands (top left), Uniform morph (U) lacking both the red dorsal stripe and black crossbands (top right) and the mimetic morph (M) with both the red dorsal stripe and black crossbands (bottom right).

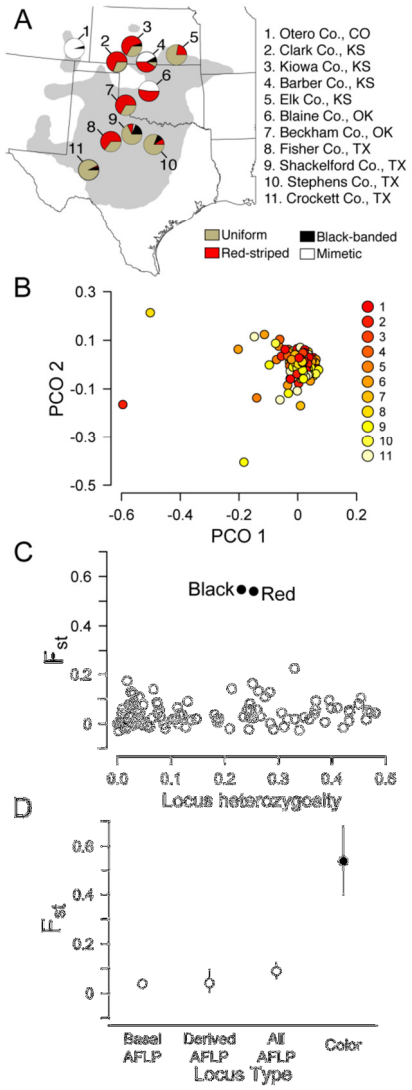

Figure 4.2. Genetic structure and spatial variation in color pattern polymorphism. a) Geographic distribution of the Great Plains clade of ground snake, with populations for the population genomic study. Pie charts represent the relative frequencies of each morph in each population. b) Principle coordinate analysis of AFLP data. Note the similar genetic clustering of all populations. c) F_{st} versus locus heterozygosity for all AFLP loci. The black and red color pattern were the only loci identified as significant outliers using Bayescan (Foll and Gaggiotti 2008) and Mcheza (Antao and Beaumont 2011). d) F_{st} for basal and derived AFLP loci, all loci combined and color pattern. Note that color pattern F_{st} was significantly greater than all other loci categories.

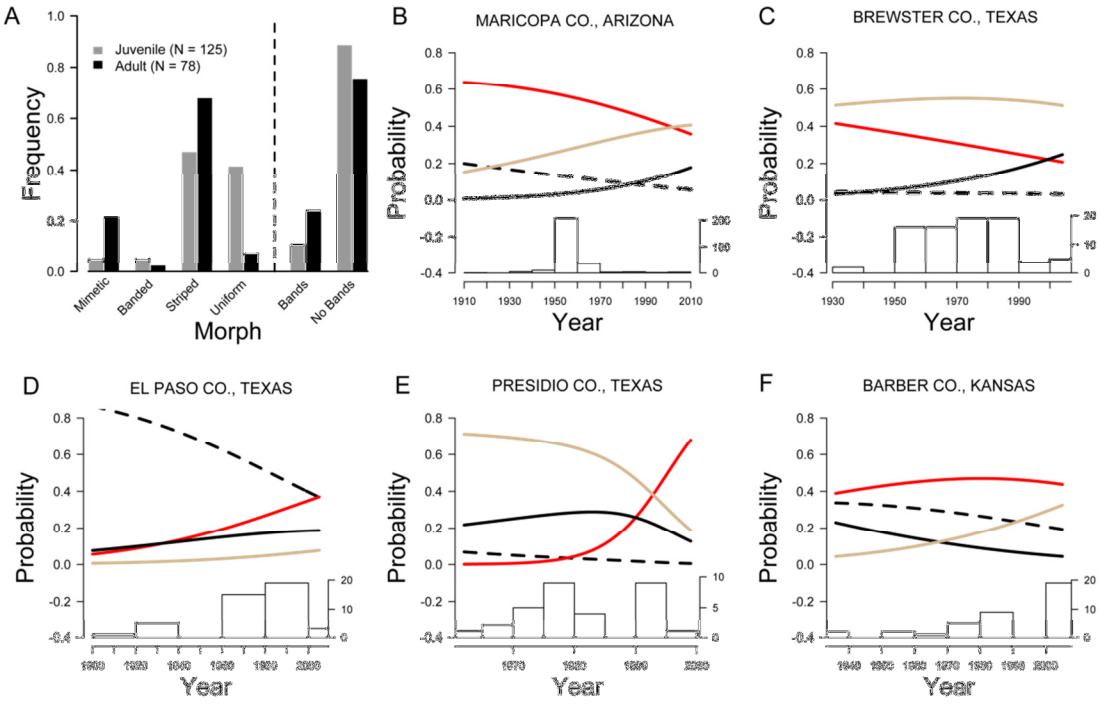

Figure 4.3. Temporal and age-class variation in color pattern. a) Frequency of color pattern morphs of juvenile and adult *S. semiannulata*. b-f) Colored lines are the probabilities of detection from multinomial logistic regression for striped (red line), banded (dashed line), uniform (tan line) and mimetic (solid black line) morphs over time for different populations. Histogram underneath trendlines is the number of individuals for each time segment.

Table 4.1. Population localities and morph compositions.

Population	Latitude	Longitude	Locality	Uniform	Red-striped	Banded	Mimetic
Barber	37.4139	-98.9656	Barber Co., KS	1	5	1	5
Barn	31.0430	-100.9838	Crockett Co., TX	38	1	2	0
ClarkKS	37.3839	-99.7862	Clark Co., KS	5	11	0	0
Geary	35.7504	-98.3952	Blaine Co., OK	0	10	0	9
Kiowa	37.4480	-99.2599	Kiowa Co., KS	7	13	1	0
LongtonKS	37.3357	-96.1076	Elk Co., KS	7	2	0	0
Roby	32.7341	-100.5711	Fisher Co., KS	6	11	0	0
SandSand	35.0649	-99.8393	Beckham Co., OK	5	10	0	0
SECO	37.7583	-103.6184	Otero Co., CO	0	0	1	22
Shack	32.6357	-99.4554	Shackleford Co., TX	21	3	6	1
Steph	32.6680	-98.5917	Stephens Co., TX	17	2	2	0

Table 4.2. Adaptor, preselective primer and selective primer sequences for AFLP analysis

Name	Sequence 5' -3' (brackets indicate selective bases)
MSE Adaptor 1	GACGATGAGTCCTGAG
MSE Adaptor 2	TACTCAGGACTCAT
EcoR1 Adaptor 1	CTCGTAGACTGCGTACC
EcoR1 Adaptor 2	AATTGGTACGCAGTCTAC
Preselective (MSE1) Primer 1	GATGAGTCCTGAGTAA[GC]
Preselective (EcoR1) Primer 2	GACTGCGTACCAATTC[G]
Selective (MSE1) Primer 1	GATGAGTCCTGAGTAA[GCC]
Selective (EcoR1) Primer 2	GACTGCGTACCAATTC[GA]

.

CHAPTER 5

THE ROLE OF SEQUENCE VARIATION IN MC1R IN COLOR PATTERN POLYMORPHISM FOR THE GROUND SNAKE (SONORA SEMIANNULATA)

5.1 Abstract

Color pattern polymorphism is widespread across animals, and has been implicated in the dynamics of mimicry complexes, sexual selection, and speciation. However, the mechanisms maintaining polymorphisms in natural populations are not well understood. Selection on color pattern polymorphism has been examined using population genetics, phylogenetics and clay model studies. However, a complementary approach is to sequence the genomic regions underlying color pattern polymorphism and measure the statistical signature of selection. We adopt this approach with the polymorphic ground snake (*Sonora semiannulata*). Ground snakes are found throughout central and western North America, and are polymorphic for black bands and a red dorsal stripe. We sequenced the melanocortin-1 receptor (*Mc1R*) of ground snake populations from across their geographic range. We also include the other species of *Sonora* and the closely related snake genera *Chionactis* and *Chilomeniscus*. *Mc1R* is an important gene in the melanin synthesis pathway, and is associated with ecological variation in color pattern in birds, mammals and other squamate reptiles. We evaluated the molecular evolution of *Mc1R*, tested amino acid variation for association with banding, and examined sequence variation for the selection. *Mc1R* nucleotide sequence

was variable, and within *S. semiannulata* there are both fixed and heterozygous nucleotide substitutions that result in an amino acid change. However, we did not detect any statistical association with banding. Selection analyses indicated that *Mc1R* sequence was likely under purifying selection. These results suggest that sequence variation in *Mc1R* does not control color pattern polymorphism in *S. semiannulata*, and highlight the limitations of traditional candidate gene approaches.

5.2 Introduction

Studying the genetic factors underlying a trait is fundamental in evolutionary biology and can provide insight into dynamics of selection and molecular basis of adaptation (Hoekstra 2006). However, for many non-model organisms, there are no genomic resources available for such studies. The candidate-gene approach involves screening sequence variation in likely "candidate" genes for association with the trait of interest and is easily applicable to non-model systems (Rosenblum et al. 2004; Hoekstra 2006). This type of study has been especially fruitful in the study of color pattern polymorphism in natural populations (Hoekstra and Nachman 2003; Nachman et al. 2003; Uy et al. 2009).

Color pattern polymorphism, or the presence of co-occurring color types within a single species or population, presents an interesting dilemma in evolutionary biology. Because random genetic drift will tend to fix neutral alleles over time, the persistence of polymorphism within populations must be explained by either adaptive (frequency dependent selection, heterosis, etc.) or stochastic (neutral drift with gene flow)

processes (Gray et al. 2007). Color polymorphism is extremely widespread across animals, and it has been implicated in the dynamics of mimicry complexes (Mallet and Joron 1999; Campbell and Lamar 2004a), sexual selection (Sinervo and Lively 1996; Gray and McKinnon 2006), speciation (Gray and McKinnon 2006; Corl et al. 2010) and adaptive background matching (Hoekstra et al. 2006; Rosenblum et al. 2010).

The ground snake, *Sonora semiannulata,* is a vertebrate with marked color pattern polymorphism (Fig 5.1). *Sonora semiannulata* exhibits four different dorsal color patterns, with individuals that are plain, red-striped, darkly banded, or both banded and red-striped. The degree of polymorphism within populations of *S. semiannulata* is geographically variable, with some populations dominated by one pattern and others containing all pattern types (Werler and Dixon 2000; Ernst and Ernst 2003). Although no breeding studies have addressed the transmission genetics of color pattern in ground snakes, banding and striping are generally under simple genetic control in snakes (Zweifel 1981; Bechtel and Whitecar 1983; Bechtel 1995). We propose to determine the role of sequence variation in *Mc1R*, a gene that controls melanistic color in squamates and other vertebrates (Hubbard et al. 2010; Rosenblum et al. 2010), in color pattern variation in *Sonora* and close relatives**.**

Although many genes have been implicated in color pattern development in vertebrates, *Mc1R* has emerged as a particularly important single gene underlying the genetic architecture of color pattern (Rosenblum et al. 2004; Mundy 2005; Hoekstra 2006; Hubbard et al. 2010). *Mc1R* is a single-copy gene that encodes for melanocortin-1 receptor, a G-protein coupled receptor with extensive transmembrane domains

(Hoekstra 2006). In mammalian systems, *Mc1R* acts as a molecular switch between eumelanin (black or brown pigment) and pheomelanin (red pigment) production based on binding with either alpha-MSH or agouti ligands, respectively (Hoekstra 2006). The melanin pathway is not well characterized in reptiles, with only one type of melanin (eumelanin) identified (Rosenblum et al. 2004). Rather than acting as a switch between eumelanin and pheomelanin, it is thought that *Mc1R* controls the amount of melanin deposited in reptiles (Rosenblum et al. 2004; Hubbard et al. 2010). The *Mc1R* gene has been implicated in color pattern polymorphism in mammals (Nachman et al. 2003; Hoekstra et al. 2006), birds (Mundy 2005; Uy et al. 2009), and reptiles (Rosenblum et al. 2004; Rosenblum et al. 2010). In addition, *Mc1R* is known to be mostly free of pleoiotropic effects, and the variation in *Mc1R* among diverse taxa may indicate that selection is free to act on this locus (Hoekstra et al. 2006).

Because of minimal pleiotropy, simple structure (a single exon that is 1 kbp in length), and excellent characterization in other vertebrates, *Mc1R* is an attractive candidate locus for controlling banding in *Sonora* and it close relatives. We sequenced *Mc1R* for 65 *S. semiannulata*, *S. aemula*, *S. mutabilis*, *S. michoacanensis*, *Chilomeniscus*, and *Chionactis* to: 1) examine the molecular evolution of the *Mc1R* gene in *Sonora* and its close relatives, 2) determine whether sequence variation in this gene is statistically associated with loss of melanic banding in *Chilomeniscus*, *Chionactis* and *Sonora* across their geographic range, and 3) test for the molecular signature of selection on this color pattern gene.

5.3 Materials and Methods

5.3.1 Taxonomic sampling

We obtained tissues for all species of *Sonora*, and representatives of the genera *Chionactis* and *Chilomeniscus* (Table 1). For *S. semiannulata*, we included individuals from across their geographic range and from multiple individuals from several populations that were polymorphic for banding (Table 1). Additionally, we included individuals with aberrant banding (e.g. faint or incomplete banding). We included two other snake species (*Thamnophis sirtalis* and *Crotalus tigris*), and three lizard species that have experienced adaptive evolution of Mc1R (*Aspidoscelis inornata*, *Sceloporus undulatus*, and *Holbrookia maculata*).

5.3.2 Molecular methods

While the internal segment of *Mc1r* is relatively conserved, the external sequence of the genes can be quite variable. Therefore, universal primers are not available and acquiring novel sequence data for *Mc1R* requires genomewalking. This is a standardized, ligation based approach for acquiring additional sequence from a known sequence. We adopted this approach using the Clontech Genomewalking Kit. First, we sequenced a ~450 bp internal fragment of *Mc1r* for a subset of Sonora tissues. We used this known sequence to design group-specific internal primers for Sonora and close relatives (Table 5.2). We then used the Clonetech kit to genomewalk the sequences upstream and downstream of the internal *Mc1R* fragment (see the Clontech Genomewalker Universal Kit User manual for more details). Briefly, we digested extracted tissue samples using one of four different restriction enzymes (Dra I, EcoR V,

Pvu II and Stu I to create four different digestion libraries. Digested libraries were purified and extracted using a phenol chloroform extraction, and were rehydrated in 1X TE buffer. Universal adaptors (see Clontech Genomewalker Universal Kit User manual for sequence) were ligated to the digested product using T4 DNA ligase (3 units) at 16 C for 12 hrs. We then amplified from the purified and digested product using the internal Mc1R primers and universal adaptor specific primers (see Clontech Genomewalker Universal Kit User manual for sequence) using a simplified two step touchdown thermal cycle (94 C for 25 sec followed by 3 min at 72 C for 7 cycles and 94 C for 25 sec followed by 3 min at 67 C for 32 cycles with final 7 min 67 C extension). We then performed a nested PCR using an aliquot of the primary PCR with similar thermal cycling parameters (94 C for 25 sec followed by 3 min at 72 C for 5 cycles and 94 C for 25 sec followed by 3 min at 67 C for 20 cycles with final 7 min 67 C extension). Results for each step were visualized on a 1-1.5% ethidium bromide stained agarose gel, and all experiment included a positive (human genomic DNA) and negative (deionized water) control. This process yielded fragments ranging from 200-1500 bp upstream and downstream of the internal *Mc1R* fragment for a total of 1700 bp spanning the approximately 1 kb coding sequence. PCR products were prepared for sequencing by the ExoSAP-IT kit (United States Biochemical) and we used the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems Inc.) to sequence the PCR products following the manufacturer's protocol. The sequenced products were precipitated using an ethanol/sodium acetate method and rehydrated in HPLC purified formamide (Hi-Di). The sample was then analyzed either on a ABI PRISM 3100xl

Genetic Analyzer in the Genomics Core Facility at the University of Texas-Arlington. Sequences were edited and assembled using Sequencher (Genes Code Corps., Inc.). We then designed 4 different primer pairs that captured the entire coding coding region, and found one primer pair that was reliable and yielded 1150 bp including the entire coding sequence (Table 5.2). These primers were used to amplify the entire Mc1R coding sequence for all tissues. Individual sequences were exported to MEGA (Tamura et al. 2011), aligned in MEGA using the CLUSTAL algorithm (Larkin et al. 2007) with default parameters and manually adjusted if necessary.

5.3.3 Analytical methods

We assessed molecular evolution of the *Mc1R* gene within *Chilomeniscus*, *Chionactis* and *Sonora* by determining the distribution of indels, synonymous and nonsynonymous mutations of the coding sequence and the amino acid sequence. Because our greatest sampling was for the genus *Sonora* and *S. semiannulata*, we also determined average percent divergence for these groups. We tested nonsysnonymous mutations for association with banding in *S. semiannulata* Fisher's Exact Test. We specifically scrutinized the amino acid residues that are association with adaptive color variation in other squamate reptiles. Finally, we tested for the presence and type of selection on *Mc1R* for *S. semiannulata* using three different tests. First, we used Tajima's D, which compares patterns of mutation in putatively neutral loci to the focal loci. Second, we used the codon-based Fisher's Exact test, which compares the proportion of synonymous and nonsynonymous mutations. Finally, we used a

maximum-likelihood approach (HyPhy) to test for the presence of selection on specific codons.

5.4 Results

5.4.1 Molecular variation

We found that the coding sequence of *Mc1R* for *Chilomeniscus*, *Chionactis* and *Sonora* was 948 bp, confirmed by the presence of a start and stop codon. Relative to the outgroups, *Chilomeniscus*, *Chionactis* and *Sonora* possess a 12 bp indel near the beginning of the coding sequence at 78 bp. *H. maculata* and *S. undulatus* possess a 3 bp indel at 54 bp relative to all other taxa. In addition, one individual of *S. mutabilis* has a single 6 bp indel at 775 bp, near the end of the coding sequence. *Mc1R* was variable, with a maximum of 0.5% sequence divergence across *Chilomeniscus*, *Chionactis* and *Sonora*. Sequence variation within the genus *Sonora* was 0.4%, and 0.1 % within *S. semiannulata*. We found that amino acid sequence variation was minimal. *S. semiannulata* possessed three apparently fixed amino acid substitutions and 8 heterozygosities that resulted in amino acid substitutions. All amino acid substitutions were rare, occurring in one or two specimens (maximum of five individuals for one substitution) out of 54 *S. semiannulata*.

5.4.2 Association analysis

Amino acid variation was not associated with banding or aberrant banding patterns for *S. semiannulata* ($P>0.05$). Additionally, we found no sequence variation in the amino acid residues controlling adaptive color pattern variation in other squamate reptiles.

5.4.3 Selection analyses

Tajima's D for Mc1R was -2.08, consistent with purifying selection. We found that *Mc1R* sequences did not differ significantly from neutrality according to the codon-based Fisher's exact test. Using HyPhy, around 258 codons (82% of the coding sequence) appeared to be evolving under purifying selection. All of the codons putatively under purifying selection were in the middle 275 codons of the coding region (Figure 5.1).

5.5 Discussion

We found that while *Mc1R* coding region was variable among *Chilomeniscus*, *Chionactis* and *Sonora*, this resulted in relatively few amino acid substitutions. These low frequency amino acid substitutions were not significantly associated with banding, and our analyses suggest that purifying selection is acting on this nuclear gene. Below we discuss the implications of our results and suggest directions for future research.

We did not detect a statistical association of *Mc1R* sequence variation with banding. This finding is supported by the fact that we detected zero nucleotide or amino acid variation in the sites associated with adaptive variation in *Mc1R* in mice and other squamate reptiles. Additionally, the major indels in *Mc1R* were not associated with banding, although one indel was common to all *Mc1R* sequences from *Chilomeniscus*, *Chionactis* and *Sonora*. Undoubtedly *Mc1R* is important for the production of melanin in snakes (Hubbard et al. 2010; Rosenblum et al. 2010), and we cannot rule out a role for *Mc1R* in banding polymorphism in *Sonora*. It is possible that sequence variation in upstream and downstream regulatory regions and transcriptional regulation are all

potential mechanisms controlling color pattern. We suggest that future studies should examine sequence variation outside of the coding sequence and that studying transcriptional regulation of *Mc1R* could clarify the role of *Mc1R* in color pattern variation in ground snakes.

Many nuclear protein-encoding genes bear the statistical signature of purifying selection (Graur and Li 2000). Given the important role of *Mc1R* in color pattern development in squamates, it is unsurprising that much of the *Mc1R* gene appears to be evolving under purifying selection. Maximum likelihood analysis suggests that the beginning and final codons are not evolving under purifying selection, and this may explain the difficulty in designing universal primers for this gene. These results are consistent with other studies that suggest that this conserved gene is subject to purifying selection (Harding et al. 2000; Hubbard et al. 2010).

The *Mc1R* gene has proved useful for understanding the molecular control of ecologically and evolutionarily important color pattern variation pattern (Rosenblum et al. 2004; Mundy 2005; Hoekstra 2006; Hubbard et al. 2010). Research within multiple study systems has identified the role of Mc1R in color pattern variation associated with background matching, mate choice and speciation (Rosenblum et al. 2004; Hoekstra et al. 2006; Uy et al. 2009). However, a great number of studies have found that sequence variation in *Mc1R* was not associated with the color pattern feature of interest (e.g., Herczeg et al. 2010; Dorn et al. 2011). Research on *Mc1R* has been important for the study of ecological genetics in a broad taxonomic context. Accordingly, publishing negative results (i.e., instances where sequence variation is not associated with the trait

of interest) is important to gain an accurate idea of the role of Mc1R variation for color pattern polymorphism or color pattern variation in natural populations. Our results provide a valuable contrast to the research that documents a role of Mc1R in color pattern variation in squamate reptiles and other vertebrates.

Although inexpensive, the research of individual candidate loci is relatively slow and laborious. The advent of high-throughput sequencing of both genomes and transcriptomes offers a more robust way to identify the molecular mechanisms underlying color pattern polymorphisms. Because of the discrete polymorphism of banding that is present within a single population, ground snakes may offer an attractive system with which to use transcriptomics to explore the genetic control of color pattern polymorphism in snakes.

Figure 5.1. Banded and unbanded ground snake morphs. Snakes are from a single population in western Texas. Note the polymorphism for melanic banding (banded individual on the far right). Photo by Alison R. Davis Rabosky.

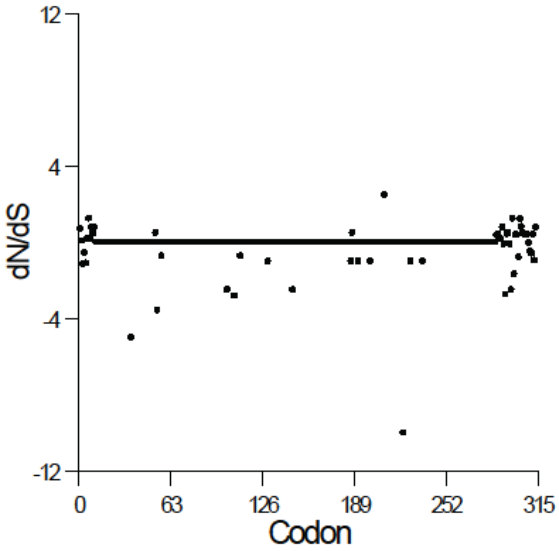

Figure. 5.2. dN/dS ratio of *Mc1R* coding sequence. Display of dN/dS ratios for each codon along the length of the Mc1R coding sequence in *Sonora semiannulata*. Note that dN/dS ratios are more variable at the extremities of the coding sequence, indicating relaxed selection on these codons.

Table 5.1. Specimen information and Genbank accession numbers for all samples in this study. FB=faint bands.

ID	Species	Country	State	County/ Munincipality	Bands	Genbank #
BAL1	*Sonora semiannulata*	USA	AZ	Yavapai	M	JX305468
CER826	*Sonora semiannulata*	USA	TX	Presidio	N	JX305469
CLC011	*Sonora semiannulata*	USA	TX	Shackleford	N	JX305470
CLC012	*Sonora semiannulata*	USA	TX	Shackleford	N	JX305471
CLC013	*Sonora semiannulata*	USA	TX	Shackleford	N	JX305472
CLC031	*Sonora semiannulata*	USA	TX	Shackleford	Y	JX305473
CLC035	*Sonora semiannulata*	USA	TX	Shackleford	Y	JX305474
CLC093	*Sonora semiannulata*	USA	TX	San Saba	Y	JX305475
CLC137	*Sonora semiannulata*	USA	TX	Stephens	Y, FB	JX305476
CLC140	*Sonora semiannulata*	USA	TX	Stephens	N	JX305477
CLC141	*Sonora semiannulata*	USA	TX	Stephens	N	JX305478
CLC142	*Sonora semiannulata*	USA	TX	Stephens	N	JX305479
CLC151	*Sonora semiannulata*	USA	TX	Shackleford	N	JX305480
CLC152	*Sonora semiannulata*	USA	TX	Shackleford	Y	JX305481
CLC204	*Sonora semiannulata*	USA	TX	Crockett	N	JX305482
CLC206	*Sonora semiannulata*	USA	TX	Crockett	N	JX305483
CLC218	*Sonora semiannulata*	USA	TX	Shackleford	Y	JX305484
CLC220	*Sonora semiannulata*	USA	TX	Shackleford	N	JX305485
CLC258	*Sonora semiannulata*	USA	CO	Otero	Y	JX305486
CLC259	*Sonora semiannulata*	USA	CO	Otero	Y	JX305487
CLC261	*Sonora semiannulata*	USA	CO	Otero	Y	JX305488
CLC262	*Sonora semiannulata*	USA	CO	Otero	Y	JX305489
CLC267	*Sonora semiannulata*	USA	CO	Otero	Y	JX305490
CLC328	*Sonora semiannulata*	USA	TX	Stephens	Y, FB	JX305491
CLC369	*Sonora semiannulata*	USA	TX	Jeff Davis	Y	JX305492
CLC371	*Sonora semiannulata*	USA	TX	Hidalgo	N	JX305493
CLC375	*Sonora semiannulata*	USA	TX	Hidalgo	N	JX305494
CLC403	*Sonora semiannulata*	MX	BCS	San Ignacio	N	JX305495
CLC428	*Sonora semiannulata*	USA	AZ	Maricopa	N	JX305496
CLC431	*Sonora semiannulata*	USA	AZ	Santa Cruz	Y	JX305497
CLC432	*Sonora semiannulata*	USA	AZ	Cochise	Y	JX305498
CLC436	*Sonora semiannulata*	USA	TX	Shackleford	N	JX305499
CLC453	*Sonora semiannulata*	USA	OK	Tulsa	Y	JX305500
CLC454	*Sonora semiannulata*	USA	OK	Tulsa	N	JX305501
CLC476	*Sonora semiannulata*	USA	KS	Elk	U	JX305502
CLC478	*Sonora semiannulata*	USA	KS	Elk	N	JX305503
CLC494	*Sonora semiannulata*	USA	OK	Blaine	N	JX305504
CLC495	*Sonora semiannulata*	USA	OK	Blaine	N	JX305505
CLC496	*Sonora semiannulata*	USA	OK	Blaine	Y	JX305506
CLC497	*Sonora semiannulata*	USA	OK	Blaine	Y	JX305507
CLC498	*Sonora semiannulata*	USA	OK	Blaine	N	JX305508
CLC500	*Sonora semiannulata*	USA	OK	Blaine	Y	JX305509
CLC501	*Sonora semiannulata*	USA	OK	Blaine	Y	JX305510
CLC503	*Sonora semiannulata*	USA	OK	Blaine	N	JX305511

Table 5.1 - continued

ID	Species	Country	State	County/ Munincipality	Bands	Genbank #
CLC505	*Sonora semiannulata*	USA	OK	Blaine	Y	JX305512
CLC506	*Sonora semiannulata*	USA	OK	Blaine	N	JX305513
CLC732	*Sonora semiannulata*	USA	TX	Crockett	Y	JX305514
CLC733	*Sonora semiannulata*	USA	TX	Crockett	U	JX305515
EBU1S	*Sonora semiannulata*	USA	NM	Sierra	N	JX305516
EBU2S	*Sonora semiannulata*	USA	NM	Sierra	N	JX305517
HIL1	*Sonora semiannulata*	USA	AZ	Yavapai	Y	JX305518
JMM660	*Chilomeniscus stramineus*	USA	AZ	NA	Y	JX305519
JMMC10	*Chionactis occipitalis*	MX	SON	Badiraguato	Y	JX305520
JMMC6	*Chionactis occipitalis*	USA	CA	Imperial	Y	JX305521
JRV127	*Sonora mutabilis*	MX	JAL	Huaxtla	Y	JX305522
JRV128	*Sonora mutabilis*	MX	JAL	Huaxtla	Y	JX305523
MJI32	*Sonora semiannulata*	USA	TX	Jeff Davis	N	JX305524
MJI84	*Sonora semiannulata*	USA	TX	Shackleford	Y	JX305525
MZFC 23956	*Sonora michoacanensis*	MX	GRO	Campo Morado	Y	JX305526
ROM RWM875	*Sonora semiannulata*	MX	BCS	San Pedro de la Presa	N	JX305527
UANL 6976	*Sonora aemula*	MX	SON	Navajoa	Y	JX305528
TS16	*Thamnophis sirtalis*	NA	NA	NA	NA	AY586157
NA	*Crotalus tigris*	NA	NA	NA	NA	EU526278
EBR50	*Aspidoscelis inornata*	NA	NA	NA	NA	AY586069
EBR88	*Aspidoscelis inornata*	NA	NA	NA	NA	AY586073
EBR91	*Aspidoscelis inornata*	NA	NA	NA	NA	AY586074
EBR358	*Holbrookia maculata*	NA	NA	NA	NA	AY586104
EBR47	*Holbrookia maculata*	NA	NA	NA	NA	AY586110
EBR53	*Holbrookia maculata*	NA	NA	NA	NA	AY586112
EBR138	*Sceloporus undulatus*	NA	NA	NA	NA	AY586150
EBR174	*Sceloporus undulatus*	NA	NA	NA	NA	AY586153
EBR98	*Sceloporus undulatus*	NA	NA	NA	NA	AY586148

Table 5.2. Primers for sequencing *Mc1R* in ground snakes.

Name	Description	Sequence (5'-3')
Mc1RLprim1	Internal Primer	CTGCTTGCCATACATGGTTGAAATCT
Mc1RRprim1	Internal Primer	CTATCACAGTATCATGACCATACAGC
Mc1RLprim2	Nested Internal Primer	AACATGTGGATGTAGAGCCCTGCAATG
Mc1RRprim2	Nested Internal Primer	ATCCTCTTCATTGTCTACGACAGCACT
Mc1RSoseUpstr2	Primer for entire coding sequence	GAAAGCTGCTGACGGAG
Mc1RDstr3	Primer for entire coding sequence	GTCACCTGCTTGCCCTGAATG

CHAPTER 6

CONCLUSIONS AND FUTURE RESEARCH

Our research has offered some initial insight into the evolution of *Sonora*, and the evolution of color pattern polymorphism within coralsnake mimicry complexes. We found that taxonomy did not agree with the phylogenetic systematics of *Sonora*, and so we revised the taxonomy of *Sonora* to better reflect evolutionary relationships. This research also highlighted the early evolution of color pattern polymorphism and mimicry in *Sonora*. We then used population genomics to study how landscape influences patterns of genetic variation and differentiation within the Great Plains regions. We found that populations were genetically homogeneous in the Great Plains, and that landscape factors did not exert a detectable influence on genetic patterns. A similar dataset was used in concert with phenotypic data from field and museum sampling to study temporal and life history shifts in morph composition and the relationship between population genetic structure and color pattern. These data strongly suggest that selection is underlying color pattern polymorphism in ground snakes, and that this selection leads to temporally and geographically variable color pattern polymorphism. Finally, we sequenced a candidate gene for banding, and found that this gene possessed nucleotide and amino acid variation that was not associated with banding. However, outstanding questions include assessing the phylogenetic structure within *S. semiannulata*, determining the relationships of *Sonora* to *Chionactis*,

Chilomeniscus, and other members of the snake tribe Sonorini, determining the genetic control of color pattern polymorphism within *Sonora*, and studying the ecological function of color pattern within *Sonora*. Below I discuss some ongoing and future research that will address these questions.

In collaboration with Alison R. Davis Rabosky, we have initiated a large-scale molecular phylogenetic analysis of the snake tribe Sonorini. Our current taxonomic sampling includes all members of the genera *Chilomeniscus*, *Chionactis*, *Gyalopion*, *Pseudoficimia*, *Stennorrhina* and range-wide sampling of *Sonora*. We also have multiple species of *Tantilla*, *Ficimia*, and relevant outgroups. Our molecular dataset for these species includes two mitochondrial (*cyt-b* and *ND4*) and two nuclear loci (*RAG-1* and *c-mos*). This study will clarify the evolutionary relationships within and among the species of the Sonorini. Importantly, this data will also offer a broader perspective on the evolution of coralsnake mimicry and color pattern polymorphism in Neotropical colubrids.

Another major ongoing research direction is to use maximum likelihood modeling and broad-scale phenotypic data to determine the geographic distribution of color pattern morphs and the likely inheritance model for color pattern. This data will inform the ancestral character state reconstruction of color pattern to map the phylogenetic gain and loss of color pattern traits.

Our future research will target both the molecular origins and ecological function of color pattern polymorphism. In order to identify the ecological function of color pattern in *S. semiannulata*, we will deploy plasticine or clay models of each

morph type in populations with different morph compositions. To assess the molecular mechanisms controlling color pattern polymorphism, we will implement whole genome and tissue-specific transcriptome high-throughput sequencing of each color pattern morphs. This approach will identify candidate genes that can be further developed using traditional PCR and Sanger sequencing.

My dissertation, ongoing and future research has integrated the fields of ecology, systematics, population genetics, molecular biology and genomics to study the evolution of color pattern polymorphism and mimicry. It is my hope that these studies will help clarify the origins of phenotypic diversity in vertebrates.

APPENDIX A

TISSUE SAMPLES FOR POPULATION GENETIC ANALYSES IN CHAPTERS 3 AND 4

ID	Population Code	Locality
MHP11224	Barber	Barber Co., KS
MHP11225	Barber	Barber Co., KS
MHP11226	Barber	Barber Co., KS
MHP11289	Barber	Barber Co., KS
MHP11290	Barber	Barber Co., KS
MHP11291	Barber	Barber Co., KS
MHP11292	Barber	Barber Co., KS
MHP11293	Barber	Barber Co., KS
MHP12886	Barber	Barber Co., KS
MHP12887	Barber	Barber Co., KS
MHP12889	Barber	Barber Co., KS
MHP8262	Barber	Barber Co., KS
CLC056	Barn	Crockett Co., TX
CLC057	Barn	Crockett Co., TX
CLC089	Barn	Crockett Co., TX
CLC090	Barn	Crockett Co., TX
CLC091	Barn	Crockett Co., TX
CLC183	Barn	Crockett Co., TX
CLC184	Barn	Crockett Co., TX
CLC185	Barn	Crockett Co., TX
CLC186	Barn	Crockett Co., TX
CLC187	Barn	Crockett Co., TX
CLC188	Barn	Crockett Co., TX
CLC189	Barn	Crockett Co., TX
CLC190	Barn	Crockett Co., TX
CLC191	Barn	Crockett Co., TX
CLC192	Barn	Crockett Co., TX
CLC193	Barn	Crockett Co., TX
CLC194	Barn	Crockett Co., TX
CLC195	Barn	Crockett Co., TX
CLC196	Barn	Crockett Co., TX
CLC197	Barn	Crockett Co., TX
CLC198	Barn	Crockett Co., TX
CLC199	Barn	Crockett Co., TX
CLC200	Barn	Crockett Co., TX
CLC202	Barn	Crockett Co., TX
CLC203	Barn	Crockett Co., TX
CLC204	Barn	Crockett Co., TX

ID	Population Code	Locality
CLC205	Barn	Crockett Co., TX
CLC206	Barn	Crockett Co., TX
CLC207	Barn	Crockett Co., TX
CLC208	Barn	Crockett Co., TX
CLC210	Barn	Crockett Co., TX
CLC211	Barn	Crockett Co., TX
CLC212	Barn	Crockett Co., TX
CLC731	Barn	Crockett Co., TX
CLC732	Barn	Crockett Co., TX
CLC733	Barn	Crockett Co., TX
CLC735	Barn	Crockett Co., TX
CLC736	Barn	Crockett Co., TX
CLC737	Barn	Crockett Co., TX
CLC738	Barn	Crockett Co., TX
CLC739	Barn	Crockett Co., TX
ARD1	ClarkKS	Clark Co., KS
ARD2	ClarkKS	Clark Co., KS
ARD4	ClarkKS	Clark Co., KS
ARD5	ClarkKS	Clark Co., KS
ARD6	ClarkKS	Clark Co., KS
CLC250	ClarkKS	Clark Co., KS
CLC251	ClarkKS	Clark Co., KS
CLC252	ClarkKS	Clark Co., KS
CLC253	ClarkKS	Clark Co., KS
MHP11313	ClarkKS	Clark Co., KS
MHP11314	ClarkKS	Clark Co., KS
MHP11315	ClarkKS	Clark Co., KS
MHP11316	ClarkKS	Clark Co., KS
MHP11317	ClarkKS	Clark Co., KS
MHP11318	ClarkKS	Clark Co., KS
MHP11319	ClarkKS	Clark Co., KS
CLC310	Edwards	Edwards Co., TX
CLC365	Edwards	Edwards Co., TX
CLC366	Edwards	Edwards Co., TX
CLC367	Edwards	Edwards Co., TX
CLC368	Edwards	Edwards Co., TX
CLC493	Geary	Blain Co., OK
CLC494	Geary	Blain Co., OK

ID	Population Code	Locality
CLC495	Geary	Blain Co., OK
CLC496	Geary	Blain Co., OK
CLC497	Geary	Blain Co., OK
CLC498	Geary	Blain Co., OK
CLC499	Geary	Blain Co., OK
CLC500	Geary	Blain Co., OK
CLC501	Geary	Blain Co., OK
CLC502	Geary	Blain Co., OK
CLC503	Geary	Blain Co., OK
CLC504	Geary	Blain Co., OK
CLC505	Geary	Blain Co., OK
CLC506	Geary	Blain Co., OK
CLC769	Geary	Blain Co., OK
CLC770	Geary	Blain Co., OK
CLC794	Geary	Blain Co., OK
CLC795	Geary	Blain Co., OK
MHP11287	Kiowa	Kiowa Co., KS
MHP12271	Kiowa	Kiowa Co., KS
MHP12272	Kiowa	Kiowa Co., KS
MHP12273	Kiowa	Kiowa Co., KS
MHP12274	Kiowa	Kiowa Co., KS
MHP12275	Kiowa	Kiowa Co., KS
MHP12276	Kiowa	Kiowa Co., KS
MHP12278	Kiowa	Kiowa Co., KS
MHP12279	Kiowa	Kiowa Co., KS
MHP12280	Kiowa	Kiowa Co., KS
MHP12281	Kiowa	Kiowa Co., KS
MHP12282	Kiowa	Kiowa Co., KS
MHP12283	Kiowa	Kiowa Co., KS
MHP12284	Kiowa	Kiowa Co., KS
MHP12285	Kiowa	Kiowa Co., KS
MHP12888	Kiowa	Kiowa Co., KS
MHP12945	Kiowa	Kiowa Co., KS
MHP12946	Kiowa	Kiowa Co., KS
MHP12947	Kiowa	Kiowa Co., KS
MHP12948	Kiowa	Kiowa Co., KS
MHP12950	Kiowa	Kiowa Co., KS
CLC470	LongtonKS	Elk Co., KS

ID	Population Code	Locality
CLC471	LongtonKS	Elk Co., KS
CLC472	LongtonKS	Elk Co., KS
CLC473	LongtonKS	Elk Co., KS
CLC474	LongtonKS	Elk Co., KS
CLC475	LongtonKS	Elk Co., KS
CLC476	LongtonKS	Elk Co., KS
CLC477	LongtonKS	Elk Co., KS
CLC478	LongtonKS	Elk Co., KS
CLC225	Roby	Fisher Co., TX
CLC226	Roby	Fisher Co., TX
CLC227	Roby	Fisher Co., TX
CLC228	Roby	Fisher Co., TX
CLC229	Roby	Fisher Co., TX
CLC230	Roby	Fisher Co., TX
CLC231	Roby	Fisher Co., TX
CLC232	Roby	Fisher Co., TX
CLC233	Roby	Fisher Co., TX
CLC234	Roby	Fisher Co., TX
CLC235	Roby	Fisher Co., TX
CLC237	Roby	Fisher Co., TX
CLC303	Roby	Fisher Co., TX
CLC304	Roby	Fisher Co., TX
CLC305	Roby	Fisher Co., TX
CLC306	Roby	Fisher Co., TX
CLC307	Roby	Fisher Co., TX
CLC281	SandSand	Beckham Co., OK
CLC282	SandSand	Beckham Co., OK
CLC283	SandSand	Beckham Co., OK
CLC284	SandSand	Beckham Co., OK
CLC285	SandSand	Beckham Co., OK
CLC286	SandSand	Beckham Co., OK
CLC287	SandSand	Beckham Co., OK
CLC288	SandSand	Beckham Co., OK
CLC289	SandSand	Beckham Co., OK
CLC290	SandSand	Beckham Co., OK
CLC291	SandSand	Beckham Co., OK
CLC292	SandSand	Beckham Co., OK
CLC293	SandSand	Beckham Co., OK

ID	Population Code	Locality
OMNH41878	SandSand	Beckham Co., OK
OMNH41879	SandSand	Beckham Co., OK
CLC093	SanSaba	San Saba Co., TX
TNHC66762	SanSaba	San Saba Co., TX
TNHC66764	SanSaba	San Saba Co., TX
CAS223560	SECO	Otero Co., CO
CAS223569	SECO	Baca Co., CO
CLC257	SECO	Otero Co., CO
CLC258	SECO	Otero Co., CO
CLC259	SECO	Otero Co., CO
CLC260	SECO	Otero Co., CO
CLC261	SECO	Otero Co., CO
CLC262	SECO	Otero Co., CO
CLC263	SECO	Otero Co., CO
CLC264	SECO	Otero Co., CO
CLC265	SECO	Otero Co., CO
CLC266	SECO	Otero Co., CO
CLC267	SECO	Otero Co., CO
CLC268	SECO	Otero Co., CO
CLC269	SECO	Otero Co., CO
CLC270	SECO	Otero Co., CO
CLC271	SECO	Otero Co., CO
CLC272	SECO	Otero Co., CO
CLC273	SECO	Otero Co., CO
CLC274	SECO	Otero Co., CO
CLC275	SECO	Otero Co., CO
CLC276	SECO	Otero Co., CO
CLC277	SECO	Otero Co., CO
CLC009	Shack	Shackleford Co., TX
CLC010	Shack	Shackleford Co., TX
CLC011	Shack	Shackleford Co., TX
CLC012	Shack	Shackleford Co., TX
CLC013	Shack	Shackleford Co., TX
CLC014	Shack	Shackleford Co., TX
CLC015	Shack	Shackleford Co., TX
CLC026	Shack	Shackleford Co., TX
CLC027	Shack	Shackleford Co., TX
CLC028	Shack	Shackleford Co., TX

ID	Population Code	Locality
CLC031	Shack	Shackleford Co., TX
CLC033	Shack	Shackleford Co., TX
CLC034	Shack	Shackleford Co., TX
CLC035	Shack	Shackleford Co., TX
CLC151	Shack	Shackleford Co., TX
CLC152	Shack	Shackleford Co., TX
CLC218	Shack	Shackleford Co., TX
CLC220	Shack	Shackleford Co., TX
CLC433	Shack	Shackleford Co., TX
CLC434	Shack	Shackleford Co., TX
CLC435	Shack	Shackleford Co., TX
CLC436	Shack	Shackleford Co., TX
CLC437	Shack	Shackleford Co., TX
CLC438	Shack	Shackleford Co., TX
CLC439	Shack	Shackleford Co., TX
CLC441	Shack	Shackleford Co., TX
JWS262	Shack	Shackleford Co., TX
MJI84	Shack	Shackleford Co., TX
MJI86	Shack	Shackleford Co., TX
MJI87	Shack	Shackleford Co., TX
MJI88	Shack	Shackleford Co., TX
CLC017	Steph	Stephens Co., TX
CLC019	Steph	Stephens Co., TX
CLC020	Steph	Stephens Co., TX
CLC021	Steph	Stephens Co., TX
CLC022	Steph	Stephens Co., TX
CLC136	Steph	Stephens Co., TX
CLC137	Steph	Stephens Co., TX
CLC138	Steph	Stephens Co., TX
CLC139	Steph	Stephens Co., TX
CLC140	Steph	Stephens Co., TX
CLC141	Steph	Stephens Co., TX
CLC142	Steph	Stephens Co., TX
CLC143	Steph	Stephens Co., TX
CLC145	Steph	Stephens Co., TX
CLC146	Steph	Stephens Co., TX
CLC325	Steph	Stephens Co., TX
CLC326	Steph	Stephens Co., TX

ID	Population Code	Locality
CLC327	Steph	Stephens Co., TX
CLC328	Steph	Stephens Co., TX
CLC442	Steph	Stephens Co., TX
CLC443	Steph	Stephens Co., TX
UTEP18436	Sutton	Sutton Co., TX
UTEP18437	Sutton	Sutton Co., TX
CLC452	Tulsa	Tulsa Co., OK
CLC453	Tulsa	Tulsa Co., OK
CLC454	Tulsa	Tulsa Co., OK
CLC455	Tulsa	Tulsa Co., OK
CLC213	ValVerde	Val Verde Co., TX
CLC214	ValVerde	Val Verde Co., TX
CLC348	ValVerde	Val Verde Co., TX
CLC349	ValVerde	Val Verde Co., TX
TNHC60972	ValVerde	Val Verde Co., TX
TNHC61357	ValVerde	Val Verde Co., TX
CLC369	West	Jeff Davis Co., TX
MJI32	West	Jeff Davis Co., TX
MJI34	West	Jeff Davis Co., TX

Specimens from Edwards, SanSaba, Sutton, Tulsa, ValVerde and West were not included in the analysis in Chapter 4.

APPENDIX B

MUSEUM SPECIMENS EXAMINED FOR CHAPTER 4

Specimen	SVL	Morph*	Year	State	County
SDNHM-30707	223	S	1938	AZ	Maricopa
SDNHM-30708	219	S	1938	AZ	Maricopa
SDNHM-30709	139	S	1938	AZ	Maricopa
SDNHM-30710	208	S	1938	AZ	Maricopa
SDNHM-41926	203	S	1952	AZ	Maricopa
SDNHM-41927	187	S	1952	AZ	Maricopa
SDNHM-40649	226	M	1950	AZ	Maricopa
SDNHM-40873	240	M	1948	AZ	Maricopa
SDNHM-41135	85	U	1951	AZ	Maricopa
SDNHM-41136	226	S	1951	AZ	Maricopa
SDNHM-41247	201	S	1951	AZ	Maricopa
SDNHM-41917	222	S	1952	AZ	Maricopa
SDNHM-41925	198	S	1952	AZ	Maricopa
SDNHM-41920	225	S	1952	AZ	Maricopa
SDNHM-41924	201	S	1952	AZ	Maricopa
SDNHM-41911	245	S	1952	AZ	Maricopa
SDNHM-41912	188	U	1952	AZ	Maricopa
SDNHM-41913	237	S	1952	AZ	Maricopa
SDNHM-41914	226	S	1952	AZ	Maricopa
SDNHM-41915	224	S	1952	AZ	Maricopa
SDNHM-41916	227	S	1952	AZ	Maricopa
SDNHM-41918	163	S	1952	AZ	Maricopa
SDNHM-41919	223	S	1952	AZ	Maricopa
SDNHM-41921	216	S	1952	AZ	Maricopa
SDNHM-41928	190	S	1952	AZ	Maricopa
SDNHM-41945	210	S	1952	AZ	Maricopa
SDNHM-41922	230	S	1952	AZ	Maricopa
SDNHM-41923	190	S	1952	AZ	Maricopa
SDNHM-41929	255	S	1952	AZ	Maricopa
SDNHM-41930	140	U	1952	AZ	Maricopa
SDNHM-41931	228	S	1952	AZ	Maricopa
SDNHM-41300	223	S	1951	AZ	Maricopa
ASU-1978	290	S	1958	AZ	Maricopa
ASU-10271	254	S	1959	AZ	Maricopa
ASU-1399	325	S	1957	AZ	Maricopa
ASU-4111	153	S	1961	AZ	Maricopa
ASU-1874	191	S	1958	AZ	Maricopa
ASU-11385	285	S	1967	AZ	Maricopa
ASU-1155	245	S	1957	AZ	Maricopa
ASU-1427	244	S	1957	AZ	Maricopa
ASU-1792	226	S	1958	AZ	Maricopa
ASU-1539	228	S	1957	AZ	Maricopa
ASU-1958	280	S	1958	AZ	Maricopa
ASU-4110	218	S	1961	AZ	Maricopa
ASU-1582	267	S	1957	AZ	Maricopa

Specimen	SVL	Morph*	Year	State	County
ASU-1913	236	S	1958	AZ	Maricopa
ASU-2300	275	S	1959	AZ	Maricopa
ASU-1383	253	S	1957	AZ	Maricopa
ASU-2271	187	S	1959	AZ	Maricopa
ASU-11321	227	U	1967	AZ	Maricopa
ASU-1407	226	S	1957	AZ	Maricopa
ASU-10275	244	S	1958	AZ	Maricopa
ASU-1191	190	S	1957	AZ	Maricopa
ASU-1643	147	S	1957	AZ	Maricopa
ASU-1644	175	S	1957	AZ	Maricopa
ASU-4170	190	S	1962	AZ	Maricopa
ASU-1915	169	S	1958	AZ	Maricopa
ASU-2303	195	S	1959	AZ	Maricopa
ASU-10274	246	S	1958	AZ	Maricopa
ASU-2447	256	S	1959	AZ	Maricopa
ASU-1962	236	S	1958	AZ	Maricopa
ASU-1888	230	S	1958	AZ	Maricopa
ASU-10279	256	S	1955	AZ	Maricopa
ASU-2837	210	S	1960	AZ	Maricopa
ASU-2226	230	S	1958	AZ	Maricopa
ASU-4563	228	U	1962	AZ	Maricopa
ASU-2262	170	U	1959	AZ	Maricopa
ASU-1961	210	S	1958	AZ	Maricopa
ASU-1912	130	U	1958	AZ	Maricopa
ASU-1127	262	S	1957	AZ	Maricopa
ASU-27537	179	U	1967	AZ	Maricopa
ASU-1102	200	S	1959	AZ	Maricopa
ASU-1425	211	S	1957	AZ	Maricopa
ASU-13571	227	S	1968	AZ	Maricopa
ASU-5654	215	S	1962	AZ	Maricopa
ASU-1914	215	S	1958	AZ	Maricopa
ASU-1037	167	S	1957	AZ	Maricopa
ASU-1122	98	U	1957	AZ	Maricopa
ASU-3193	97	U	1960	AZ	Maricopa
ASU-1153	163	S	1957	AZ	Maricopa
ASU-2126	118	S	1958	AZ	Maricopa
ASU-1910	107	U	1958	AZ	Maricopa
ASU-1100	134	U	1959	AZ	Maricopa
ASU-1120	101	U	1957	AZ	Maricopa
ASU-1637	153	S	1957	AZ	Maricopa
ASU-2068	112	U	1958	AZ	Maricopa
ASU-10272	125	U	1958	AZ	Maricopa
ASU-969	111	U	1956	AZ	Maricopa
ASU-1408	188	S	1957	AZ	Maricopa
ASU-10278	126	S	1959	AZ	Maricopa

Specimen	SVL	Morph*	Year	State	County
ASU-15502	123	B	1975	AZ	Maricopa
ASU-8829	210	S	1968	AZ	Maricopa
ASU-1165	129	S	1957	AZ	Maricopa
ASU-1546	101	U	1957	AZ	Maricopa
ASU-15596	96	U	1965	AZ	Maricopa
ASU-2209	110	U	1958	AZ	Maricopa
ASU-1426	134	U	1957	AZ	Maricopa
ASU-2304	187	S	1959	AZ	Maricopa
ASU-11280	245	S	1967	AZ	Maricopa
ASU-1423	121	S	1957	AZ	Maricopa
ASU-1795	244	S	1958	AZ	Maricopa
ASU-1098	119	U	1959	AZ	Maricopa
ASU-2031	99	U	1958	AZ	Maricopa
ASU-11453	80	M	1965	AZ	Maricopa
ASU-1192	111	S	1957	AZ	Maricopa
ASU-1619	126	S	1957	AZ	Maricopa
ASU-1332	120	U	1957	AZ	Maricopa
ASU-129	90	U	1953	AZ	Maricopa
ASU-2252	137	U	1958	AZ	Maricopa
ASU-1151	235	S	1957	AZ	Maricopa
ASU-1889	110	U	1958	AZ	Maricopa
ASU-11454	105	U	1965	AZ	Maricopa
ASU-1870	108	U	1958	AZ	Maricopa
ASU-1101	102	U	1959	AZ	Maricopa
ASU-1121	107	U	1957	AZ	Maricopa
ASU-127	86	S	1953	AZ	Maricopa
ASU-10273	125	U	1958	AZ	Maricopa
ASU-1118	87	U	1957	AZ	Maricopa
ASU-258	86	U	1955	AZ	Maricopa
ASU-549	102	U	1956	AZ	Maricopa
ASU-3864	222	U	1958	AZ	Maricopa
ASU-9258	172	U	1967	AZ	Maricopa
ASU-1119	255	S	1957	AZ	Maricopa
ASU-875	278	S	1956	AZ	Maricopa
ASU-3211	242	S	1960	AZ	Maricopa
ASU-350	171	S	1955	AZ	Maricopa
ASU-547	252	S	1956	AZ	Maricopa
ASU-118	284	S	1953	AZ	Maricopa
ASU-4243	237	S	1962	AZ	Maricopa
ASU-190	195	S	1955	AZ	Maricopa
ASU-2975	268	B	1960	AZ	Maricopa
ASU-3884	266	M	1958	AZ	Maricopa
ASU-109	256	S	1953	AZ	Maricopa
ASU-3419	205	S	1961	AZ	Maricopa
ASU-655	269	S	1956	AZ	Maricopa

Specimen	SVL	Morph*	Year	State	County
ASU-230	224	U	1955	AZ	Maricopa
ASU-34542	278	M	2004	AZ	Maricopa
ASU-4244	229	S	1962	AZ	Maricopa
ASU-1934	250	M	1958	AZ	Maricopa
ASU-4475	242	M	1962	AZ	Maricopa
ASU-668	262	M	1956	AZ	Maricopa
ASU-1556	231	B	1957	AZ	Maricopa
ASU-761	239	S	1956	AZ	Maricopa
ASU-200	210	U	1953	AZ	Maricopa
ASU-4492	167	B	1962	AZ	Maricopa
ASU-892	288	S	1956	AZ	Maricopa
ASU-2597	215	M	1955	AZ	Maricopa
ASU-759	300	U	1956	AZ	Maricopa
ASU-915	276	S	1956	AZ	Maricopa
ASU-673	317	M	1956	AZ	Maricopa
ASU-205	184	U	1954	AZ	Maricopa
ASU-670	280	S	1956	AZ	Maricopa
ASU-758	265	M	1956	AZ	Maricopa
ASU-548	320	S	1956	AZ	Maricopa
ASU-3014	233	S	1960	AZ	Maricopa
ASU-546	249	S	1956	AZ	Maricopa
ASU-594	242	U	1956	AZ	Maricopa
ASU-9178	245	U	1967	AZ	Maricopa
ASU-1430	266	M	1957	AZ	Maricopa
ASU-9034	250	S	1967	AZ	Maricopa
ASU-108	245	U	1953	AZ	Maricopa
ASU-498	174	S	1956	AZ	Maricopa
ASU-317	300	S	1955	AZ	Maricopa
ASU-254	256	S	1955	AZ	Maricopa
ASU-209	245	U	1955	AZ	Maricopa
ASU-167	254	S	1955	AZ	Maricopa
ASU-575	248	U	1956	AZ	Maricopa
ASU-208	252	M	1955	AZ	Maricopa
ASU-9099	241	S	1968	AZ	Maricopa
ASU-958	236	S	1956	AZ	Maricopa
ASU-2668	182	M	1959	AZ	Maricopa
ASU-1467	282	M	1957	AZ	Maricopa
ASU-3866	278	M	1958	AZ	Maricopa
ASU-110	240	S	1953	AZ	Maricopa
ASU-760	283	S	1956	AZ	Maricopa
ASU-3058	242	M	1960	AZ	Maricopa
ASU-713	250	S	1956	AZ	Maricopa
ASU-714	264	S	1956	AZ	Maricopa
ASU-9082	168	S	1968	AZ	Maricopa
ASU-3013	270	M	1960	AZ	Maricopa

Specimen	SVL	Morph*	Year	State	County
ASU-210	185	S	1955	AZ	Maricopa
ASU-191	222	M	1955	AZ	Maricopa
ASU-596	230	S	1956	AZ	Maricopa
ASU-597	242	S	1956	AZ	Maricopa
ASU-1959	240	M	1958	AZ	Maricopa
ASU-656	282	S	1956	AZ	Maricopa
ASU-1307	263	M	1957	AZ	Maricopa
ASU-790	254	S	1956	AZ	Maricopa
ASU-1956	274	M	1958	AZ	Maricopa
ASU-188	194	S	1955	AZ	Maricopa
ASU-455	262	S	1956	AZ	Maricopa
ASU-13430	258	S	1968	AZ	Maricopa
ASU-306	212	S	1955	AZ	Maricopa
ASU-3183	226	U	1960	AZ	Maricopa
ASU-187	188	S	1955	AZ	Maricopa
ASU-304	240	U	1955	AZ	Maricopa
ASU-216	227	S	1955	AZ	Maricopa
ASU-998	220	S	1956	AZ	Maricopa
ASU-3194	200	S	1960	AZ	Maricopa
ASU-8969	260	S	1965	AZ	Maricopa
ASU-211	216	U	1955	AZ	Maricopa
ASU-4506	244	M	1962	AZ	Maricopa
ASU-2387	153	B	1959	AZ	Maricopa
ASU-3210	210	S	1960	AZ	Maricopa
ASU-3865	240	M	1958	AZ	Maricopa
ASU-1570	187	B	1957	AZ	Maricopa
ASU-206	185	M	1955	AZ	Maricopa
ASU-499	145	S	1956	AZ	Maricopa
ASU-672	135	U	1956	AZ	Maricopa
ASU-321	115	U	1955	AZ	Maricopa
ASU-422	160	B	1956	AZ	Maricopa
ASU-3212	102	U	1960	AZ	Maricopa
ASU-9257	135	U	1967	AZ	Maricopa
ASU-557	106	U	1956	AZ	Maricopa
ASU-3460	96	U	1961	AZ	Maricopa
ASU-276	85	U	1955	AZ	Maricopa
ASU-1581	84	B	1957	AZ	Maricopa
ASU-3187	91	B	1960	AZ	Maricopa
ASU-130	93	M	1953	AZ	Maricopa
ASU-417	107	U	1955	AZ	Maricopa
ASU-115	117	U	1953	AZ	Maricopa
ASU-201	82	B	1953	AZ	Maricopa
ASU-349	100	U	1955	AZ	Maricopa
ASU-1002	90	U	1956	AZ	Maricopa
ASU-9256	132	U	1967	AZ	Maricopa

Specimen	SVL	Morph*	Year	State	County
ASU-4171	91	U	1962	AZ	Maricopa
ASU-5046	93	U	1964	AZ	Maricopa
ASU-319	103	U	1955	AZ	Maricopa
ASU-310	78	U	1955	AZ	Maricopa
ASU-595	95	U	1956	AZ	Maricopa
ASU-284	87	U	1955	AZ	Maricopa
UAZ-56451	226	U	2006	AZ	Maricopa
UAZ-55742	322	S	1988	AZ	Maricopa
UAZ-55743	265	U	1988	AZ	Maricopa
UAZ-40523	238	S	1952	AZ	Maricopa
UAZ-26345	181	S	1952	AZ	Maricopa
UAZ-26352	236	S	1958	AZ	Maricopa
UAZ-26359	195	U	1958	AZ	Maricopa
UAZ-26349	221	S	1958	AZ	Maricopa
UAZ-26353	201	U	1965	AZ	Maricopa
UAZ-26346	147	U	1958	AZ	Maricopa
UAZ-40519	226	S	1949	AZ	Maricopa
UAZ-26350	208	S	1943	AZ	Maricopa
UAZ-26362	209	S	1965	AZ	Maricopa
UAZ-36784	203	U	1957	AZ	Maricopa
UAZ-36785	196	M	1958	AZ	Maricopa
UAZ-26363	211	U	1958	AZ	Maricopa
UAZ-43278	178	S	1979	AZ	Maricopa
UAZ-28567	248	M	1968	AZ	Maricopa
UAZ-40520	223	M	1953	AZ	Maricopa
UAZ-40522	299	S	1952	AZ	Maricopa
UAZ-40401	307	S	1975	AZ	Maricopa
UAZ-47357	253	M	1949	AZ	Maricopa
UAZ-26351	119	U	1958	AZ	Maricopa
UAZ-45662	129	B	1984	AZ	Maricopa
UAZ-26356	NA	M	1965	AZ	Maricopa
UAZ-40518	95	U	1954	AZ	Maricopa
UTEP-17729	265	S	1998	AZ	Maricopa
UTEP-17728	227	S	1998	AZ	Maricopa
MSB-37844	341	S	1982	AZ	Maricopa
CAS-17550	231	M	1910	AZ	Maricopa
CAS-80677	223	U	1941	AZ	Maricopa
KU-22864	234	M	1942	AZ	Maricopa
KU-49671	253	S	1955	AZ	Maricopa
KU-14166	191	S	1931	AZ	Maricopa
KU-22865	204	S	1942	AZ	Maricopa
KU-22873	207	S	1942	AZ	Maricopa
KU-68942	238	S	1956	AZ	Maricopa
KU-68941	277	M	1956	AZ	Maricopa
KU-22866	222	S	1942	AZ	Maricopa

Specimen	SVL	Morph*	Year	State	County
UTA-CLC-428	NA	S	2010	AZ	Maricopa
UAZ-40515	183	B	1975	TX	Brewster
UAZ-42370	260	U	1975	TX	Brewster
UAZ-40514	225	S	1975	TX	Brewster
UAZ-40512	219	U	1975	TX	Brewster
UAZ-41695	176	U	1975	TX	Brewster
UAZ-42144	209	B	1975	TX	Brewster
UAZ-40513	202	U	1975	TX	Brewster
UAZ-32528	NA	U	1970	TX	Brewster
UTEP-10655	243	U	1985	TX	Brewster
UTEP-2859	213	S	1976	TX	Brewster
UTEP-10654	202	U	1985	TX	Brewster
UTEP-10653	273	B	1985	TX	Brewster
UTEP-2856	228	U	1976	TX	Brewster
UTEP-10651	255	B	1985	TX	Brewster
NMSU-6912	195	U	1991	TX	Brewster
NMSU-6911	225	U	1991	TX	Brewster
NMSU-6910	183	S	1991	TX	Brewster
NMSU-6705	236	U	1992	TX	Brewster
NMSU-6657	182	U	1987	TX	Brewster
NMSU-6658	182	U	1987	TX	Brewster
NMSU-6511	195	S	1985	TX	Brewster
NMSU-6515	212	U	1985	TX	Brewster
NMSU-6517	227	U	1985	TX	Brewster
NMSU-6516	207	S	1985	TX	Brewster
NMSU-6513	168	U	1985	TX	Brewster
NMSU-6510	200	U	1985	TX	Brewster
NMSU-6512	231	S	1985	TX	Brewster
NMSU-6514	191	U	1985	TX	Brewster
NMSU-6509	217	S	1985	TX	Brewster
NMSU-6508	225	U	1985	TX	Brewster
NMSU-3160	274	B	1973	TX	Brewster
NMSU-3213	245	M	1975	TX	Brewster
NMSU-3161	210	U	1973	TX	Brewster
NMSU-3205	234	U	1975	TX	Brewster
NMSU-3208	255	S	1975	TX	Brewster
NMSU-3204	203	U	1975	TX	Brewster
NMSU-3206	230	U	1975	TX	Brewster
NMSU-3209	254	S	1975	TX	Brewster
NMSU-3207	210	U	1975	TX	Brewster
MSB-22891	147	S	1969	TX	Brewster
MSB-20806	252	U	1968	TX	Brewster
MSB-71646	225	B	2004	TX	Brewster
MSB-71626	220	B	2004	TX	Brewster
MSB-66855	153	U	2003	TX	Brewster

Specimen	SVL	Morph*	Year	State	County
MSB-32976	185	U	1968	TX	Brewster
MSB-9902	233	S	1958	TX	Brewster
MSB-6559	255	U	1961	TX	Brewster
MSB-9909	136	S	1960	TX	Brewster
MSB-19724	106	U	1968	TX	Brewster
MSB-9896	189	S	1957	TX	Brewster
MSB-71622	167	S	2004	TX	Brewster
MSB-26383	240	U	1967	TX	Brewster
MSB-9904	197	M	1958	TX	Brewster
MSB-9897	175	B	1957	TX	Brewster
MSB-71623	147	U	2004	TX	Brewster
MSB-32977	209	B	1968	TX	Brewster
MSB-9901	197	S	1958	TX	Brewster
MSB-9894	210	U	1957	TX	Brewster
MSB-9903	190	U	1958	TX	Brewster
MSB-9900	183	S	1957	TX	Brewster
MSB-9899	167	S	1957	TX	Brewster
MSB-9907	216	S	1958	TX	Brewster
MSB-9895	200	U	1957	TX	Brewster
MSB-20868	139	U	1969	TX	Brewster
MSB-9905	186	S	1958	TX	Brewster
MSB-20815	178	S	1968	TX	Brewster
MSB-9898	205	S	1957	TX	Brewster
MSB-9906	204	U	1958	TX	Brewster
MSB-20787	227	B	1968	TX	Brewster
MSB-19758	293	U	1968	TX	Brewster
CAS-169508	193	S	1988	TX	Brewster
CAS-190362	162	S	1981	TX	Brewster
CAS-190363	207	U	1978	TX	Brewster
CAS-190365	190	M	1987	TX	Brewster
KU-176906	244	S	1966	TX	Brewster
KU-176907	175	U	1966	TX	Brewster
KU-176909	219	U	1969	TX	Brewster
KU-97839	203	U	1957	TX	Brewster
KU-176908	93	U	1968	TX	Brewster
KU-14164	190	U	1931	TX	Brewster
KU-14165	93	U	1931	TX	Brewster
SDNHM-3384	83	B	1930	TX	El Paso
SDNHM-5026	207	M	1931	TX	El Paso
SDNHM-5027	215	M	1931	TX	El Paso
SDNHM-5028	241	M	1931	TX	El Paso
UTEP-2447	343	M	1973	TX	El Paso
UTEP-6255	258	S	1979	TX	El Paso
UTEP-498	192	M	1970	TX	El Paso
UTEP-18668	225	B	2001	TX	El Paso

Specimen	SVL	Morph*	Year	State	County
UTEP-13675	200	M	1990	TX	El Paso
UTEP-173	NA	M	1970	TX	El Paso
UTEP-19294	185	B	2005	TX	El Paso
UTEP-2692	180	S	1975	TX	El Paso
UTEP-14081	230	M	1991	TX	El Paso
UTEP-18554	212	S	2000	TX	El Paso
UTEP-22	204	M	1968	TX	El Paso
UTEP-18429	300	B	1999	TX	El Paso
UTEP-11206	201	S	1986	TX	El Paso
UTEP-11012	299	S	1986	TX	El Paso
UTEP-226	190	M	1970	TX	El Paso
UTEP-1347	266	S	1966	TX	El Paso
UTEP-15718	166	M	1994	TX	El Paso
UTEP-10775	180	S	1985	TX	El Paso
UTEP-786	221	M	1961	TX	El Paso
UTEP-16312	236	B	1996	TX	El Paso
UTEP-15705	190	U	1994	TX	El Paso
UTEP-10776	244	S	1985	TX	El Paso
UTEP-12103	173	B	1988	TX	El Paso
UTEP-11094	250	M	1986	TX	El Paso
UTEP-10998	226	M	1986	TX	El Paso
UTEP-8791	NA	M	1967	TX	El Paso
UTEP-569	203	M	1969	TX	El Paso
UTEP-5007	204	U	1973	TX	El Paso
UTEP-2493	193	M	1974	TX	El Paso
UTEP-16106	196	M	1995	TX	El Paso
UTEP-10774	187	S	1985	TX	El Paso
UTEP-570	173	S	1969	TX	El Paso
UTEP-10790	NA	S	1985	TX	El Paso
UTEP-83	120	M	1969	TX	El Paso
UTEP-17493	90	M	1997	TX	El Paso
UTEP-18719	105	M	2002	TX	El Paso
UTEP-9401	98	M	1982	TX	El Paso
MSB-9908	234	M	1900	TX	El Paso
KU-301289	155	B	1931	TX	El Paso
UAZ-32725	NA	U	1970	TX	Presidio
UAZ-32525	229	B	1970	TX	Presidio
UTEP-10652	239	B	1999	TX	Presidio
NMSU-3237	267	U	1975	TX	Presidio
NMSU-5079	221	B	1977	TX	Presidio
NMSU-5076	226	U	1977	TX	Presidio
NMSU-5088	192	U	1977	TX	Presidio
NMSU-6917	216	S	1991	TX	Presidio
NMSU-5078	212	M	1977	TX	Presidio
NMSU-2947	222	U	1971	TX	Presidio

Specimen	SVL	Morph*	Year	State	County
NMSU-6916	243	U	1991	TX	Presidio
NMSU-5074	207	U	1977	TX	Presidio
NMSU-2948	204	B	1971	TX	Presidio
NMSU-5075	205	U	1977	TX	Presidio
NMSU-3219	214	U	1975	TX	Presidio
NMSU-5073	207	B	1977	TX	Presidio
NMSU-5850	203	U	1981	TX	Presidio
NMSU-5849	218	U	1981	TX	Presidio
NMSU-5851	259	B	1981	TX	Presidio
NMSU-5852	187	U	1981	TX	Presidio
NMSU-6723	259	U	1992	TX	Presidio
NMSU-6724	217	B	1992	TX	Presidio
NMSU-4908	200	B	1976	TX	Presidio
NMSU-6913	262	S	1991	TX	Presidio
NMSU-5077	211	U	1977	TX	Presidio
NMSU-3220	180	U	1975	TX	Presidio
NMSU-6908	230	U	1991	TX	Presidio
NMSU-6069	280	S	1991	TX	Presidio
NMSU-6907	258	S	1991	TX	Presidio
NMSU-6722	161	U	1992	TX	Presidio
KU-72768	NA	U	1962	TX	Presidio
MHP-1376	234	M	1964	KS	Barber
MHP-5377	180	S	1971	KS	Barber
MHP-12889	157	S	2006	KS	Barber
MHP-12886	228	B	2006	KS	Barber
MHP-12888	204	B	2006	KS	Barber
MHP-12887	244	M	2006	KS	Barber
MHP-8262	223	M	2004	KS	Barber
MHP-5296	205	S	1971	KS	Barber
MHP-11225	204	S	2005	KS	Barber
MHP-8263	94	S	2004	KS	Barber
MHP-11224	221	S	2005	KS	Barber
MHP-11293	239	S	2005	KS	Barber
MHP-11290	203	M	2005	KS	Barber
MHP-11226	95	U	2005	KS	Barber
MHP-11291	109	S	2005	KS	Barber
MHP-5376	NA	S	1971	KS	Barber
KU-192286	223	M	1982	KS	Barber
KU-192288	227	S	1982	KS	Barber
KU-192285	202	B	1982	KS	Barber
KU-192287	221	M	1982	KS	Barber
KU-207308	186	S	1987	KS	Barber
KU-192284	195	S	1982	KS	Barber
KU-188872	NA	U	1962	KS	Barber
KU-206321	224	M	1986	KS	Barber

Specimen	SVL	Morph*	Year	State	County
KU-20213	154	S	1936	KS	Barber
KU-20212	115	M	1936	KS	Barber
KU-188870	234	S	1957	KS	Barber
KU-155264	188	B	1974	KS	Barber
KU-155265	190	S	1974	KS	Barber
KU-155263	212	B	1974	KS	Barber
KU-188871	NA	M	1956	KS	Barber

*U=Uniform, S=Striped, B=Banded, M=Mimetic

REFERENCES

Abbot, J. K., S. Bensch, T. P. Gosden, and E. I. Svensson. 2008. Patterns of differentiation in a color polymorphism and in neutral markers reveal rapid genetic changes in natural damselfly populations. Molecular Ecology 17:1597-1604.

Amaral, A. d. 1929. Estudos sobre ophidios neotropicos XVIII. Lista remissiva dos ophidios da regiao neotropica. Memoir Institut Butantan 4:126-271.

Andres, J. A., R. A. Sanchez-Guillen, and A. C. Rivera. 2000. Molecular evidence for selection on female color polymorphism in the damselfly *Ischnura graellsii*. Evolution 54:2156-2161.

Antao, T., and M. A. Beaumont. 2011. Mcheza: A workbench to detect selection using dominant markers. Bioinformatics available online April 2011:1-2.

Bagnara, J. T. 1983. Developmental aspects of vertebrate chromatophores. American Zoologist 23:465-478.

Bagnara, J. T., J. Matsumoto, W. Ferris, S. K. Frost, W. A. Turner, T. T. Tchen, and J. D. Taylor. 1979. Common origin of pigment cells. Science 203:410-415.

Barrett, J. A. 1976. The maintenance of non-mimetic forms in a dimorphic Batesian mimic species. Evolution 30:82-85.

Bates, H. W. 1862. XXXII. Contribution to an insect fauna of the Amazon Valley. Lepidoptera. Transactions of the Linnean Society of London 23:495-566.

Beaumont, M. A., and D. J. Balding. 2004. Identifying adaptive genetic divergence among populations from genome scans. Molecular Ecology 13:969-980.

Bechtel, E. R., and T. Whitecar. 1983. Genetics of striping in the gopher snake, *Pituophis melanoleucus*. Journal of Herpetology 17:362-370.

Bechtel, H. B. 1978. Color and pattern in snakes (Reptilia:Serpentes). Journal of Herpetology 12:521-532.

—. 1995, Reptile and amphibian variants: color, patterns and scales. Malabar, Florida, Krieger Publishing Company.

Bechtel, H. B., and E. Bechtel. 1962. Heridit of albinism in the corn snake, *Elaphe guttatta guttatta*, demonstrated in captive breedings. Copeia 1962:148-149.

—. 1978. Heredity of pattern mutation in the corn snake, *Elaphe guttatta guttatta*, demonstrated in captive breedings. Copeia 1978:719-721.

Bogert, C. M., and J. A. Oliver. 1945. A preliminary analysis of the herpetofauna of Sonora. Bulletin of the American Museum of Natural History 83:297-426.

Bonnet, E., and Y. van de Peer. 2002. zt: a software tool for simple and partial Mantel tests. Journal of Statistical Software 7:1-12.

Boulenger, G. A. 1896, Catalogue of the snakes in the British Museum (Natural History), British Museum (Natural History).

Bouzat, J. L., H. H. Cheng, H. A. Lewin, R. L. Westmeier, J. D. Brawn, and K. N. Paige. 1998. Genetic evaluation of a demographic bottleneck in the greater prairie chicken. Conservation Biology 12:836-843.

Bouzat, J. L., and K. Johnson. 2004. Genetic structure among closely spaced leks in a peripheral population of lesser prairie chickens. Molecular Ecology 13.

Branch, L. C., A. M. Clark, P. E. Moler, and B. W. Bowen. 2003. Fragmented landscapes, habitat specificity and conservation genetics of three lizards in the Florida scrub. Conservation Genetics 4:199-212.

Brodie, E. D. 1993. Differential avoidance of coral snake banded patterns by free-ranging avian predators in Costa Rica. Evolution 47:227-235.

Brodie, E. D., and E. D. Brodie. 2004. Venomous snake mimicry, Pages vii + 870pp *in* J. A. Campbell, and W. W. Lamar, eds. The venomous reptiles of the Western hemisphere. Ithaca, Comstock Publishing Associates.

Brodie, E. D., and F. Janzen. 1995. Experimental studies of coral snake mimicry: generalized avoidance of ringed snake patterns by free-ranging avian predators. Functional Ecology 9:186-190.

Brodie, E. D., and A. J. Moore. 1995. Experimental studies of coral snake mimicry: do snakes mimic millipedes? Animal Behaviour 49:534-536.

Brodie, E. D., B. J. Ridenhour, and E. D. B. III. 2002. The evolutionary response of predators to dangerous prey: hotspots and coldspots in the geographic mosaic of coevolution between garter snakes and newts. Evolution 56:2067-2082.

Brown, K. S., and W. W. Benson. 1974. Adaptive polymorphism associated with multiple Mullerian mimicry in *Heliconius numata* (Lepid. Nymph.). Biotropica 6:205-228.

Bryant, H. C. 1916. Habitats and food of the roadrunner in California. University of California Publications in Zoology 17:21-58.

Bryson, R. W., R. W. Murphy, A. Lathrop, and D. Lazcano-Villareal. 2011. Evolutionary drivers of phylogeographic diversity in the highlands of Mexico: a case study of the *Crotalus triseriatus* species group of monatane rattlesnakes. Journal of Biogeography 38:697-710.

Burbrink, F. T., R. Lawson, and J. B. Slowinski. 2000. Mitochondrial DNA phylogeography of the polytypic North American rat snake (*Elaphe obsoleta*): a critique of the subspecies concept. Evolution 54:2107-2118.

Burbrink, F. T., H. Yao, M. Ingrasci, R. W. Bryson, T. J. Guiher, and S. Ruane. 2011. Speciation at the Mogollon Rim in the Arizona mountain kingsnake (*Lampropeltis pyromelana*). Molecular Phylogenetics and Evolution 60:445-454.

Burke, I. C., F. G. F. Kittel, W. K. Laurenoth, P. Snook, C. M. Yonker, and W. J. Parton. 1991. Regional analysis of the central Great Plains. Bioscience 41:685-692.

Campbell, J. A., and W. W. Lamar. 2004a, The venomous reptiles of the western hemisphere, v. 2. Ithaca, New York, Cornell University Press.

—. 2004b, The venomous reptiles of the western hemisphere, v. 1. Ithaca, New York, Cornell University Press.

Ceccarelli, F. S., and R. H. Crozier. 2007. Dynamics of the evolution of Batesian mimicry: molecular phylogenetic analysis of ant-mimicking *Myrmarachne*

(Aranae:Salticidae) species and their ant models. Journal of Evolutionary Biology 20:286-295.

Charlesworth, D., and B. Charlesworth. 1975a. Theoretical genetics of Batesian mimicry I. Single locus models. Journal of Theoretical Biology 55:283-303.

—. 1975b. Theoretical genetics of Batesian mimicry II: Evolution of supergenes. Journal of Theoretical Biology 55:305-324.

—. 1975c. Theoretical genetics of Batesian mimicry III: evolution of dominance. Journal of Theoretical Biology 55:325-337.

Clarke, C. A., P. M. Shepard, and I. W. B. Thornton. 1968. The genetics of the mimetic butterfly *Papilio memmon*. Philosophical Transactions of the Royal Society of London B Biology 254:37-89.

Conner, J. K., and D. L. Hartl. 2004, A primer of ecological genetics. Sunderland, MA, USA, Sinauer Associates, Inc.

Cope, E. D. 1879. Eleventh contribution to the herpetology of tropical America. Proceedings of the American Philosophical Society 18:261-277.

—. 1885. Twelfth contribution to the herpetology of tropical America. Proceedings of the American Philosophical Society 22:167-194.

—. 1895. The classification of the Ophidia. Transactions of the American Philosophical Society 18:186-219.

—. 1896. The geographical distribution of Batrachia and Reptilia in North America (continued). The American Naturalist 30:1003-1026.

Corl, A., A. R. Davis, S. R. Kuchta, and B. Sinervo. 2010. Selective loss of polymorphic mating types is associated with rapid phenotypic evolution during morphic speciation. Proceedings of the National Academy of Science 107:4254-4259.

Cox, C. L., A. R. D. Rabosky, J. Reyes-Velasco, P. Ponce-Campos, E. N. Smith, O. Flores-Villela, and J. A. Campbell. 2012. Molecular systematics of the genus Sonora (Squamata: Colubridae) in central and western Mexico. Systematics and Biodiversity 10:93-108.

Croucher, P. J., G. S. Oxford, A. Lam, and R. G. Gillespie. 2011. Stabilizing selection maintains colour polymorphism in the spider Theridion californicum (Araneae, Theridiidae). Molecular Ecology 20:206-218.

de Queiroz, K. 1998. The general lineage concept of species, species criteria, and the process of speciation: A conceptual unification and terminological recommendations*in* D. J. Howard, and S. H. Berlocher, eds., Endless forms: species and speciation. New York, Oxford University Press.

Demastes, J. W., T. A. Spradlin, M. S. Hafner, D. J. Hafner, and D. L. Reed. 2002. Systematics and phylogeography of pocket gophers in the genera *Cratogeomy* and *Pappogeomys*. Molecular Phylogenetics and Evolution 22:144-154.

Devitt, T. J., T. J. Laduc, and J. A. McGuire. 2008. The *Trimorphodon biscutatus* (Squamata: Colubridae) species complex revisited: A multivariate statistical analysis of geographic variation. Copeia 2008:370-387.

Dorn, A., E. Ng'oma, K. Janko, K. Reichwald, M. Polacik, M. Platzer, A. Cellerino et al. 2011. Phylogeny, genetic variability and colour polymorphism of an emerging animal model: the short-lived annual *Nothobranchius* fishes of southern Mozambique. Molecular Phylogenetics and Evolution 61:739-749.

Dowling, H. G. 1975. A provisional classification of snakes*in* H. G. Dowling, ed., Yearbook of Herpetology. New York, Hiss Publications.

Dowling, H. G., and W. E. Duellman. 1978, Systematic herpetology: a synopsis of families and higher categories. New York, Hiss Publications.

Dowling, H. G., and J. M. Savage. 1960. A guide to the snakes hemipenes: A survey of basic structure and systematic characteristics. Zoologica 45:17-28.

Drummond, A. J., and A. Rambaut. 2007. BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evolutionary Biology:214.

DuVal, E. H., H. W. Greene, and K. L. Manno. 2006. Laughing Falcon (*Herpetotheres cahinnans*) predation on coral snakes (*Micrurus nigrocinctus*). Biotropica 38:566-568.

Echternacht, A. C. 1973. The color pattern of *Sonora michoacanensis* (Dugés) (Serpentes, Colubridae) and its bearing on the origin of the species. Breviora of the Museum of Comparative Zoology 410:1-18.

Edmunds, M. 2000. Why are there good and poor mimics? Biological Journal of the Linnean Society 70:569-466.

Ehrich, D. 2006. AFLPdat: a collection of R functions for convenient handling of AFLP data Molecular Ecology Notes 6:603-604.

Ernst, C. H., and E. M. Ernst. 2003, Snakes of the United States and Canada. Washington and London, Smithsonian Books.

Ferrusquia-Villafranca, I. 2007. En sayo sobre la caracterización y significancia biológica, Pages 7-23 *in* I. Luna, J. J. Morrone, and D. Espinosa, eds. Biodiversidad de la Faja Volcánica Transmexicana. Distrito Federal, Mexico, UNAM.

Fitch, H. S. 1999, A Kansas snake community: composition and chages over 50 years. Malabar, Florida, Krieger Publishing Company.

Flores-Villela, O., and L. Canseco-Márquez. 2004. Nuevas especies y cambios taxonómicos para la Herpetofauna de México. Acta Zoológica Mexicana (Nueva Serie) 20:115-144.

Flores-Villela, O., and P. Gerez. 1994, Biodiversidad y conservación en México: vertebrados, vegetación y uso del suelo. Distrito Federal, México, CONABIO/UNAM.

Foll, M., and O. Gaggiotti. 2008. A genome-scan method to identify selected loci appropriate for both dominant and codominant markers: a Bayesian perspective. Genetics 180:977-993.

Fontenot, B. E., R. Makowsky, and P. T. Chippindale. 2011. Nuclear-mitochondrial discordance and gene flow in a recent radiation of toads. Molecular Phylogenetics and Evolution 59:66-80.

Gilbert, L. E. 2003. Adaptive novelty through introgression in Heliconius wing patterns: Evidence from a shared gnetic "toolbox" from synthetic hybrid zones and a

theory of diversification, Pages 281-318 *in* C. L. Boggs, W. B. Watt, and P. R. Ehrlich, eds. Butterflies:ecology and evolution taking flight. Chicago, University of Chicago Press.

Gillespie, R. G., and G. S. Oxford. 1998. Selection on the color polymorphism in Hawaiian happy-face spiders: evidence from genetic structure and temporal fluctuations. Evolution 52:775-783.

Gómez-Tuena, A., and G. Carrasco-Núñez. 2000. Cerro Grande volcano: the evolution of a Miocene stratocone in the early Trans-Mexican Volcanic Belt. Tectonophysics 318:249-280.

Goosens, B., L. Chikhi, M. Ancrenaz, I. Lackman-Ancrenaz, P. Andau, and M. W. Bruford. 2006. Genetic signature of anthropogenic population collapse in orang-utans. PLoS Biology 4:e25.

Goynechea, I. 2009. Relaciones filogenéticas de las serpientes del género *Conopsis* con base en la morfología. Revista Mexican de Biodiversidad 80:721-725.

Graur, D., and W.-H. Li. 2000, Fundamentals of Molecular Evolution. Sunderland, Massachusetts, Sinauer Associates, Inc. .

Gray, S. M., L. M. Dill, and J. S. McKinnon. 2007. Cuckoldry incites cannibalism: Male fish turn to cannibalism when perceived certainty of paternity decreases. American Naturalist 169:258-263.

Gray, S. M., and J. S. McKinnon. 2006. Linking color polymorphism maintenance and speciation. TRENDS in Ecology and Evolution 22:71-79.

Greenbaum, E., E. N. Smith, and R. O. d. Sá. 2011. Molecular systematics of the Middle American genus *Hypopachus* (Anura:Microhylidae). Molecular Phylogenetics and Evolution 61:265-277.

Greene, H. W. 1997, Snakes: the evolution of mystery in nature. Berkeley, CA, University of California Press.

Greene, H. W., and R. W. McDiarmid. 1981. Coral snake mimicry: Does it occur? Science 213:1207-1212.

Greene, H. W., and W. F. Pyburn. 1973. Comments on aposematism and mimicry among corals snakes. Biologist 55:144-148.

Gunther, A. 1895, Reptiles and Batrachia: Biologi Centrali-Americana. London, Dulau and Co.

Harding, R. M., E. Healy, A. J. Ray, N. S. Ellis, N. Flanagan, C. Todd, C. Dixon et al. 2000. Evidence for variable selective pressures at Mc1R. American Journal of Human Genetics` 66:1351-1361.

Hare, M. P. 2001. Prospects for nuclear gene phylogeography. TRENDS in Ecology and Evolution 12:700-706.

Harper, G. R., and D. W. Pfennig. 2007. Mimicry on the edge: Why do mimics vary in resemblance to their mode in different parts of their geographic range? Proceedings of the Royal Society of London B 274:1955-1961.

—. 2008. Selection overrides gene flow to break down maladaptive mimicry. Nature 451:1103-1106.

Heled, J., and A. J. Drummond. 2010. Bayesian inference of species trees from multilocus data. Molecular Biology and Evolution 27:570-580.

Herczeg, G., C. Matsuba, and J. Merila. 2010. Sequence variation in the melanocortin-1 receptor does not explain variation in the degree of melanism in a widespread amphibian. Annales Zoologici Fennici 47:37-45.

Hoekstra, H. E. 2006. Genetics, development and evolution of adaptive pigmentation in vertebrates. Heredity 97:222-234.

Hoekstra, H. E., R. J. Hirschmann, R. A. Bundley, P. A. Insel, and J. P. Crossland. 2006. A single amino acid mutation contributes to adaptive beach mous color pattern. Science 313:101-104.

Hoekstra, H. E., and M. W. Nachman. 2003. Different genes underlie adaptive melanism in different populations of pocket mice. Molecular Ecology 12:1185-1194.

Hoffman, E. A., F. W. Schueler, A. G. Jones, and M. S. Blouin. 2006. An analysis of selection on a colour polymorphism in the northern leopard frog. Molecular Ecology 15:2627-2641.

Holm, P. A. 2008. Phylogenetic biology of the burrowing snake tribe Sonorini (Colubridae), Pages 1-240, unpublished dissertation, University of Arizona.

Hubbard, J. K., J. A. C. Uy, M. E. Hauber, H. E. Hoekstra, and R. J. Safran. 2010. Vertebrate pigmentation: from underlying genes to adaptive function. Trends in Genetics 26:231-239.

Huelsenbeck, J. P., B. Larget, R. E. Miller, and F. Ronquist. 2002. Potentiall applications and pitfalls of Bayesian inference of phylogeny. Systematic Biology 51:673-688.

Huelsenbeck, J. P., and F. Ronquist. 2001. MRBAYES: Bayesian inference of phylogeny. Bioinformatics 17:754-755.

Ibrahim, K. I., R. A. Nichols, and G. M. Hewitt. 1996. Spatial patterns of genetic variation generated by different forms of dispersal during range expansion. Heredity 77:282-291.

Jaeger, J. R., B. R. Riddle, and D. F. Bradford. 2005. Cryptic Neogene vicariance and Quaternary dispersal of the red-spotted toad (*Bufo punctatus*): insights on the evolution of North American warm desert biotas. Molecular Ecology 14:3033-3048.

Jiang, Z. J., T. A. Castoe, C. C. Austin, F. T. Burbrink, M. D. Herron, J. A. McGuire, C. L. Parkinson et al. 2007. Comparative mitochondrial genomics of snakes: extraordinary sustitution rate dynamics and functionality of the duplicate control region. BMC Evolutionary Biology 7:123.

Johnson, J. A., J. E. Toepfer, and P. O. Dunn. 2003. Contrasting patterns of mitochondrial and microsatellite population structure in fragmented populations of greater prairie chickens. Molecular Ecology 12:3335-3347.

Jorgensen, T. H., D. S. Richardson, and S. Andersson. 2006. Comparative analyses of population structure in two subspecies of Nigella degenii: evidence for diversifying selection on pollen-color dimorphisms. Evolution 60:518-528.

Joron, M., and J. L. B. Mallet. 1998. Diversity in mimicry: paradox or paradigm? TRENDS in Ecology and Evolution 13:461-466.

Kanehisa, M., S. Goto, M. Furumichi, and M. Tanabe. 2012. KEGG for integration and interpretation of large-scale molecular data sets. Nucleic Acids Research 40:D109-D114.

Kassing, E. F. 1961. A life history study of the Great Plains Ground Snake, *Sonora episcopa episcopa* (Kennicott). The Texas Journal of Science 13:185-203.

Kikuchi, D. W., and D. W. Pfennig. 2010a. High model abundance may permit the gradual evolution of Batesian mimicry: an experimental test. Proceedings of the Royal Society of London B 277:1041-1048.

—. 2010b. Predator cognition permits imperfect coral snake mimicry. The American Naturalist 176:830-834.

King, R. B. 1993. Color-pattern variation in Lake Erie water snakes: prediction and measurement of natural selection. Evolution 47:1819-1833.

King, R. B., and R. Lawson. 1995. Color-pattern variation in Lake Erie water snakes: the role of gene flow. Evolution 49:885-896.

Knowles, L. L., and L. S. Kubatko. 2010, Estimating species trees: practical and theoretical aspects, v. ix + 216 pp. Hoboken, NJ, Wiley-Blackwell.

Kondo, S., and H. Shirota. 2009. Theoretical analysis of mechanisms that generate the pigmentation pattern of animals. Seminars in Cell and Developmental Biology 20:82-89.

Krammer, J. C. 1990. Largest rivers in the United States, Pages 1-2, United States Geological Survey.

Kunte, K. 2009. The diversity and evolution of Batesian mimicry in *Papilio* swallowtail butterflies. Evolution 63:2707-2716.

Larkin, M. A., G. Blackshields, N. P. Brown, R. Chenna, P. A. McGettigan, H. McWilliam, F. Valentin et al. 2007. ClustalW and Clustal X version 2.0. Bioinformatics 23:2947-2948.

LaRue, M. A., and C. K. Nielsen. 2008. Modelling potential dispersal corridors for cougars in midwestern North America using least-cost path methods. Ecological Modelling 212:372-381.

Leache, A. D., and M. K. Fujita. 2010. Bayesian species delimitation in West African forest geckos (*Hemidactylus fasciatus*). Proceedings of the Royal Society of London B 277:3071-3077.

Leache, A. D., and B. Rannala. 2011. The accuracy of species tree estimation under simulation: a comparison of methods. Systematic Biology 60:126-137.

Lemos-Espinal, J. A., D. Chiszar, M. J. Ingrasci, and H. M. Smith. 2004a. Year 2002 turtles and snakes from Chihuahua, Mexico. Bulletin of the Chicago Herpetological Society 39:82-87.

Lemos-Espinal, J. A., D. Chiszar, and H. M. Smith. 2004b. Variation in *Procinura aemula*, the File-tailed Groundsnake of Mexico. . Bulletin of the Maryland Herpetological Society 40:61-69.

Lemos-Espinal, J. A., H. M. Smith, and D. Chiszar. 2004c, Introduccíon a los anfidios y reptiles del estado de Chihuahua, México. D.F. México, Universidad Nacional Autonoma de México y Conabio.

Madison, W. P., and L. L. Knowles. 2006. Inferring phylogeny despite incomplete lineage sorting. Systematic Biology 55:21-30.

Makowsky, R., J. Chesser, and L. J. Rissler. 2009. A striking lack of genetic diversity across the wide-ranging amphibian *Gastrophryne carolinensis* (Anura:Microhylidae). Genetica 135:169-183.

Makowsky, R., C. L. Cox, C. Roelke, and P. T. Chippindale. 2010. Analyzing the relationship between sequence divergence and nodal support using Bayesian phylogenetic analyses. Molecular Phylogenetics and Evolution 57:485-494.

Mallet, J., and M. Joron. 1999. Evolution of diversity in warning color and mimicry: polymorphisms, shifting balance and speciation. Annual Reviews of Ecology and Systematics 30:201-233.

Mateos, M. 2005. Comparative phylogeography of livebearing fishes in the genera *Poeciliopsis* and *Poecilia* (Poeciliidae: Cyprinodontiformes) in central Mexico. Journal of Biogeography 32:775-780.

Matthews, E. G. 1977. Signal-based frequency-dependent defense strategies and the evolution of mimicry. The American Naturalist 111:213-222.

Miller, E. T., H. F. Greeney, and U. Valdez. 2010. Breeding behavior of the Laughing Falcon (Herpetotheres cachinnans) in southwestern Ecuador and northwestern Peru. Ornitologia Colombiana 10:43-50.

Mittermeier, R. A., P. R. Gil, M. Hoffman, J. Pilgrim, C. G. Mittermeier, J. Lamoreux, and G. A. B. d. Fonseca. 2005, Hotspots revisited: Earth's biologically richest and most endangered terrestrial ecoregions. Washington, D.C., Conservation International.

Mulcahy, D. G., and J. R. Mendelson. 2000. Phylogeography and speciation of the morphologically variable, widespread species *Bufo valliceps*, based on molecular evidence from mtDNA. Molecular Phylogenetics and Evolution 17:173-189.

Müller, F. 1879, *Ituna* and *Thyridia*: a remarkable case of mimicry in butterflies: Proceedings of the Royal Entomological Society of London. London.

Mundy, N. I. 2005. A window on the genetics of evolution: Mc1R and plumage coloration in birds. 272:1633-1640.

Myers, C. W., and J. E. Cadle. 2003. On the snake hemipenis, with notes of *Psomophis* and techniques of eversion: a response to Dowling. Herpetological Review 34:295-302.

Nachman, M. W., H. E. Hoekstra, and S. L. D'Agostino. 2003. The genetic basis of adaptive melanism in pocket mice. Proceedings of the National Academy of Science 100:5268-5273.

Nickerson, M. A., and H. L. Heringhi. 1966. Three noteworthy colubrids from southern Sonora, Mexico. Great Basin Naturalist 26:136-140.

Nijhout, H. F. 2003. Polymorphic mimicry in Papilio dardanus: mosaic dominance, big effects and origins. Evolution and Development 5:579-592.

Noonan, B. P., and P. T. Chippindale. 2006. Dispersal and vicariance: the complex evolutionary history of boid snakes. Molecular Phylogenetics and Evolution 40:347-358.

Nur, U. 1970. Evolutionary rates of models and mimics in Batesian mimicry. The American Naturalist 104:477-486.

Omernik, J. M. 1987. Map Supplement: Ecoregions of the conterminous United States. Annals of the Association of American Geographers 77:118-125.

Peakall, R., and P. E. Smouse. 2006. GENALEX 6: Genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes 6:288-295.

Peterson, A. T., O. A. Flores-Villela, L. S. Leon-Paniagua, J. E. Llorente-Bousquets, M. A. Luis-Martinez, A. G. Navarro-Siguenza, M. G. Torres-Chavez et al. 1993. Conservation priorities in northern Middle America: moving up in the world. Biodiversity Letters 1:33-38.

Pfennig, D. W., W. R. Harcombe, and K. S. Pfennig. 2001. Frequency dependent Batesian mimicry. Nature 410:323.

Pfennig, D. W., and S. P. Mullen. 2010. Mimics without models: causes and consequences of allopatry in Batesian mimicry. Proceedings of the Royal Society of London B 277:2577-2585.

Ponce-Campos, P., H. M. Smith, H. S. Harris, and D. Chiszar. 2004. A review of the taxonomic status of the members of the *Sonora michoacanensis* group

(Serpentes:Colubridae). Bulletin of the Maryland Herpetological Society 40:144-151.

Pyron, R. A., F. T. Burbrink, G. R. Colli, A. N. M. d. Oca, L. J. Vitt, C. A. Kuczynski, and J. J. Wiens. 2011. The phylogeny of advanced snakes (Colubroidea), with discovery of a new subfamily and comparison of support methods for likelihood trees. Molecular Phylogenetics and Evolution 58:329-342.

R Development Core Team. 2008.R: A language and environment for statistical computing.R Foundation for Statistical Computing, Vienna, Austria.

Ramamoorthy, T., R. Bye, A. Lot, and J. Fa. 1993, Biological diversity of Mexico: origins and distribution. Oxford, Oxford University Press.

Rambaut, A. 2007. FigTree, a graphical viewer of phylogenetic trees.

Ray, N., M. Currat, and L. Excoffier. 2003. Intra-deme molecular diversity in spatially expanding populations. Molecular Biology and Evolution 20:76-86.

Riddle, B. R., and D. J. Hafner. 2006. A step-wise approach to integrating phylogeographic and phylogenetic biogeographic perspectives on the history of core North American warm deserts biota. Journal of Arid Environments 66:435-461.

Rosenblum, E. B. 2006. Convergent evolution and divergent selection: lizards at the White Sands ecotone. The American Naturalist 167:1-16.

Rosenblum, E. B., H. E. Hoekstra, and M. W. Nachman. 2004. Adaptive reptile color variation and the evolution of the *MC1R* gene. Evolution 58:1794-1808.

Rosenblum, E. B., H. Rompler, T. Shoneberg, and H. E. Hoekstra. 2010. Molecular and functional basis of phenotypic convergence in white lizards at White Sands. Proceedings of the National Academy of Science 107:2113-2117.

Ruiz-Martinez, V. C., M. L. Osete, J. I. Núñez-Aguilar, J. Urrutia-Facugauchi, and D. H. Tarling. 2000. Paleomagnetism of late Miocene to Quaternary volcanics from the eastern segment of the Trans-Mexican Volcanic Belt. Tectonophysics 318:217-233.

Samson, F. B., and F. L. Knopf. 1994. Prairie conservation in North America. Bioscience 44:418-421.

Samson, F. B., F. L. Knopf, and W. R. Ostlie. 2004. Great Plains ecosystems: past, present, and future. . Wildlife Society Bulletin 32:6-15.

Savage, J. M. 1982. The enigma of the Central American herpetofauna: dispersals or vicariance. Annals of the Missouri Botanical Garden 69:464-547.

Savage, J. M., and J. B. Slowinski. 1992. The colouration of venomous coral snakes (family Elapidae) and their mimics (families Aniliidae and Colubridae). Biological Journal of the Linnean Society 45:235-254.

Savitzky, A. H. 1983. Coadapted character complexes among snakes: fossoriality, piscivory, and durophagy. American Zoologist 23:397-409.

Schargel, W. E., G. A. Rivas, R. Makowsky, J. C. Seňaris, M. A. Natera, T. R. Barros, C. R. Molina et al. 2010. Phylogenetic systematics of the genus *Gonatodes* (Squamata: Sphaerodactylidae) in the Guyana region, with description of a new species from Venezuela. Systematics and Biodiversity 8:321-339.

Sherbrooke, W. C., and M. F. Westphal. 2006. Responses of greater roadrunners during attacks on sympatric venomous and nonvenomous snakes. The Southwestern Naturalist 51:41-47.

Sinervo, B., and C. M. Lively. 1996. The rock-paper-scissors game and the evolution of alternative male strategies. Nature 380:240-243.

Smith, H. M., and E. H. Taylor. 1945. An annotated checklist and key to the snakes of Mexico. U.S. National Museum Bulletin 187:iv + 239 pp.

—. 1950. An annotated checklist and key to reptiles of Mexico exclusive of snakes. Bulletin of the United States National Museum 199:1-253.

Smith, S. M. 1975. Innate recognition of coral snake pattern by a possible avian predator. Science 187:759-760.

—. 1977. Coral snake pattern recognition and stimulus generalisation by naive great kiskadees (Aves: Tyrranidae). Nature 265:535-536.

Spear, S. F., N. Balkenhol, M.-J. Fortin, B. H. McRae, and K. Scribner. 2010. Use of resistance surfaces for landscape genetic studies: considerations for parameterization and analysis Molecular Ecology 19:3576-3591.

Specht, G., E. P. Mesquita, and F. A. Santos. 2008. Breeding biology of Laughing Falcon Herpetotheres cachinnans (Linnaeus, 1758)(Falconidae) in southeastern Brazil. Revista Brasileira de Ornitologia 16:155-159.

Stamatakis, A. 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22:2688-2690.

Stickel, W. H. 1938. The snakes of the genus *Sonora* in the United States and Lower California. Copeia 1938:182-190.

—. 1943. The Mexican snakes of the genera *Sonora* and *Chionactis* with notes on the status of other colubrid genera. Proceedings of the Biological Society of Washington 56:109-128.

Storfer, A., M. A. Murphy, J. S. Evans, C. S. Goldberg, S. Robinson, S. F. Spear, R. Dezzani et al. 2007. Putting the "landscape" in landscape genetics. Heredity 98:128-142.

Symula, R., R. Schulte, and K. Summers. 2001. Molecular phylogenetic evidence for a mimetic radiation in Peruvian poison frogs support a Mullerian mimicry hypothesis. Proceedings of the Royal Society of London B 268:2415-2421.

Tamura, K., D. Peterson, G. Stecher, M. Nei, and S. Kumar. 2011. MEGA5: Molecular evolutionary genetics analysis using likelihood, evolutionary distance and maximum parsimony methods. Molecular Biology and Evolution 28:2731-3739.

Taylor, E. H. 1937. A new snake of the genus *Sonora* from Mexico, with comments on *S. michoacanensis*. Herpetologica 1:69-74.

Thompson, J. N. 1994, The coevolutionary process. Chicago, The University of Chicago Press.

—. 2005, The geographic mosaic of coevolution. Chicago, University of Chicago Press.

Townsend, T. M., A. Larson, E. Louis, and J. R. Macey. 2004. Molecular phylogenetics of squamata: the position of snakes, amphisbaenids and dibamids and the root of the squamate tree. Systematic Biology 53:735-757.

Turner, J. R. G. 1978. Why male butterflies are non-mimetic: natural selection, sexual selection, group selection, modification and sieving. Biological Journal of the Linnean Society 10:385-432.

Uy, J. A. C., R. G. Moyle, C. E. Filardi, and Z. A. Cheviron. 2009. Difference in plumage color used in species recognition between incipient species is linked to a single amino acid substitution in the melanocortin-1 receptor. The American Naturalist 17:244-254.

Vawter, L., and W. M. Brown. 1986. Nuclear and mitochondrial DNA comparisons reveal extreme rate variation in the molecular clock. Science 234:194-196.

Vidal, N., and S. B. Hedges. 2009. The molecular evolutionary tree of lizards, snakes, and amphisbaenians. Comptes Rendus Biologies 332:129-139.

Vos, P., R. Hogers, M. Bleeker, M. Reijans, T. v. d. Lee, M. Hornes, A. Frijters et al. 1995. AFLP: a new technique for DNA fingerprinting. Nucleic Acids Research 23:4407-4414.

Waldbauer, G. P. 1988. Asynchrony between Batesian mimics and their models. The American Naturalist 131:S103-S121.

Wang, I. J. S., W. K., and H. B. Shaffer. 2009. Landscape genetics and least-cost path analysis reveal unexpected dispersal routes in the California tiger salamader (*Ambystoma californiense*). Molecular Ecology 18:1365-1374.

Wegman, D., M. Currat, and L. Excoffier. 2006. Molecular diversity after a range expansion in heterogeneous environments. Genetics 174:2009-2020.

Werler, J. E., and J. R. Dixon. 2000, Texas snakes: identification, distribution and natural history. Austin, TX, University of Texas Press.

Whitlock, R., H. Hipperson, H. Mannerelli, R. K. Butlin, and T. Burke. 2008. An objective, rapid and reproducible method for scoring AFLP peak-height data that minimizes genotyping error. Molecular Ecology Resources 8:725-735.

Wickler, W. 1968, Mimicry in plants and animals. New York, McGraw-Hill.

Wiley, E. O. 1978. The evolutionary species concept reconsidered. Systematic Biology 27:88-92.

Wilgenbusch, J. C., D. L. Warren, and D. L. Swofford. 2004. A system for graphical exploration of MCMC convergence in Bayesian phylogenetic inference. Florida State University.

Wisely, S. M., S. W. Buskirk, M. A. Fleming, D. B. Mcdonald, and E. A. Ostrander. 2002. Genetic diversity and fitness in black-footed ferrets before and during a bottleneck. Journal of Heredity 93:231-237.

Wuster, W., C. S. E. Allum, I. B. Bjargardottir, K. L. Bailer, K. J. Dawson, J. Guenioui, J. Lewis et al. 2004. Do aposematism and Batesian mimicry require bright colours? A test, using European viper markings. Proceedings of the Royal Society of London B 271:2495-2499.

Yang, Z., and B. Rannala. 2010. Bayesian species delimitation using multilocus sequence data. Proceedings of the National Academy of Science 107:9264-9269.

Yeh, F. C., and T. J. B. Boyle. 1997. Population genetic analysis of co-dominant and dominant markers and quantitative traits. Belgian Journal of Botany 129:157.

Zaher, H., and A. L. C. Prudente. 2003. Hemipenes of *Siphlophis* (Serpentes, Xerodontinae) and techniques of hemipenial preparation in snakes: a response to Dowling. Herpetologial Review 34:302-307.

Zweifel, R. G. 1981. Genetics of color pattern polymorphism in the California kingsnake. Journal of Heredity 72:238-244.

Zweifel, R. G., and K. S. Norris. 1955. Contributions to the herpetology of Sonora, Mexico. American Midland Naturalist 54:230-249.

BIOGRAPHICAL INFORMATION

Christian L. Cox was born in Laramie, Wyoming to Robert and Susan Cox, an entomologist and an English teacher, respectively. Christian graduated with a Bachelors of Science from Iowa State University in 2005, majoring in Zoology with a minor in Genetics. He continued his education at the University of Alabama in the laboratory of Dr. Stephen Secor, graduating in 2007 with a Master's degree in Biological Sciences. Christian began his degree at the University of Texas-Arlington in 2007 under the supervision of Dr. Jonathan A. Campbell and Dr. Paul T. Chippindale, and graduated with a PhD. in Quantitative Biology in 2012. His research interests are broadly based in ecology, physiology, and evolution. Christian has been active in fieldwork, assisting with the collection of over 5000 specimens from mainland Mexico. In addition, he has personally collected over 900 specimens from Costa Rica, Baja California and the United States which are deposited in the Instituto Clodomiro Picado (UCR), Museo de Zoologia de la Facultad de Ciencias (UNAM), and the Amphibian and Reptile Diversity Research Center (UTA). Christian has 14 peer-reviewed publications published or in press, and has served as a reviewer for nine scientific journals. In recognition of exemplary research and teaching, he has received the T.E. Kennerley Excellence in Teaching Award and the Outstanding Graduate Research Award. Christian will continue his research in evolutionary biology as a postdoctoral fellow at the University of Virginia.