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Evolutionary Linkage of Mimetic and Non-Mimetic Color Traits in a Coral Snake Mimicry Complex

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EVOLUTIONARY LINKAGE OF MIMETIC AND NON-MIMETIC COLOR TRAITS IN A CORAL SNAKE MIMICRY COMPLEX

by

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(Under the Direction of Christian L. Cox)

ABSTRACT

Color polymorphism in aposematic mimicry systems is a perplexing phenomenon for evolutionary biologists, as theoretically the benefits of converging on a model phenotype should constrain the evolution of phenotypic diversity in these systems (i.e., color polymorphism should not occur). Nevertheless, color polymorphism in mimicry systems is prevalent throughout many taxa. In some of these systems, the evolution of color polymorphism results in the existence of non-mimetic morphs, such as those that are cryptic. The case of ground snakes (*Sonora semiannulata*) is unique in that color polymorphism encompasses both mimetic and cryptic morphs, as well as individual mimetic and non-mimetic traits. In this study, I used ground snakes to investigate the evolutionary drivers of polymorphic non-mimetic traits within a mimicry system. With a robust dataset of 1240 individuals from 49 populations, I assessed spatial patterns of color traits and associations among them. In addition, I utilized high-throughput DNA sequencing to generate 2,125 neutral single-nucleotide polymorphisms (SNPs) shared among 109 individuals, which allowed me to conduct population genetic analyses that, in turn, shed light on selective processes. I demonstrated that mimetic and non-mimetic polymorphic traits are spatially linked with one another, but that they appear to be influenced by different patterns of selection. These results, when taken together, offer support for genetic linkage between these different types of color polymorphism. Such findings present a novel mechanism by which

phenotypic diversity can be maintained, which has major implications for color pattern diversity across the tree of life.

INDEX WORDS: Color polymorphism, Evolution, Mimicry, Population genetics

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by

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tests were run with 99,999 iterations. Partial Mantel tests that included all $N = 31$ populations are displayed here, but no significant correlations were recovered when simple Mantel tests were used, when populations with one individual were excluded, or when populations with two individuals were excluded.

Table 2.4: Results of Spearman's rank correlation analyses testing for the association between genetic diversity and color trait diversity (both measured as Shannon's Information Index, I) among $N = 23$ populations (populations with genetic data for one individual not included). No correlations were significant at $\alpha = 0.05$ regardless of the diversity measure used (Shannon's I, heterozygosity, or unbiased heterozygosity).

Table 2.5: The results of contingency analyses using Fisher's exact test, showing all significant associations between any SNPs and any of the four color traits. Significance was assessed using the Benjamini-Hochberg procedure with the false discovery rate set to 10%. Note that no SNPs share a significant relationship with more than one color trait.

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values of K, the log likelihood of each independent run was calculated. (a) The average log likelihood for 12 potential values of K. (b) The estimation of ΔK, calculated using the methods of Evanno et al. (2005). The K value with the highest ΔK is the most likely number of genetic clusters given the data. (c) A *STRUCTURE* plot based on the results of the most likely run with a K value of two.

Figure 2.2: Distribution of genotypes across the landscape, based on analyses in *STRUCTURE*. Each pie chart represents the average proportions of genotypes assigned to a genetic cluster within a population.

Figure 2.3: Among-population F_{ST} values for color patterns with 95% confidence intervals, presented with (a) color traits separated or (b) grouped into their respective type (mimetic or non-mimetic). The dashed line indicates the mean F_{ST} value for neutral SNPs, and the gray bar represents the 95% confidence interval. Note that in both panels, mimetic and non-mimetic color traits had significantly higher F_{ST} values than did the neutral genetic markers. When (c) F_{ST} was calculated for each locus individually, color trait F_{ST} values are higher on average than most SNP F_{ST} values (frequencies shown in dark gray).

GENERAL INTRODUCTION

Adaptive coloration in animals

One of the most fundamental and extensively-studied questions in biology is how to explain the patterns of phenotypic diversity observed in nature, especially with regards to color (Bennett *et al.* 1994; Cott 1940; Poulton 1890). In animals, adaptive coloration (as opposed to neutral or maladaptive coloration, such as albinism or leucism) can be broadly categorized as having one to three non-mutually exclusive evolutionary functions: (a) intraspecific communication and sexual signaling, (b) physiological regulation, and (c) predator-prey interactions.

Sexual selection is responsible for some of the brightest and most conspicuous colors seen in the animal kingdom, such as those found in the plumages of birds of paradise (Irestedt *et al.* 2009), the dewlaps of anole lizards (Sigmund 1983), and the scales and fins of guppies (Godin and McDonough 2003; Kodric-Brown 1985). In systems in which color evolution is driven primarily by sexual selection, bright colors are often used by one sex to signal to members of the opposite sex, advertising their suitability as a potential mate (Kodric-Brown and Brown 1984). Additionally, sexually-selected colors may be used as means of communication among members of the same sex, often in aggressive territorial disputes or as signals of dominance or submission (Höglund *et al.* 2002; Losos 1985). In some systems, sexual selection favors colors that make a male resemble a female, allowing the "sneaker" male to gain access to females without running the risk of being perceived as a threat by more dominant males (Brantley *et al.* 1993; Sinervo and Lively 1996). Because bright colors implicated with sexual selection are directly linked to an individual's reproductive output, the selection pressure can be intense,

despite the potential survival cost of being highly conspicuous to predators (Endler 1983; Godin and McDonough 2003; Zuk and Kolluru 1998).

Color may also serve as a physiological adaptation for the regulation of temperature (Clusella Trullas *et al.* 2007; Majerus 1998; Rosenblum *et al.* 2004). Particularly in heliothermic ectotherms, lighter-colored individuals are often better at reflecting heat, while darker-colored individuals are better at absorbing it (Bittner *et al.* 2002; Clusella Trullas *et al.* 2007). In many cases, this results in diurnal, darker-colored species inhabiting cooler environments than lighter-colored species (Clusella Trullas *et al.* 2007). However, some animals are able to promote thermoregulation through dynamic physiological regulation of color, including lizards, frogs, fishes, and crustaceans (Fernandez and Bagnara 1991; Norris 1965; Stuart-Fox and Moussalli 2009).

Perhaps the most widespread function of color is for use in predator-prey interactions. Most animals are cryptic, usually possessing muted colors that provide camouflage and allow them to escape detection by a potential predator or potential prey (Endler 1978; Endler and Greenwood 1988). However, bright coloration can provide a number of adaptive advantages in predator-prey interactions as well. Bright colors are used by some species with decoy coloration, in which an animal possesses a brightly colored limb or tail, which attracts the attention of predators and is not critical to survival if lost (Bateman *et al.* 2014). Other prey species utilize startle coloration or flash coloration (also called deimatic coloration), in which they flash a bright color, eye spots, etc. at a predator and then remove it from sight, thereby effectively intimidating or confusing the predator (Schlenoff 1985; Williams *et al.* 2000). Predators themselves can use color as well; some species use brightly-colored appendages to lure prey towards them (Laurenson *et al.* 2004; Neill 1960). Finally, aposematic species possess bright colors that

dissuade a potential predator from pursuing by advertising danger, such as venom or toxin (Mappes *et al.* 2005). In mimicry systems, which are often characterized by aposematism, one species receives some sort of protective advantage against predators by possessing colors that imitate a distasteful or dangerous species (Ruxton *et al.* 2004).

Mimicry and color polymorphism

Mimicry is often characterized as either Müllerian or Batesian. In Müllerian mimicry, a group of aposematic toxic species converge on a similar phenotype, such that predators learn to avoid all of them by learning to avoid one of them (Brower 1958; Kapan 2001; Mallet and Gilbert Jr 1995; Müller 1879b; O'Donald and Pilecki 1970). In the case of Batesian mimicry systems, a harmless species can take advantage of the benefits of aposematism and deceitfully imitate a dangerous species for its own protection (Bates 1862; Ceccarelli and Crozier 2007; Emlen 1968; Ohsaki 1995; Pfennig *et al.* 2001). In both types of mimicry systems, species may exhibit a phenomenon known as color polymorphism, in which two or more discrete color types (deemed "morphs") exist concurrently within a population (Cox and Davis Rabosky 2013b; Gray and McKinnon 2006). In part because of this, mimicry may be considered a driver of phenotypic diversity (Cox and Davis Rabosky 2013; Davis Rabosky *et al.* 2016b; Jiggins *et al.* 2001; Joron *et al.* 2011; Maan and Cummings 2011; Wang and Shaffer 2008).

Coral snake mimicry is a classic case of Batesian mimicry, in which relatively harmless mimics possess the aposematic coloration of venomous coral snakes, which sends a false signal of danger to potential predators (Bates 1862; Greene and McDiarmid 1981). Unlike the distastefulness associated with some aposematic butterflies (Müller 1879; Ruxton *et al.* 2004), attacking a true coral snake can be deadly for a predator, and such strong selection pressure has led many predators to avoid anything communicating the coral snake signal (Brodie III 1993;

Brodie III and Janzen 1995; Greene and McDiarmid 1981). In many birds, for example, the recognition and avoidance of dowels painted with red, yellow, and black rings is innately present (Smith 1975; 1977). Additionally, even mimics that are imprecise, such as those with only two of the three colors of a coral snake, those with a different order of rings, etc., are avoided (Kikuchi and Pfennig 2010). This may be due to predators avoiding anything that looks remotely close to a coral snake, or because the mimic is exploiting the cognitive abilities of the predator, possessing only the most necessary components of the coral snake signal (Davis Rabosky *et al.* 2016a; Kikuchi and Pfennig 2010). Although one might expect to see this pattern only in areas where predators frequently encounter coral snakes, research investigating the effect of coral snake sympatry or allopatry on mimics has shown mixed outcomes. In some systems, the protective advantage of coral snake mimicry breaks down in allopatry with coral snakes (Pfennig *et al.* 2001; Ruxton *et al.* 2004), while in others, mimics continue to be avoided by predators well outside the coral snakes' range (Pfennig and Mullen 2010). This may occur because potential predators, such as birds, have large home ranges or migratory routes that encompass areas with coral snakes and as such, they have learned or inherited the avoidance behavior (Holmes *et al.* 2017; Pfennig and Mullen 2010). Finally, some coral snake mimics, like those in the genera *Sonora, Chionactus,* and *Chilomeniscus*, exhibit pronounced color polymorphism, in which some individuals strongly resemble coral snakes, while other individuals possess few or none of the signal components associated with coral snake mimicry (Cox and Davis Rabosky 2013; Savage and Slowinski 1992; Stebbins 2003).

Color polymorphism in general can be highly variable both among and within species and populations, and this high degree of variation is often derived from a multitude of sources (Cox and Davis Rabosky 2013). Populations that exhibit color polymorphism can differ

markedly in the number of morphs, with some possessing only two (Andrén and Nilson 1981; King 1988) and others exhibiting greater than ten (referred to as "exuberant" color polymorphisms; Croucher *et al.* 2011; Franks and Oxford 2009). Differences between the color patterns of morphs can also vary from relatively subtle to so drastic that the morphs can be mistaken for separate species (Cox *et al.* 2012; Ford 1955; Forshaw 1978; Rowell 1972). In addition, some polymorphic species vary in the conspicuousness of morphs, such that some morphs are highly cryptic (Bond 2007; King and Lawson 1995) while others exhibit aposematism (Brodie III and Brodie Jr. 2004; Noonan and Comeault 2009). Finally, color polymorphism can vary across the landscape (within- versus among-population variation) and through time (transient versus stable polymorphism) (Cox and Davis Rabosky 2013).

Experimental Framework and Study System

Previous work has shown that color polymorphism in mimicry systems can encompass both mimetic and non-mimetic (e.g., cryptic) morphs (Nijhout 2003; Ohsaki 1995; Vences *et al.* 2003; Wang and Shaffer 2008), and some have suggested possible explanations for why these non-mimetic morphs may arise. Such explanations include sexual selection (Ohsaki 1995), shifts in predator avoidance strategies from aposematism to crypsis (Rudh 2013; Wang and Shaffer 2008), and the decoupling of the aposematic color and the danger it signals (e.g., toxin; Wang 2011). However, no studies have addressed color polymorphism of non-mimetic *traits* within mimetic or non-mimetic morphs. There is a significant gap in the literature as to how color polymorphism of these non-mimetic traits might evolve, as well as how their evolution might compare to that of color polymorphism in mimetic traits.

This thesis research utilized the ground snake (*Sonora semiannulata*; Serpentes: Colubridae), a small, semi-fossorial snake found throughout central and western North America

that possesses both mimetic and non-mimetic color traits (Cox and Davis Rabosky 2013; Davis Rabosky *et al.* 2016b). This species exhibits pronounced color polymorphism with regards to red and black pigmentation, such that four distinctive color morphs can be found throughout its range: 1) individuals with a red longitudinal dorsal stripe, 2) individuals with black dorsal crossbands, 3) individuals with both a red stripe and black crossbands, and 4) individuals possessing neither black or red pigmentation, resulting in a uniform gray to brown coloration (Cox and Chippindale 2014b; Cox and Davis Rabosky 2013; Davis Rabosky *et al.* 2016b). All four color morphs can be found in coexistence in some populations, while other populations possess only a single morph (Cox and Chippindale 2014; Cox and Davis Rabosky 2013). Red and black coloration in this species has an evolutionary origin in coral snake mimicry (Cox *et al.* 2012), so the red and black morph is considered to be a coral snake mimic (Cox and Davis Rabosky 2013; Davis Rabosky *et al.* 2016b; Savage and Slowinski 1992). The uniform morph, which possesses neither of the two mimetic traits, is considered to be a cryptic morph (Cox and Davis Rabosky 2013). Previous research has found that temporally and spatially variable selection, including frequency dependence, governs the evolution of these color traits in this species (Cox and Davis Rabosky 2013). Sexual selection is unlikely to influence color variation in this species, as snakes in general have limited color vision (Sillman *et al.* 1999). Moreover, there is no evidence for sexual dichromatism or assortative mating in ground snakes (Cox and Chippindale 2014; Cox and Davis Rabosky 2013), which would be expected for sexually-selected color. Red and black pigmentation are likely controlled by separate loci, with no support for linkage disequilibrium (Davis Rabosky *et al.* 2016b). Finally, the genetic control of pigmentation in ground snakes is currently unknown, although we do know that it is not

controlled by the *Mc1r* gene (Cox *et al.* 2013) as it is in many other reptiles (Rosenblum *et al.* 2004).

While polymorphism of the red and black patterns has received some attention in previous studies (Cox and Davis Rabosky 2013; Davis Rabosky *et al.* 2016a; Davis Rabosky *et al.* 2016b), the ground snake is also polymorphic for two traits that have remained virtually unstudied in this species: a black cap and a black nuchal collar (a single band of pigment located several scales posterior to the parietal scales on the top of the head; Figure 1.2). The function of these traits is currently unknown, but they are not exclusive to ground snakes; black caps are characteristic of a number non-mimetic snake species, such as many of those in the genus *Tantilla* (Powell *et al.* 2016), and the nuchal collar is found in both mimetic (Liner 1960) and non-mimetic (Powell *et al.* 2016; Sawaya and Sazima 2003) snake species as well. While this black pigment may aid in thermoregulation (Andrén and Nilson 1981; Bittner *et al.* 2002; Luiselli 1992) by attracting heat to the head without completely exposing the snake, it may also or instead be more of a type of background color matching or disruptive coloration (Stevens 2007), which would make the head less likely to be the focal point of attack from a predator. Although one could argue that the Texas coral snake (*Micrurus tener*), which is sympatric with ground snakes throughout much of their range, possesses a black head and a black band posterior to the head (Powell *et al.* 2016; Stebbins 2003), this would not explain the persistence of these traits in species that have no known implications with mimicry. As such, though the exact function is unknown, I consider the black cap and nuchal collar to be non-mimetic traits in ground snakes.

This study takes two different but related approaches to ask questions about color polymorphism and mimicry. The first approach entails quantifying morph frequencies across the

landscape. Using a robust dataset comprised of individuals from many populations, I ask three broad questions: 1) how do color traits vary spatially, 2) are color traits statistically associated across populations, and 3) does mimetic trait diversity predict non-mimetic trait diversity? The second approach I use to investigate mimicry and color polymorphism makes use of population genetics. I use a combination of both genotypic and phenotypic data to draw inferences about presence and pattern of selection acting on each color trait and color trait type (mimetic or non-mimetic), as well as test for genetic linkage among traits. In this second approach, I address four broad questions: 1) does genetic structure explain color distribution, 2) does genetic diversity within or among populations predict color trait diversity, 3) what patterns of selection (if any) are influencing mimetic and non-mimetic color polymorphism, and 4) can I identify any loci as being linked to any color trait? The first and second approach to answering such questions about color polymorphism are addressed in Chapters 1 and 2 of this thesis, respectively.

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CHAPTER 1

SPATIAL VARIATION OF MIMETIC AND NON-MIMETIC COLOR POLYMORPHISM IN THE GROUND SNAKE

ABSTRACT

Mimicry, in which an animal closely resembles a dangerous or toxic model for a protective advantage, is a prevalent form of phenotypic diversity found in nature. While the fitness benefits of mimicry often lead to convergence on a single color pattern, some species exhibit color polymorphism, in which two or more discrete color patterns co-occur in a population. In many taxa, the evolution of this color polymorphism has resulted in the presence of both mimetic and non-mimetic (e.g., cryptic) morphs. Although some research has investigated how these non-mimetic morphs originate and persist, we know very little about how the evolutionary dynamics of non-mimetic traits compare to those of mimetic traits. I directly addressed this by studying spatial variation in the presence/absence and frequency of mimetic (a red dorsal stripe and black crossbands) and non-mimetic (a black cap and a black nuchal collar) color traits in the polymorphic ground snake, a putative coral snake mimic. Using a dataset comprising 1240 individuals from 49 populations across the range of ground snakes, I assessed patterns of spatial distribution, looked for associations with geographic variables (including latitude, longitude, and coral snake sympatry/allopatry), and tested for statistical associations among traits. I found that mimetic and non-mimetic traits had similar patterns of spatial distribution, with some traits exhibiting the mosaic type of arrangement, others exhibiting variation along a latitudinal cline, and none being associated with longitude or coral snake sympatry. I also found that mimetic and non-mimetic traits were significantly associated with one another. These findings suggest that polymorphism in mimetic and non-mimetic traits is

evolutionarily linked in ground snakes, with either similar selection among populations or genetic linkage between these two types of traits. More broadly, it appears that the evolutionary processes that maintain one type of color polymorphism can simultaneously maintain polymorphisms of color traits with other functions.

INTRODUCTION

The processes responsible for the vast diversity of phenotypes found in nature have captivated evolutionary biologists since the time of Charles Darwin (Darwin 1872). One important driver of phenotypic diversity is signal evolution. Signals for inter- and intra-specific communication can have dramatic impacts on fitness, and as such traits evolve in a spatially and temporally variable environment, diversity can be generated (Endler 1992). In many systems, this signaling-driven diversity comes in the form of colors and patterns (Endler 1978; Endler and Greenwood 1988). For example, in mimicry systems, an animal closely resembles a dangerous or distasteful species and honestly or deceitfully signals to predators that it, too, may be costly to attack (Bates 1862; Endler 1981; Müller 1879; Ruxton *et al.* 2004). While the protective advantage of such colors has led some species to be fixed for a mimetic phenotype, especially when in sympatry with the model (Greene and McDiarmid 1981; Pfennig *et al.* 2001), other species exhibit color polymorphism (the phenomenon in which two or more color patterns exist concurrently in a population; Cox and Davis Rabosky 2013; Endler 1981; Gray and McKinnon 2006). In a number of mimicry complexes, this color polymorphism can encompass both mimetic and cryptic (i.e., non-mimetic) color morphs (Nijhout 2003; Ohsaki 1995; Vences *et al.* 2003; Wang and Shaffer 2008). Although mimicry has previously been shown to be a potent driver of phenotypic diversity (Cox and Davis Rabosky 2013; Davis Rabosky *et al.* 2016b; Jiggins *et al.* 2001; Joron *et al.* 2011; Maan and Cummings 2011; Wang and Shaffer 2008), the

impact of mimicry-related selection on non-mimetic color polymorphism remains to be addressed.

Depending on the function, color polymorphism can arise and persist under alternate types of selection and can exhibit variable evolutionary dynamics (Forsman *et al.* 2008; Gray and McKinnon 2006; Roulin 2004). In most mimicry systems, color polymorphism of mimicry traits is maintained by predator-driven frequency-dependent selection (Bonansea and Vaira 2012; Holmes *et al.* 2017; O'Donald and Pilecki 1970). Species such as the aposematic *Heliconius* butterflies and dendrobatid poison dart frogs, two classic examples of Müllerian mimicry, often experience positive frequency-dependent selection, in which rare color morphs are at a disadvantage as predators disproportionately avoid familiar, common morphs (Joron and Mallet 1998; Langham 2004; Symula *et al.* 2001). In contrast, Batesian mimics like those that mimic coral snakes often experience negative frequency-dependent selection, in which predators disproportionately consume the most common morphs, giving rare morphs an advantage (Gray and McKinnon 2006; Holmes *et al.* 2017). In both Müllerian and Batesian mimicry systems, the evolution of color polymorphism can produce non-mimetic morphs, but these are often selected against because a deviation from the aposematic signal that a predator avoids renders such color morphs unprotected (Joron *et al.* 2011; Ohsaki 1995). Nevertheless, non-mimetic morphs can be maintained by sexual dimorphism (Ohsaki 1995), shifts in predator avoidance strategies from aposematism to crypsis (Rudh 2013; Wang and Shaffer 2008), or the decoupling of the aposematic color and the danger it signals (e.g., toxin; Wang 2011). While these factors give us some idea of how non-mimetic morphs might persist in mimicry systems, it is equally important to understand how color polymorphism *within* these morphs may evolve. We can gain insight

into this phenomenon by studying the geographic distribution of mimetic and non-mimetic color morphs across the landscape.

The spatial distribution of color morphs can be quite informative about the type of selection responsible (Holmes *et al.* 2017). If all morphs are present ubiquitously across populations at similar frequencies, it is likely the result of an equally ubiquitous pattern of density-dependent selection (Gosden *et al.* 2011; Svensson and Abbott 2005). In contrast, if gradual ecological changes are tracked by gradual changes in morph frequencies, the spatial arrangement of color morphs is considered to be that of the clinal type (Hegna *et al.* 2013). If instead color morphs form a mosaic across the landscape that cannot be explained by geography or environment, the underlying selective forces may be highly variable in strength, direction, or type (Cox and Davis Rabosky 2013), or polymorphism may be driven by regional-scale predation and negative frequency-dependent selection (Holmes *et al.* 2017). Beyond these three types of spatial arrangements, if traits are found to be spatially associated with one another, it may suggest genetic linkage, such that selection favoring one trait will also favor the other through the non-random assortment of alleles (Hartl and Clark 2007), or that these traits are subject to the same type of selection (Cox and Davis Rabosky 2013; Holmes *et al.* 2017). Focusing on the geographic distribution of mimetic and non-mimetic traits can tell us whether such traits are linked, as well as what type of selection is acting upon them.

In this study, I compared the evolutionary dynamics of mimetic and non-mimetic color polymorphisms in the ground snake (*Sonora semiannulata*). Specifically, I assessed the spatial distribution of both types of color traits and tested whether morph frequencies were influenced by latitude, longitude, and coral snake sympatry. I then tested for statistical associations between mimetic and non-mimetic traits by asking whether the frequency of mimetic traits predicts the

frequency of non-mimetic traits across all populations and whether mimetic trait diversity predicts non-mimetic trait diversity within populations.

METHODS

Study species

The ground snake (*Sonora semiannulata*) is a small, semi-fossorial snake that inhabits parts of central and western North America (Cox and Davis Rabosky 2013; Powell *et al.* 2016). It is polymorphic for two color traits that are associated with coral snake mimicry: a red dorsal stripe and black crossbands (Figure 1.1). The presence or absence of these traits yields four distinct color morphs: red-striped, black-banded, mimetic (having both the red stripe and black crossbands), and uniformly brown (Cox and Chippindale 2014; Cox and Davis Rabosky 2013; Davis Rabosky *et al.* 2016a). Additionally, the ground snake is polymorphic for the presence or absence of a black cap and a black nuchal collar (a single black band located behind the head; Figure 1.2), both of which have been noted but unstudied in the literature (Frost 1983; Powell *et al.* 2016). Although the function of the black cap and nuchal collar is unknown, I consider them to be non-mimetic, as they are found in a number of non-mimicking snake species, such as those in the genera *Tantilla, Diadophis*, and *Storeria* (Powell *et al.* 2016; Sawaya and Sazima 2003).

Phenotypic scoring

My dataset consisted of 1240 specimens from 49 populations spanning the geographic range of the Great Plains clade (C.L. Cox, unpublished data) of *Sonora semiannulata* (Table 1.1; Figure 1.3). These specimens were obtained from a number of museum collections and personal collections (Appendix I). All individuals were photographed from multiple angles, and photographs were scored based on the presence or absence of a red dorsal stripe, black crossbands, a black cap, and a black nuchal collar. Upon first examination, it was impossible to

discern whether black-banded and mimetic individuals possessed a black nuchal collar, so I counted the number of scales between the parietal scales and the first (or only) dark band for all individuals. Although the average number of scales differed for banded/mimetic individuals and un-banded individuals with a nuchal collar, the number of scales between the parietals and the first band or the nuchal collar frequently overlapped. This confirmed that it was indeed impossible to determine whether banded and mimetic animals truly possessed a nuchal band. I elected to score banded and mimetic animals as lacking the nuchal collar because, from a functional standpoint, an animal with a single nuchal band differs quite markedly from an animal with bands along the entire length of the body. Nevertheless, I also ran all analyses involving the nuchal collar under the alternate scenario (animals with bands do possess a nuchal collar) to ensure that I was not biasing my results (see Appendices II and III).

Population designation

Following Cox and Davis Rabosky (2013), I designated populations as the U.S. county in which individuals were originally collected, and the geographic "location" of each county was calculated by finding the center GPS point of the county's polygon using ArcGIS (ESRI 2017). For the location of the Coahuila, Mexico population, I calculated a GPS point based on the center of the samples' specific locality information, rather than the center of large state of Coahuila. Additionally, a few counties in Texas were grouped together to achieve a higher sample size, but this was only done if (a) the counties were neighboring, and (b) the combined size of the group of counties was smaller than the majority of single counties in other states. For these county groups, the "location" was calculated as the average of each county's latitude and longitude. To determine whether populations were sympatric with the Texas coral snake (*Micrurus tener*;

Serpentes: Elapidae), I followed Powell *et al.* (2016) and the species range map provided by the IUCN (Hammerson *et al.* 2007).

Spatial distribution analyses

To assess what type of spatial distribution characterized each type of color polymorphism, I calculated trait frequencies within each population and plotted them on a map. I investigated the effect of latitude or longitude on each color trait and on the Shannon diversity index of each type of color trait within populations using non-parametric Spearman's rank correlational analyses (the data were not normally distributed). The effect of coral snake allopatry or sympatry on these traits was assessed using a non-parametric Mann-Whitney-Wilcoxon analysis (these data were also not normally distributed). For these 18 tests of the effect of spatial variables (latitude, longitude, and coral snake sympatry) on color variables (the four color traits, the diversity of mimetic traits, and the diversity of non-mimetic traits), I applied a Bonferroni-corrected *P*-value of 0.00278. The above statistical tests were conducted in JMP (Version 11; SAS 2014).

Trait association tests

To investigate the association of mimetic traits and non-mimetic traits across all populations, I conducted Model 1 contingency analyses using likelihood ratios. To investigate the relationship between mimetic and non-mimetic trait diversity within populations, I used the non-parametric Spearman's rank correlation analysis (the data were not normally distributed). This test, as well as the contingency analyses, were conducted in JMP (Version 11; SAS 2014). I also used the program GenAlEx (Peakall and Smouse 2006; 2012) to generate distance matrices for all color traits, coding the presence or absence of a trait as a 1 or 0 in a binary fashion. These distance matrices were analyzed using partial Mantel tests (Manly 1986; Smouse *et al.* 1986)

with 99,999 iterations in the program zt (Bonnet and de Peer 2002). Partial Mantel tests (controlling for geographic distance) were used rather than full Mantel tests because the distance matrix of at least one trait in each analysis was significantly correlated with geographic distance, indicating spatial autocorrelation (Legendre 1993). Because I ran six of these partial Mantel tests, I applied a Bonferroni-corrected critical *P*-value of 0.00833.

RESULTS

Trait frequencies and spatial distributions

I found that frequencies of both mimetic and non-mimetic traits varied considerably across the landscape, with some populations apparently fixed for one morph and others exhibiting all morphs (Figure 1.4). When I tested for the relationship between spatial variables and color traits within populations, I found that no color traits were significantly associated with longitude or coral snake sympatry (Table 1.2). The percentage of individuals with the red stripe and the percentage of those with the nuchal collar were associated with latitude (Spearman's $\rho = 0.478$; $P = 0.008$ and Spearman's $\rho = 0.463$; $P = 0.0012$, respectively); as latitude increased, so did the proportions of the red stripe and the nuchal collar. None of the other color variables shared these significant relationships with latitude.

Trait and diversity associations

I found that mimetic and non-mimetic color traits were statistically associated with one another (Figure 1.5). When color trait frequencies were summed for all individuals across all populations, I found a significant association between the red stripe and the black cap ($\chi^2 = 99.361$; $P < 0.0001$), between crossbands and the black cap ($\chi^2 = 309.992$; $P < 0.0001$), between the red stripe and the nuchal collar ($\chi^2 = 34.119$; $P < 0.0001$), and between the black cap and the nuchal collar ($\chi^2 = 48.665$; $P < 0.0001$). I also found that the diversity of mimicry traits was

significantly positively correlated with the diversity of non-mimicry traits (Spearman's ρ = 0.436; P = 0.0024; Figure 1.6). Although the latter analysis excluded populations with five or fewer individuals, re-running with the inclusion of these populations did not affect the significance of these results.

Color trait distance matrices were found to be correlated for some traits, but not others (Table 1.3). Using partial Mantel tests (accounting for geographic distance), I found significant correlations between red stripe distance and black cap distance (r = 0.364; $P < 0.0001$) and between crossband distance and black cap distance (r = 0.446; $P < 0.0001$). However, after applying a Bonferroni correction for multiple tests (critical $P_{Bonferroni}$ = 0.00833), red stripe distance and nuchal collar distance were not significantly correlated (r = 0.199; P = 0.0335), and neither were black cap distance and nuchal collar distance (r = 0.146; P = 0.0186). Finally, I found a significant correlation between mimetic trait diversity distance and non-mimetic trait diversity distance using a partial Mantel test accounting for geographic distance (r = 0.337; P = 0.0017). These results are based on analyses that excluded populations with less than five individuals (N = 47), but these trends are consistent if no populations are excluded, as well as if populations with less than 10 individuals are excluded.

DISCUSSION

I found spatial patterns that suggest evidence of evolutionary linkage between mimetic color traits and non-mimetic color traits, which has major implications for the origin and maintenance of phenotypic diversity. This spatial association may suggest that these different types of traits are genetically linked, such that selection favoring one trait will also favor the other through the non-random assortment of alleles (Hartl and Clark 2007). Alternatively, it may indicate that both types of traits are subjected to the same type of selection (Cox and Davis

Rabosky 2013; Holmes *et al.* 2017). Regardless, it appears that the evolutionary processes that produce and maintain one type of color polymorphism can simultaneously maintain polymorphisms of color traits with other functions. This evolutionary linkage is particularly interesting because it generates an enormous amount of phenotypic diversity, which is the raw material for selection and adaptation. As color polymorphism can serve as a precursor to speciation (Corl *et al.* 2010; Holmes *et al.* 2017; Hugall and Stuart-Fox 2012), the linkage of multiple types of color polymorphism could also accelerate lineage diversification.

My study offers new insights into the evolution of color polymorphism in mimicry systems. While much previous work in this field focuses primarily on the persistence of multiple mimetic phenotypes (Jiggins *et al.* 2001; Noonan and Comeault 2009; Plowright and Owen 1980), other work has focused on the origin and maintenance of non-mimetic morphs in mimicry systems (Ohsaki 1995; Rudh 2013; Wang 2011; Wang and Shaffer 2008). In ground snakes, the prevalence of non-mimetic color morphs and the presence of color polymorphism for non-mimetic traits suggests that non-mimics are generally not selected against, which is the opposite case for many butterflies (Joron *et al.* 2011; Ohsaki 1995). Ground snakes may be more similar to poison dart frogs, in which evolutionary losses of mimetic coloration likely reflect shifts in predator avoidance strategies from aposematism to crypsis (Wang and Shaffer 2008), especially considering the ancestor of ground snakes had a mimetic phenotype (Davis Rabosky *et al.* 2016b). Although a switch from aposematism to crypsis might explain the persistence of cryptic morphs in ground snakes, we would not necessarily predict non-mimetic polymorphism, and such an explanation would not address why mimetic and non-mimetic traits are associated with one another. Furthermore, we do not currently know whether non-mimetic traits in ground

snakes can be considered cryptic. As such, I present a novel means by which non-mimetic color polymorphism is maintained: via evolutionary linkage with mimetic color polymorphism.

My study also contributes to the steadily growing body of work on ground snakes. I found positive relationships between mimetic polymorphic traits (a red dorsal stripe and black crossbands) and non-mimetic polymorphic traits (a black cap and a black nuchal collar), such that individuals with higher numbers of mimetic traits are more likely to also have higher numbers of non-mimetic traits and vice versa. As mentioned, this finding may be indicative of either genetic linkage or similar selective regimes. Although the red stripe and black crossbands are themselves unlinked (Davis Rabosky *et al.* 2016a), it is certainly possible that some of the genes responsible for the molecular pathway associated with the black crossbands are also responsible for the production of the black cap and black nuchal collar; in many reptiles, a single gene is responsible for variation in pigmentation (Rosenblum *et al.* 2004). Alternatively, this spatial linkage might suggest that both types of traits are subject to the same negative frequency-dependent selection, as has been shown previously for the mimetic traits (Cox and Davis Rabosky 2013; Holmes *et al.* 2017). If this were indeed the case, it could be that the non-mimetic traits are beneficial for crypsis, as cryptic systems often experience negative frequency dependence (Endler and Greenwood 1988).

The spatial distribution of the crossbands and black cap are suggestive of the mosaic type (rather than the ubiquitous or clinal type) of spatial arrangement, in which the high variation of trait presence or absence cannot easily be explained by geography (Holmes *et al.* 2017; McLean and Stuart-Fox 2014). This was supported by the findings that morph frequencies vary widely among populations and that crossbands and the black cap were not associated with latitude or longitude. This arrangement may result from variation in selective regimes across time and space

(Cox and Davis Rabosky 2013), or by ubiquitous negative frequency-dependence driven by a wide-ranging predator (Holmes *et al.* 2017). Such hypotheses have previously been supported for the two mimicry-linked traits (Cox and Davis Rabosky 2013; Holmes *et al.* 2017). However, the same patterns found for non-mimetic traits further supports an evolutionary linkage between mimetic and non-mimetic traits. Additionally, by finding no evidence of an effect of coral snake allopatry or sympatry on mimetic traits, this may suggest that selection is being driven by a predator with a wide range, such as birds (Holmes *et al.* 2017; Pfennig and Mullen 2010). As the black cap and nuchal collar are assumed to be unrelated to mimicry, it is unsurprising that these traits were not associated with coral snake sympatry or allopatry.

I found evidence that the frequencies of the red stripe and nuchal collar may instead be driven by adaptation to an environmental gradient, as their relationships with latitude indicate the clinal type of spatial arrangement (Holmes *et al.* 2017; McLean and Stuart-Fox 2014). As such, geographic variation in the frequencies of these traits may be explained by environmental factors that change gradually with longitude, causing the red stripe and nuchal collar to be increasingly favored by selection with increases in latitude (Hegna *et al.* 2013). For example, if soils are redder along this latitudinal gradient, red-striped individuals may gain an added protective advantage via crypsis, thus increasing in frequency at higher latitudes. The finding of this clinal spatial pattern is particularly surprising for the red stripe, as a previous study found no relationship between the red stripe and any geographical variables (Cox and Davis Rabosky 2013). However, my study focused on the Great Plains clade of *Sonora semiannulata*, rather than the entire species, which possesses a range that extends far northward and westward.

Many of my spatial comparisons utilized partial Mantel tests, which, despite being commonly used, are somewhat controversial (Castellano and Balletto 2002; Legendre and Fortin

2010; Raufaste and Rousset 2001). One of the major criticisms of using partial Mantel tests is a loss of statistical power compared to other methods (Legendre and Fortin 2010). However, I recovered significant results in four of my six partial Mantel tests. The two tests that were not significant after a Bonferroni correction (the relationship between the red stripe and the nuchal collar and the relationship between the black cap and the nuchal collar) were also the two that had the weakest, yet significant, relationships in contingency analyses (Figure 1.5). This may suggest that the relationships indeed exist, but that my statistical power was simply too low to detect them using partial Mantel tests or that the Bonferroni correction was too conservative.

While similar spatial patterns and statistical associations between two different types of color polymorphism tell us about evolutionary linkage, geographic distribution alone does not allow us to distinguish between similar patterns of selection and genetic linkage between trait types. To disentangle these evolutionary forces, DNA sequencing, such as ddRADseq (Peterson *et al.* 2012), allows for population genetic analyses, which could be used to infer patterns of selection on mimetic and non-mimetic traits (Abbot *et al.* 2008; Cox and Davis Rabosky 2013; Gillespie and Oxford 1998). Additionally, genetic association studies can be used to test whether any loci are statistically associated with any color traits and whether any of those loci overlap for any traits (suggesting genetic linkage; Rosenblum *et al.* 2004). In any case, this study presents compelling evidence that evolutionary linkage among multiple types of color traits is associated with the maintenance of multiple types of color polymorphism.

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Table 1.1: Populations of ground snakes (*Sonora semiannulata*) used to assess geographic patterns of color polymorphism.

Country	State	Population	Latitude	Longitude	*N*
Mexico	Coahuila	Coahuila	27.79371	-101.671	14
U.S.A.	Colorado	Otero County	37.89501	-103.709	25
	Kansas	Barber County	37.22591	-98.6826	38
		Chautauqua County	37.14724	-96.2374	13
		Clark County	37.23134	-99.8205	45
		Comanche County	37.18762	-99.2713	5
		Cowley County	37.23709	-96.8279	88
		Elk County	37.44869	-96.2363	9
		Greenwood County	37.87383	-96.2275	14
		Kiowa County	37.55479	-99.2800	31
		Russell County	38.90825	-98.7560	17
		Wilson County	37.55615	-95.7417	8
	Missouri	Taney County	36.65097	-93.0491	16
	New Mexico	Eddy County	32.47545	-104.295	23
		Guadalupe County	34.86521	-104.777	10
		San Miguel County	35.48868	-104.804	89
	Oklahoma	Beckham County	35.27020	-99.6785	36
		Blaine County	35.87634	-98.4315	23
		Carter County	34.25569	-97.2807	33
		Cleveland County	35.20965	-97.3258	11
		Comanche County	34.66493	-98.4728	87
		Creek County	35.90881	-96.3684	10
		Garvin County	34.71362	-97.3061	36
		Greer County	34.93558	-99.5584	27
		Harmon County	34.74243	-99.8421	10
		Kay County	36.82007	-97.1445	13
		Logan County	35.92232	-97.4419	16
		Love County	33.95190	-97.2396	4
		Marshall County	34.03367	-96.7632	19
		Murray County	34.48982	-97.0621	18
		Osage County	36.63355	-96.4029	12
		Payne County	36.08317	-96.9759	10
		Tulsa County	36.12863	-95.9443	42
		Woods County	36.76583	-98.8591	8
	Texas	Bandera County	29.75537	-99.2604	15
		Bosque County	31.90683	-97.6347	11
		CCCE (Callahan, Coleman, Comanche, and Eastland Counties)	32.09097	-99.0533	7
		Crockett County	30.72617	-101.410	63
		EKR (Edwards, Kimble, and Real Counties)	30.10262	-99.9610	7
		Fisher County	32.73938	-100.406	18
		Hood County	32.43274	-97.8293	15
		JDR (Jeff Davis and Reeves Counties)	31.02257	-103.918	4
		Palo Pinto County	32.75601	-98.3079	57
		Parker County	32.77888	-97.8001	30
		Shackleford County	32.73915	-99.3553	30
		Stephens County	32.73858	-98.8345	22
		Tarrant County	32.77624	-97.2871	70
		Throckmorton County	33.17827	-99.2094	10
		Val Verde County	29.89228	-101.147	21
				Total	1240

Table 1.2: Results of Spearman's rank correlation analyses (latitude and longitude) and Mann-Whitney-Wilcoxon analyses (coral snake sympatry/allopatry) testing color-trait associations with spatial variables within $N = 46$ populations (populations with five or fewer individuals not included). Diversity of mimicry and non-mimicry traits was assessed using the Shannon Index (H). Bold *P*-values indicate significance when compared to a Bonferroni-adjusted critical *P*-value accounting for multiple tests (0.05/18 = 0.00278).

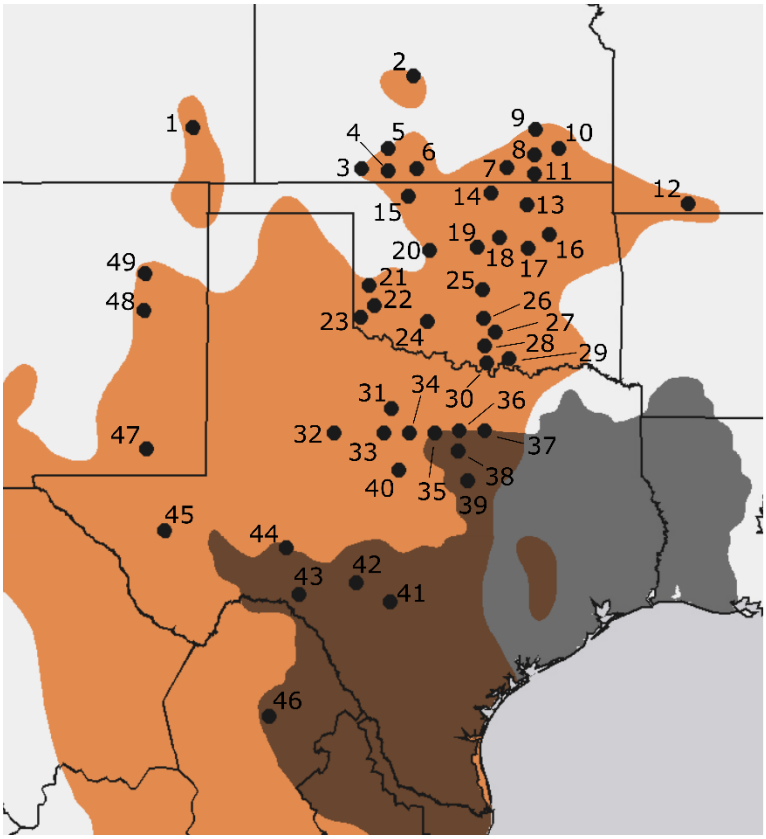
Spatial variable	Color trait variable	Test statistic	*P*-value
Latitude	% Red stripe	Spearman $\rho = 0.4779$	**0.0008**
	% Crossbands	Spearman $\rho = 0.2530$	0.0898
	% Black cap	Spearman $\rho = 0.2735$	0.0659
	% Nuchal collar	Spearman $\rho = 0.4629$	**0.0012**
	H mimicry traits	Spearman $\rho = 0.3168$	0.0320
	H non-mimicry traits	Spearman $\rho = 0.2691$	0.0706
Longitude	% Red stripe	Spearman $\rho = -0.2353$	0.1154
	% Crossbands	Spearman $\rho = -0.1682$	0.2639
	% Black cap	Spearman $\rho = -0.1641$	0.2758
	% Nuchal collar	Spearman $\rho = 0.2663$	0.0736
	H mimicry traits	Spearman $\rho = 0.0540$	0.7216
	H non-mimicry traits	Spearman $\rho = 0.0621$	0.6821
Coral snake sympatry/allopatry	% Red stripe	$Z = -2.53317$	0.0113
	% Crossbands	$Z = -0.20498$	0.8376
	% Black cap	$Z = -0.39959$	0.6895
	% Nuchal collar	$Z = -2.16889$	0.0301
	H mimicry traits	$Z = -1.10557$	0.2689
	H non-mimicry traits	$Z = -0.99882$	0.3179

Table 1.3: Results of partial Mantel tests (accounting for geographic distance) assessing correlations among color trait distance matrices and between color trait diversity (H) distance matrices within $N = 47$ populations (populations with fewer than five individuals not included). All tests were run with 99,999 iterations. Bold *P*-values indicate significance when compared to a Bonferroni-adjusted critical *P*-value accounting for multiple tests (0.05/6 = 0.00833).

Variable 1	Variable 2	Correlation coefficient (*r*)	*P*-value
Red stripe	Black cap	0.363619	**0.00001**
Red stripe	Nuchal collar	0.199318	0.03345
Crossbands	Black cap	0.446193	**0.00001**
Crossbands	Red stripe	0.488333	**0.00005**
Black cap	Nuchal collar	0.146082	0.01856
H mimicry traits	H non-mimicry traits	0.336822	**0.00170**

Figure 1.1: The four mimicry-linked color morphs of *Sonora semiannulata*: (a) uniform, (b) red-striped, (c) banded, and (d) mimetic. Photos by C.L. Cox.

Figure 1.2: *Sonora semiannulata* with a non-mimetic black cap and black nuchal collar, for which this species is also polymorphic. Photo by J.D. Curlis.



1 Otero Co., CO
2 Russell Co., KS
3 Clark Co., KS
4 Comanche Co., KS
5 Kiowa Co., KS
6 Barber Co., KS
7 Cowley Co., KS
8 Elk Co., KS
9 Greenwood Co., KS
10 Wilson Co., KS
11 Chautauqua Co., KS
12 Taney Co., MO
13 Osage Co., OK
14 Kay Co., OK
15 Woods Co., OK
16 Tulsa Co., OK
17 Creek Co., OK
18 Payne Co., OK
19 Logan Co., OK
20 Blaine Co., OK
21 Beckham Co., OK
22 Greer Co., OK
23 Harmon Co., OK
24 Comanche Co., OK
25 Cleveland Co., OK
26 Garvin Co., OK
27 Murray Co., OK
28 Carter Co., OK
29 Marshall Co., OK
30 Love Co., OK
31 Throckmorton Co., TX
32 Fisher Co., TX
33 Shackleford Co., TX
34 Stephens Co., TX
35 Palo Pinto Co., TX
36 Parker Co., TX
37 Tarrant Co., TX
38 Hood Co., TX
39 Bosque Co., TX
40 CCCE, TX
41 Bandera Co., TX
42 EKR, TX
43 Val Verde Co., TX
44 Crockett Co., TX
45 JDR, TX
46 Coahuila, MEX
47 Eddy Co., NM
48 Guadalupe Co., NM
49 San Miguel Co., NM

Figure 1.3: Geographic distribution of *Sonora semiannulata* (orange on map) and list of sampling locations (see Table 1.1 for explanations of abbreviations and sample sizes for each population). The range of Texas coral snakes (*Micrurus tener*) is shown in gray, and areas of sympatry with ground snakes is shown in brown.

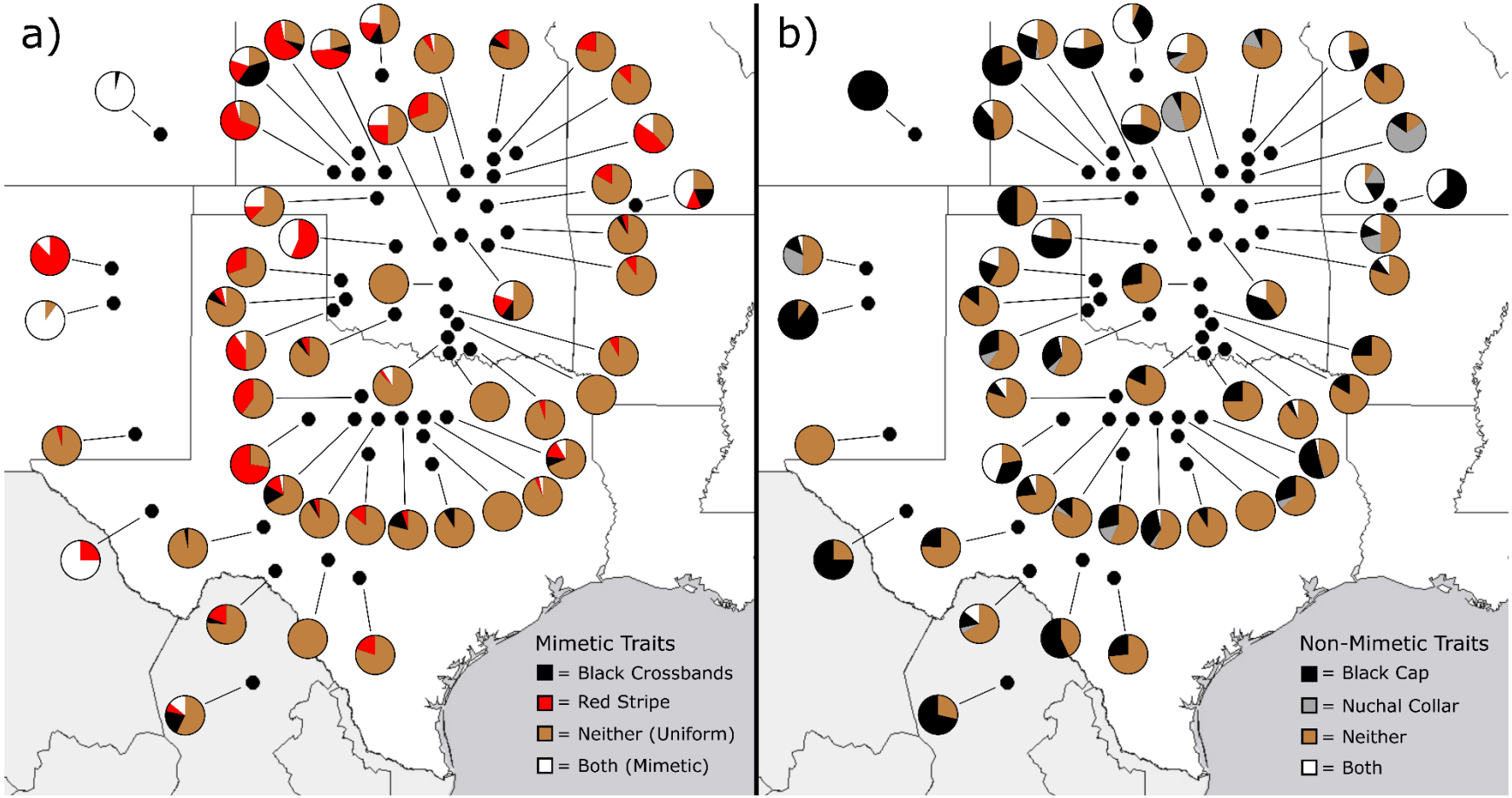


Figure 1.4: Proportions of (a) mimetic color traits and (b) non-mimetic color traits within 49 populations sampled across the range of *Sonora semiannulata* (see Table 1.1 for list of populations and sample sizes).

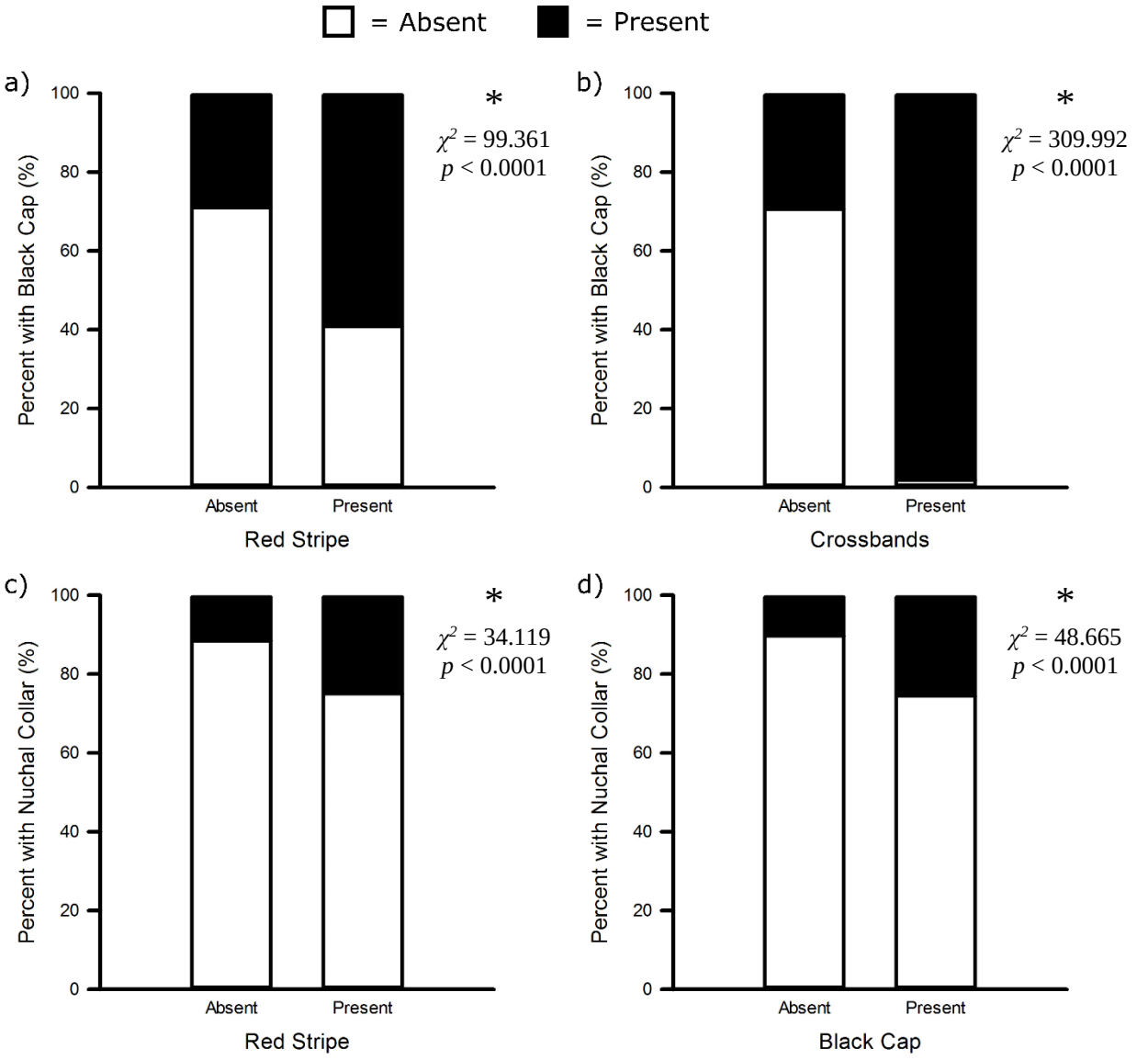


Figure 1.5: Model 1 contingency analyses using likelihood ratios, showing the association between (a) the red stripe and the black cap, (b) crossbands and the black cap, (c) the red stripe and the nuchal band, and (d) the black cap and the nuchal band when color morph frequencies are summed across all populations (N = 1240 individuals). All relationships were significant at $\alpha = 0.05$.

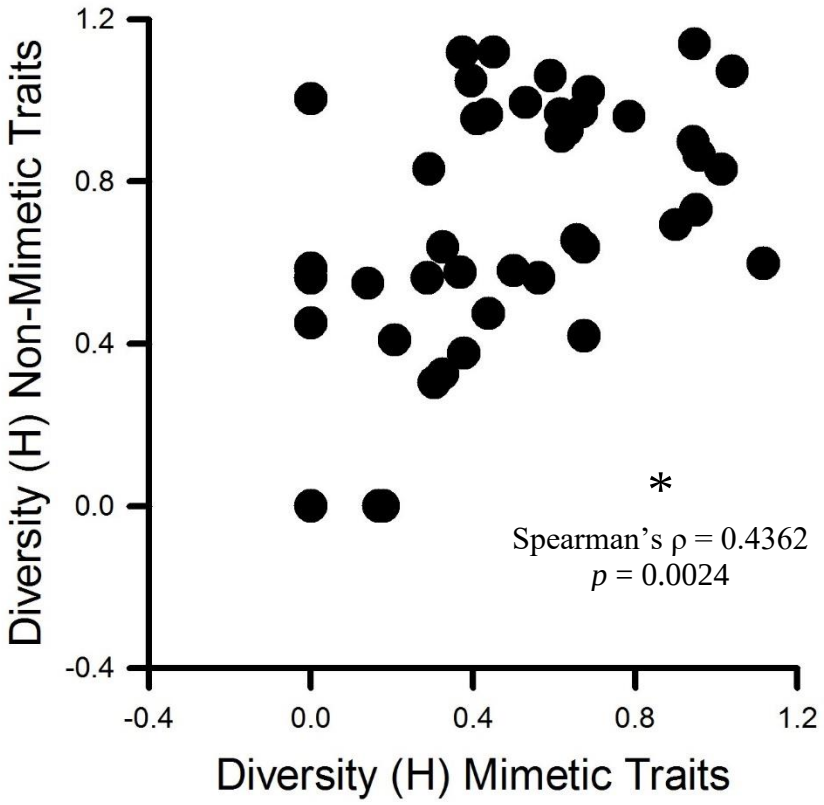


Figure 1.6: Relationship between the Shannon diversity index (H) of mimicry traits and the Shannon diversity index of non-mimicry traits within populations. Diversity (H) of mimicry traits is positively correlated with H of non-mimicry traits across $N = 46$ populations (populations with less than five individuals not included).

CHAPTER 2:

PATTERNS OF SELECTION IN MIMETIC AND NON-MIMETIC COLOR POLYMORPHISM IN THE GROUND SNAKE

ABSTRACT

Although mimicry has been studied for over a century, many questions remain unaddressed, particularly with regard to color polymorphism that encompasses both mimetic and non-mimetic phenotypes. While some studies have addressed how non-mimetic morphs may evolve, the maintenance of non-mimetic color polymorphism and how it relates to mimetic color polymorphism have not been studied. To compare the evolutionary dynamics of these two types of color polymorphism, I took a population genetics approach by generating ddRADseq SNP libraries and asking 1) does genetic structure explain color distribution, 2) does genetic diversity within or among populations predict color trait diversity, 3) what patterns of selection (if any) are influencing mimetic and non-mimetic color polymorphism, and 4) can I identify any loci as being linked to any color trait? I found evidence of two genetic clusters, but they had little explanatory power when applied to color morph distributions. I also found that genetic diversity both within and among populations was not predictive of color trait diversity, suggesting the presence of selection acting on color. Using F_{ST} comparisons, I found further evidence for the presence of selection, as well as a substantial discrepancy between F_{ST}-values for mimetic and non-mimetic traits, suggesting that selection is acting differently on these two types of color polymorphism. When combined with evidence for spatial association between mimetic and non-mimetic traits (see Chapter 1), this difference in the pattern of selection is likely reflective of genetic linkage between these traits, although I was unable to confirm this with SNP association analyses. Regardless, such findings present a novel mechanism by which phenotypic diversity

can be maintained, and this has major implications for color pattern diversity in mimicry systems and beyond.

INTRODUCTION

Mimicry is the phenomenon in which an organism gains protection by honestly or dishonestly signaling danger to predators via color patterns that are similar to those of a harmful or toxic species (Bates 1862; Endler 1981; Müller 1879). Although mimicry has been studied extensively in many taxa, such as butterflies (Brower 1958; Clarke and Sheppard 1960; Mallet and Gilbert Jr 1995; Punnett 2016), poison dart frogs (Noonan and Comeault 2009; Rudh *et al.* 2007; Wang and Shaffer 2008), and coral snakes (Brodie III and Janzen 1995; Greene and McDiarmid 1981; Pfennig *et al.* 2001), there are still many outstanding questions. One topic that has spurred a substantial amount of debate and research is the existence of color polymorphism in mimicry systems (Joron and Mallet 1998), in which a mimetic species exhibits multiple color morphs within a population (Cox and Davis Rabosky 2013; Gray and McKinnon 2006). While this color polymorphism can occur in the form of multiple mimetic color morphs, it can encompass both mimetic and non-mimetic (e.g., cryptic) color morphs (Nijhout 2003; Ohsaki 1995; Vences *et al.* 2003; Wang and Shaffer 2008). Comparing the evolutionary drivers of these different types of color polymorphisms can allow us to ask and answer questions about the maintenance of phenotypic diversity.

Previous work in mimicry systems has shown a high degree of variability in the evolutionary dynamics of mimetic color polymorphism. In many mimic species, color polymorphism is maintained by predator-mediated frequency-dependent selection (Holmes *et al.* 2017; Noonan and Comeault 2009; O'Donald and Pilecki 1970; Pfennig *et al.* 2001). Other evolutionary explanations include regulation via supergenes (Jones *et al.* 2011; Joron *et al.*

2011), sympatry with multiple divergent models (Mallet and Gilbert Jr 1995), assortative mating and sexual selection (Jiggins *et al.* 2001; Maan and Cummings 2009), and honest signaling of toxicity (Maan and Cummings 2011). In contrast, the evolution and maintenance of non-mimetic color polymorphism in mimicry complexes is poorly understood. Several species have been noted to possess cryptic morphs, and some hypotheses for why these morphs might evolve have been postulated, including sexual selection (Ohsaki 1995) or shifts in predator avoidance strategies from aposematism to crypsis (Rudh 2013; Wang and Shaffer 2008). However, no studies have investigated the mechanisms by which color polymorphism might be maintained *within* cryptic morphs in mimicry systems or how this may be influenced by selection on mimetic color polymorphism. In order to make inferences about the relationship of evolutionary forces acting on mimetic and non-mimetic color traits, we can utilize approaches that couple color trait frequencies with genetic information from populations across the landscape.

Population genetic methods represent powerful tools for studying evolutionary biology, especially due to recent advances in DNA sequencing. These sequencing techniques allow for unprecedented volumes of high-quality sequence data to be recovered (Peterson *et al.* 2012; Shendure and Ji 2008). This, in turn, results in a much finer-scale insight into genetic structuring among individuals and populations, which can be applied to a wide range of evolutionary concepts. Relationships between genotypes and phenotypes are commonly used to infer the pattern of selection, if any, that is acting upon phenotypic traits (Andres *et al.* 2000; Cox and Davis Rabosky 2013; Gillespie and Oxford 1998). For example, a significant relationship between genetic diversity (measured as heterozygosity, Shannon's I, etc.) and color diversity within populations would suggest that neutral processes (such as genetic drift or local gene flow) are responsible for color variation, rather than selection. Similarly, a significant relationship

between genetic distance and color trait distance among populations would also suggest that selection is not acting on color. On the other hand, finding no relationship in either of these two tests would indicate that selection is indeed responsible (Cox and Davis Rabosky 2013). Another way in which to test for selection on color is via comparisons of F_{ST} (as well as its analogs), a test statistic that measures differentiation or sub-structuring among populations (Nei 1972; Weir and Cockerham 1984; Wright 1951). If F_{ST} values are equal for neutral genetic markers and color traits, it is likely that neutral genetic processes are at work. However, a mismatch between these F_{ST} values suggests that selection is involved, and the nature of the mismatch can lend insight into the underlying pattern of selection (Cox and Davis Rabosky 2013). If population subdivision is significantly higher for neutral genetic data than for color data ($F_{ST\_SNP} > F_{ST\_color}$), this indicates balancing selection. Balancing selection is characterized by populations being driven towards similar morph compositions, despite high genetic differentiation among populations. In contrast, if population subdivision is significantly lower for neutral genetic data than for color data ($F_{ST\_SNP} < F_{ST\_color}$), this suggests diversifying selection. Diversifying selection drives populations towards different morph frequencies, despite low genetic differentiation among populations. Lastly, to investigate whether a color trait is statistically linked to a particular genetic marker, association studies can be used (Rosenblum *et al.* 2004). If the same marker appears to be associated with multiple color traits, it may imply that these phenotypic traits are genetically linked.

In this study, I compared the evolutionary drivers of mimetic and non-mimetic color polymorphism in the ground snake (*Sonora semiannulata*). First, I assessed genetic structuring by testing for population clusters across the range of ground snakes. I then used correlations between measures of genetic and color trait diversity and correlations between genetic and color

trait distance matrices to test for evidence of neutral genetic processes driving the distribution of color traits. I also compared neutral genetic structure to color polymorphism structure for each type of color trait among populations, such that the presence and/or nature of a mismatch in F_{ST} values would allow me to infer presence and patterns of selection. Finally, I tested whether any genetic markers were statistically associated with any color traits, as well as whether any markers found to be linked overlapped among color traits, interpreting an overlap as genetic linkage between traits.

METHODS

Study species

The ground snake (*Sonora semiannulata*) is a small, semi-fossorial species with dramatic color polymorphism in both mimetic and non-mimetic traits (Cox and Chippindale 2014; Cox and Davis Rabosky 2013; Davis Rabosky *et al.* 2016a). Mimetic color polymorphism is present in the form of red and black coloration, two colors with an evolutionary origin in coral snake mimicry (Brodie III and Brodie Jr. 2004; Greene and McDiarmid 1981). Different combinations of red and black produce four color morphs in ground snakes: black-banded, red-striped, uniform (with neither black bands nor red stripe), and mimetic (with both black bands and red stripe). In addition, the presence or absence of a black cap and the presence or absence of a black nuchal collar leads to four color combinations that can be considered non-mimetic, as these two traits are found in a variety of snakes that have no association with coral snake mimicry (Powell *et al.* 2016; Sawaya and Sazima 2003). While the four mimicry-related color morphs have been studied previously (Cox and Chippindale 2014; Cox and Davis Rabosky 2013; Davis Rabosky *et al.* 2016a), the black cap and nuchal collar have received very little attention in the literature, other than being briefly mentioned by Frost (1983) and noted in field guides (Powell *et al.* 2016).

Tissue sampling and DNA extraction

I obtained a tissue sample (comprised of liver, brain, or tail) for 142 individuals from 32 populations across the range of the Great Plains clade of ground snakes (Table 2.1) and stored each in 95% ethanol, RNAlater, or lysis buffer. I extracted DNA from each sample using a Qiagen DNEasy Blood & Tissue Kit (Qiagen, Venlo, the Netherlands), following the manufacturers' protocols for tissue samples with the exceptions of an increased enzyme digestion time (overnight) and the use of pure water instead of buffer before the final spin in the centrifuge (this was done to eliminate the addition of salt that normally accompanies the buffer in the final sample). I measured the concentration of DNA in each sample using a Qubit Fluorometer (Qubit 2.0 HS DNA assay; Invitrogen, Life Technologies, Carlsbad, California, USA). Samples with a stock concentration of less than 1.0 ng/μL or greater than 600 ng/μL were either resampled or discarded. Samples with a stock concentration between 1.0 ng/μl and 10 ng/μl were placed in a Speedvac with the drying rate set to low until they reached a stock concentration greater than 10 ng/μL.

Sequencing

I conducted double digest restriction-site associated DNA sequencing (ddRADseq) following the protocol set forth in Peterson *et al.* (2012). As described, I first annealed adapters P1 and P2 and used the provided ddRAD ligation molarity calculator to prepare final working concentrations. Double digest was conducted using the restriction enzymes *EcoR1* and *MSP1*, and I used Ampure XP beads (Beckman Coulter Genomics, Danvers, Massachusetts, USA) with a SPRIPlate Super Magnet Plate for cleaning. Next, I combined Adapters P1 and P2 with the digested DNA, then combined this with a "master mix" of T4 DNA ligase, T4 DNA ligase buffer, and water, which I subsequently incubated and heat-killed for the appropriate amounts of

time in an Eppendorf thermocycler. I pooled DNA with 24 unique Illbarcodes and then size-selected 294 to 394 base pairs using Sage Science Pippin-Prep (Sage Science, Inc., Beverly, Massachusetts, USA). I conducted PCR amplification of DNA with a Phusion High-Fidelity Polymerase kit (New England BioLab, Ipswich, Massachusetts, USA) followed by one final cleaning step and DNA quantification. The products were sequenced in two lanes of an Illumina HiSeq 2500 System (Illumina, Inc., San Diego, California, USA) at the University of Michigan, which produced 150-bp paired-end reads.

SNP discovery and genotyping

Raw sequences were demultiplexed using the program *pyRAD* (Eaton 2013), and the resulting fast-Q files were run through the *dDocent* v.2.2.16 pipeline (Puritz *et al.* 2014). *dDocent* is specifically designed for paired-end RAD data and utilizes multiple bioinformatics software packages (Puritz *et al.* 2014). Briefly, *dDocent* uses the program *Trimmomatic* v.0.36 (Bolger *et al.* 2014) to remove adapter sequences and bases with low quality scores. It then uses the program *Rainbow* v.2.0.4 (Chong *et al.* 2012) to cluster reads based on similarity and assemble them into reference contigs. Next, *CD-HIT* v.4.6.1 (Fu *et al.* 2012; Li and Godzik 2006) is used to cluster the reference contigs based on 90% similarity, after which only the longest contig from each cluster is retained. *BWA* v.0.7.13 (Li and Durbin 2010) then maps the quality-trimmed reads to the reference contigs, using a match score of one, a mismatch score of three, and a gap-opening penalty of five. Finally, *dDocent* uses the program *FreeBayes* (Garrison and Marth 2012) to call variants including SNPs, indels, and complex polymorphisms, which it outputs as a VCF file.

SNP filtering

The VCF file outputted from *dDocent* underwent a number of filtering steps to obtain a dataset of neutral SNPs shared among many individuals. All filtering steps were achieved through the use of the software programs *VCFtools* v.0.1.15 (Danecek *et al.* 2011) and *vcflib* (a program included in *FreeBayes*) and were modeled after the SNP filtering protocol on the *dDocent* GitHub page (https://github.com/jpuritz/dDocent/; Brauer *et al.* 2016). First, loci that were recovered in less than 60% of individuals, loci that had a minor allele count of less than three, and loci with a quality score less than 30 were removed. The complex variants produced by FreeBayes were decomposed into SNPs and indels, and indels were removed. I then calculated the percentage of missing data for each individual and excluded individuals with greater than 80% missing data. I applied a filter that retained only biallelic SNPs, as well as one that retained a single SNP per locus with a minor allele frequency of at least 0.05. I then applied a six-step filtering process that removed SNPs on the basis of allele balance, read orientation, mapping quality, paired reads, read quality, and read depth (https://github.com/jpuritz/dDocent/; Brauer *et al.* 2016). Finally, I used the program *BayeScan* v.2.1 (Foll and Gaggiotti 2008) to identify and remove loci likely to be under selection, using default settings with the prior odds set to 10,000 and a false discovery rate of 0.1. The final dataset consisted of 2,125 putatively neutral SNPs from 109 individuals. The number of SNPs retained after each filtering step can be found in Table 2.1.

Phenotypic Scoring

For analyses of color pattern, I treated each color trait (crossbands, red stripe, black cap, and nuchal collar) like a separate dominant marker and coded the presence or absence of the trait in an individual as 1 or 0 (see Cox and Davis Rabosky 2013; Davis Rabosky *et al.* 2016a).

Because I also had the phenotypic dataset of 1,240 individuals from Chapter 1, comparisons of the color data with genetic data were done using: a) only individuals for which I had both color and genetic data, 2) only populations for which I had both color data and genetic data, and 3) all individuals for which I had color data and all individuals for which I had genetic data.

Population genetic analyses

Population clustering analyses among populations

To assess patterns of genetic structure in ground snakes, I used the program *STRUCTURE* (Pritchard *et al.* 2000). This program utilizes a Bayesian MCMC to detect the number of underlying genetic clusters (K) in a dataset and to calculate the proportion of each individual's genome that can be assigned to each of those clusters. I implemented a model that included genetic admixture and correlated allele frequencies, and it was run for 50,000 iterations after a burn-in of 10,000. I ran this model for K-values of one through ten, with 20 independent replicates of each K. The files produced by *STRUCTURE* were concatenated into a single zipped file and inputted to the program *Structure Harvester* (Earl and vonHoldt 2012), which employed the Evanno *et al.* (2005) method to determine the K-value with the highest likelihood. Finally, I used the program *CLUMPAK* (Kopelman *et al.* 2015) to graphically visualize population clustering for the most likely K-value.

Assessing the role of gene flow among and within populations

To test for a relationship between neutral gene flow and among-population variation in color pattern, I used GenAlEx (Peakall and Smouse 2006; 2012). First, I generated genetic distance matrices (using SNP data) and color trait distance matrices (coding the presence/absence of a trait as binary, producing a Euclidean distance matrix) for each population pair. I then compared the genetic distance matrix to each color trait distance matrix using simple and partial

Mantel tests (controlling for geographic distance) in the program zt (Bonnet and de Peer 2002) with 99,999 iterations. While the use of Mantel tests (especially partial Mantel tests) is controversial (Castellano and Balletto 2002; Raufaste and Rousset 2001), they are considered appropriate when comparing genetic distances and Euclidean distances (Legendre and Fortin 2010).

To test for a relationship between genetic diversity and color trait diversity within populations, I calculated several diversity indices in GenAlEx (Peakall and Smouse 2006; 2012). A significant relationship between genetic diversity and color trait diversity would indicate the role of gene flow, while no relationship would suggest the presence of selection acting on color traits (Cox and Davis Rabosky 2013). I calculated Shannon's I, heterozygosity, and unbiased heterozygosity for SNP data and for each color trait (coded as a binary locus). I then assessed correlations between each measure of genetic diversity and each measure of color trait diversity for color traits separately and grouped into mimetic or non-mimetic.

Population structure and patterns of selection

To assess the influence of neutral processes or selection on geographic variation in color patterns, I compared population subdivision for neutral SNPs and color traits (Abbot *et al.* 2008; Andres *et al.* 2000; Cox and Davis Rabosky 2013; Gillespie and Oxford 1998). I did this by calculating analogs of F_{ST} (θ and Φ_{PT}, hereafter referred to as F_{ST}), which measure population sub-structuring (Peakall and Smouse 2006; Weir and Cockerham 1984; Wright 1951). Using an analysis of molecular variance (AMOVA) in GenAlEx, I calculated a global F_{ST} value for all neutral SNPs (and for each SNP individually) and for each color trait separately, and I generated 95% confidence intervals by running 9,999 permutations.

Linking phenotypes to genomic sequences

I conducted association analyses to test for significant relationships between any of the four color traits (the black crossbands, the red stripe, the black cap, and the nuchal collar) and any SNP in the dataset. Because this type of analysis does not require loci to be selectively neutral, the 15 outlier SNPs identified by BayeScan were added to the 2,125 neutral SNPs. The resulting 2,140 SNPs were then analyzed using Fisher's exact tests in contingency analyses for each color trait separately. I assessed significance using the Benjamini-Hochberg procedure (Benjamini and Hochberg 1995), setting the false discovery rate to 10%. Contingency analyses were conducted in JMP (Version 11; SAS 2014).

RESULTS

Genotyping and outlier detection

After forward and reverse reads produced from Illumina sequencing of 142 DNA samples were run through *dDocent*, a VCF file containing 753,623 variable sites was created. The VCF file was subjected to multiple filtering criteria, such that 2,140 SNPs from 109 individuals were retained. Fifteen loci identified as outliers by BayeScan were removed, resulting in a putatively neutral dataset of 2,125 SNPs (Table 2.2; see Table 2.1 for list of sample sizes after some individuals were removed).

Population clustering

In exploratory analyses of the number of genetic clusters (K), I found that likelihood increased with increasing values of K (Figure 2.1a). However, after employing the Evanno *et al.* (2005) method, I found strong support for two population clusters (Figure 2.1b). These clusters corresponded well with geographic regions, such that one relatively distinct cluster occurred in Kansas, Colorado, and northern Oklahoma, while the other was found in in southern Oklahoma, most of Texas, and southern New Mexico (Figure 2.1c; Figure 2.2). As expected, some

individuals exhibited intermediate genotypes in populations between these two regions and around the periphery.

Genetic distance and color trait distance

I found that genetic distance was not correlated with crossband distance, red stripe distance, black cap distance, nuchal collar distance, mimetic trait distance, or non-mimetic trait distance (all *P*-values > 0.05; Table 2.3). Although I present results for partial Mantel tests (controlling for geographic distance) using all populations, these trends held true when I repeated all tests with simple Mantel tests and when I iteratively excluded populations containing one or two individuals.

Genetic diversity and color trait diversity

I found no significant correlations between genetic diversity and diversity of any color trait (all *P*-values > 0.05; Table 2.4). These non-significant trends were recovered regardless of whether traits were assessed separately or grouped into mimetic or non-mimetic, as well as regardless of which measure of diversity was used (Shannon's I, heterozygosity, or unbiased heterozygosity).

Genetic F_{ST}, color trait F_{ST}, and patterns of selection

I found a mismatch between genetic structuring and color polymorphism structuring among populations for both mimetic and non-mimetic traits (Figure 2.3). Global F_{ST} was relatively low for neutral genetic markers, while F_{ST} for mimetic traits and non-mimetic traits were both significantly higher (Figure 2.3a-b). When F_{ST} was analyzed on a per-locus basis, both mimetic and non-mimetic color traits were found to be in the trailing end of the frequency distribution (Figure 2.3c-d). Because the calculation of F_{ST} incorporates within-population variation, populations with only one individual were excluded from this analysis (N=101 individuals from 23 populations). It is worth noting that Figure 2.3 represents analyses conducted

using all *populations* for which I had genetic and color data. In this case, genetic F_{ST} was calculated using 101 individuals and color trait F_{ST} was calculated using 718 individuals, but the same 23 populations were used. The same results were recovered when these F_{ST} comparisons were conducted using only the 101 *individuals* for which I had both genetic and color data, as well as when I used all individuals in the phenotypic dataset (1240 individuals from 49 populations).

SNP association tests

I identified 28 SNPs that were statistically associated with color traits after the application of the Benjamini-Hochberg procedure to account for multiple tests (Table 2.5). The SNPs that produced significant results included one for crossbands, 21 for the red stripe, five for the black cap, and one for the nuchal collar. However, none of these SNPs were fixed for any color traits, as all 28 were present in individuals with and without each color trait. Additionally, none of these SNPs were found to be significantly associated with more than one color trait.

DISCUSSION

I found substantial variation in the mismatch between neutral genetic variation and color pattern variation for two different types of color polymorphism, suggesting that selection differs between mimetic and non-mimetic traits. Combined with the finding that mimetic and non-mimetic traits are spatially linked (see Chapter 1), I interpret this difference in selection between trait types to be a result of genetic linkage between them. In this case, strong selection driving variation in mimetic traits could simultaneously drive variation in non-mimetic traits through the non-random assortment of alleles; essentially, non-mimetic traits get dragged along in the wake of diversifying selection for mimetic traits, maintaining color polymorphism of both types of traits. Such a finding has important implications for the evolution of phenotypic diversity and

speciation, both of which can result from color polymorphism (Gray and McKinnon 2006; Joron and Mallet 1998).

My results present novel contributions to previous work on color polymorphism in coral snake mimicry systems. Studies by Cox and Davis Rabosky (2013) and Holmes *et al.* (2017) suggested that spatial and temporal heterogeneity in selection and/or negative frequency-dependent selection could be responsible for the pattern of diversifying selection observed for mimetic color polymorphism across populations. My results for mimetic traits are congruent with this pattern of selection. However, this seems to be an insufficient explanation for the non-mimetic traits, which appear to be under very weak diversifying selection at best. Instead, this weak selection is likely a product of genetic linkage with selection for mimetic traits. Although several other coral snake mimics beyond ground snakes have been noted to be polymorphic (Davis Rabosky *et al.* 2016b), it is difficult to draw meaningful comparisons with them because the evolutionary dynamics of their color polymorphisms have not yet been assessed.

I also recovered support for two genetic clusters across the range of ground snakes. This contrasts with previous research that found little evidence of any genetic clustering (Cox and Chippindale 2014), but may be attributed to greater genomic coverage in my study (2,125 SNPs vs 112 AFLPs). Nevertheless, the mechanisms responsible for this clustering remain to be determined. Patterns of genetic structure are often influenced by geography, so the clusters I found could reflect differences in habitat type or geographic barriers to gene flow (Cox and Chippindale 2014; Cox *et al.* 2012; Manel *et al.* 2003). Differences in habitat type may be unlikely, as there are likely more habitat differences within the range of each cluster than there are between them. In addition, there may be some scope for a barrier in the form of the Canadian River that flows east-west across Oklahoma through north Texas to eastern New Mexico.

However, the Great Plains region upon which this study focused is generally considered to lack large geographic barriers that could impact gene flow (Cox and Chippindale 2014). The genetic clusters I observed could perhaps more plausibly be explained by rapid range expansion from a single population following a genetic bottleneck, which could have occurred in response to historical glacial cycles (Makowsky *et al.* 2009; Streicher *et al.* 2012).

Although some populations used in this study had very small sample sizes, it is unlikely that their inclusion biased the results. The use of ddRADseq to recover SNPs from DNA samples yielded deep genomic coverage and produced a massive amount of information about each individual, allowing a fine-scale understanding of how ground snakes are related within and among populations. Including small populations increased the resolution of the geographic distribution of color trait frequencies across the landscape, and they were consistent with population designations in Chapter 1, which generally had larger sample sizes. In addition, I re-ran many of my analyses excluding populations with the smallest sample sizes (1 and 2 individuals) and found inconsequential differences in the trends I observed. I also repeated all F_{ST} tests assigning individuals from unambiguous populations to their respective genetic cluster, and I recovered very similar values of F_{ST}. These genetic clusters are likely the closest measure of true "populations" that I would be able to resolve. Unfortunately, small population sizes did limit some of my analyses; populations with 1 individual had to be removed for calculating F_{ST}, and some indices of genetic diversity within populations could not be assessed because they required at least three individuals per population per locus. However, the inclusion of these small populations in the analyses that I did conduct appears to have been sufficient for detecting the patterns in which I was interested.

In my previous studies, I found that mimetic and non-mimetic traits were spatially linked, suggesting either similar patterns of selection or genetic linkage among these two types of traits. I found a lack of evidence for similar patterns of selection based on F_{ST} comparisons, as differentiation of mimetic traits was much higher than that of non-mimetic traits. While this renders genetic linkage to be the more likely driver, I did not recover any SNPs in my association studies that were associated with multiple color traits. Nevertheless, the absence of these linked SNPs does not indicate that they do not exist; I may have simply not been able to detect them with a dataset of 2,140 loci. Quantitate trait locus (QTL) analysis or an annotation of the entire genome of ground snakes would most certainly shed light on the answers I seek.

Given that mimicry can generate color polymorphism that encompasses both mimetic and non-mimetic (e.g., cryptic) diversity (Davis Rabosky *et al.* 2016b; Nijhout 2003; Ohsaki 1995; Wang and Shaffer 2008), my findings suggest that diversifying selection on mimetic traits can also drive the diversity of genetically-linked non-mimetic traits. This could serve as an explanation for the persistence of non-mimetic color polymorphism in species that have lost their association with mimicry. This may be the case in the *Sonora semiannulata taylori* clade, in which populations are fixed for the uniform morph, yet polymorphic for the black cap (C.L. Cox, unpublished data). More broadly, the finding that selection maintaining one type of color polymorphism can maintain another presents a previously-unexplored mechanism by which phenotypic diversity can be generated. This can have far-reaching implications for not only mimicry-related species, but also any species with multiple types of color polymorphism.

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Table 2.1: List of populations used for genetic analyses and sample sizes of populations before and after removing individuals with > 80% missing data. All populations sampled in the United States unless otherwise noted.

Population	*N* before Filtering	*N* after Filtering
Barber, KS	7	6
Beckham, OK	8	6
Blaine, OK	8	7
Callahan Co., Coleman Co., and Eastland Co., TX	6	4
Carter, OK	3	3
Clark, KS	5	3
Coahuila, Mexico	1	0
Comanche, KS	1	1
Comanche, OK	3	3
Cooke, TX	2	1
Crockett, TX	8	6
Eddy, NM	1	1
Edwards Co., Kimble Co., and Real Co., TX	7	6
Elk, KS	8	4
Fisher, TX	8	6
Jeff Davis Co. and Reeves Co., TX	2	2
Kiowa, KS	7	5
Llano, TX	1	1
Love, OK	2	1
Menard, TX	1	1
Otero, CO	10	6
Palo Pinto, TX	6	5
Parker, TX	2	2
Russell, KS	8	8
San Miguel, NM	2	2
San Saba, TX	3	2
Shackleford, TX	7	6
Stephens, TX	2	1
Sutton, TX	2	2
Taney, MO	1	1
Tulsa, OK	4	3
Val Verde, TX	6	4
Total	142	**109**

Table 2.2: The number of single-nucleotide polymorphisms (SNPs) retained after each filtering step.

Filtering step	SNP count
Raw SNP catalogue	753,623
Genotyped in >60% of individuals, base quality ≥30, minor allele count 3	42,012
Decomposition of complex variants, indels removed	51,462
Removal of individuals with > 80% missing data	
Biallelic SNPs only	48,937
Single SNP per locus, minor allele frequency ≥ 0.05	4,204
Allele balance	3,326
Read orientation	2,761
Mapping quality	2,419
Paired reads	2,378
Read quality	2,344
Read depth	2,140
Removal of BayeScan-identified outliers	2,125
Final, putatively neutral dataset	**2,125**

Table 2.3: Results of partial Mantel tests (accounting for geographic distance) assessing correlations between the genetic distance matrix and each color trait distance matrix. All tests were run with 99,999 iterations. Partial Mantel tests that included all $N = 31$ populations are displayed here, but no significant correlations were recovered when simple Mantel tests were used, when populations with one individual were excluded, or when populations with two individuals were excluded.

Variable 1	Variable 2	Correlation coefficient (*r*)	*P*-value
Genetic distance	Crossband distance	0.029760	0.41384
	Red stripe distance	0.144129	0.08608
	Black cap distance	0.082198	0.07687
	Nuchal collar distance	0.223427	0.08813
	Mimetic trait distance	0.109388	0.20202
	Non-mimetic trait distance	0.197155	0.05908

Table 2.4: Results of Spearman's rank correlation analyses testing for the association between genetic diversity and color trait diversity (both measured as Shannon's Information Index, I) among $N = 23$ populations (populations with genetic data for one individual not included). No correlations were significant at $\alpha = 0.05$ regardless of the diversity measure used (Shannon's I, heterozygosity, or unbiased heterozygosity).

Variable 1	Variable 2	Test statistic	*P*-value
Genetic diversity	Crossband diversity	Spearman $\rho = -0.2100$	0.3362
	Red stripe diversity	Spearman $\rho = -0.0243$	0.9122
	Black cap diversity	Spearman $\rho = 0.2138$	0.3273
	Nuchal collar diversity	Spearman $\rho = -0.2503$	0.2493
	Mimetic trait diversity	Spearman $\rho = -0.0504$	0.8193
	Non-mimetic trait diversity	Spearman $\rho = -0.1527$	0.4866

Table 2.5: The results of contingency analyses using Fisher's exact test, showing all significant associations between any SNPs and any of the four color traits. Significance was assessed using the Benjamini-Hochberg procedure with the false discovery rate set to 10%. Note that no SNPs share a significant relationship with more than one color trait.

Color trait	Locus	Fisher's exact test *P*-value
Crossbands	SNP #230	$5.19*10^{-6}$
Red stripe	SNP #126	0.0002
	SNP #138	$4.42*10^{-5}$
	SNP #234	$1.23*10^{-5}$
	SNP #251	0.0005
	SNP #296	0.0004
	SNP #315	0.0001
	SNP #403	$3.86*10^{-5}$
	SNP #800	0.0004
	SNP #1059	0.0002
	SNP #1140	$4.16*10^{-5}$
	SNP #1144	$5.56*10^{-8}$
	SNP #1193	$7.21*10^{-5}$
	SNP #1245	$4.96*10^{-5}$
	SNP #1318	0.0005
	SNP #1441	0.0005
	SNP #1467	0.0005
	SNP #1562	0.0002
	SNP #1598	0.0002
	SNP #1727	0.0006
	SNP #1921	0.0007
	SNP #1971	0.0004
Black cap	SNP #59	0.0002
	SNP #287	0.0002
	SNP #685	$7.06*10^{-5}$
	SNP #882	0.0002
	SNP #1093	0.0002
Nuchal collar	SNP #1150	$1.65*10^{-5}$

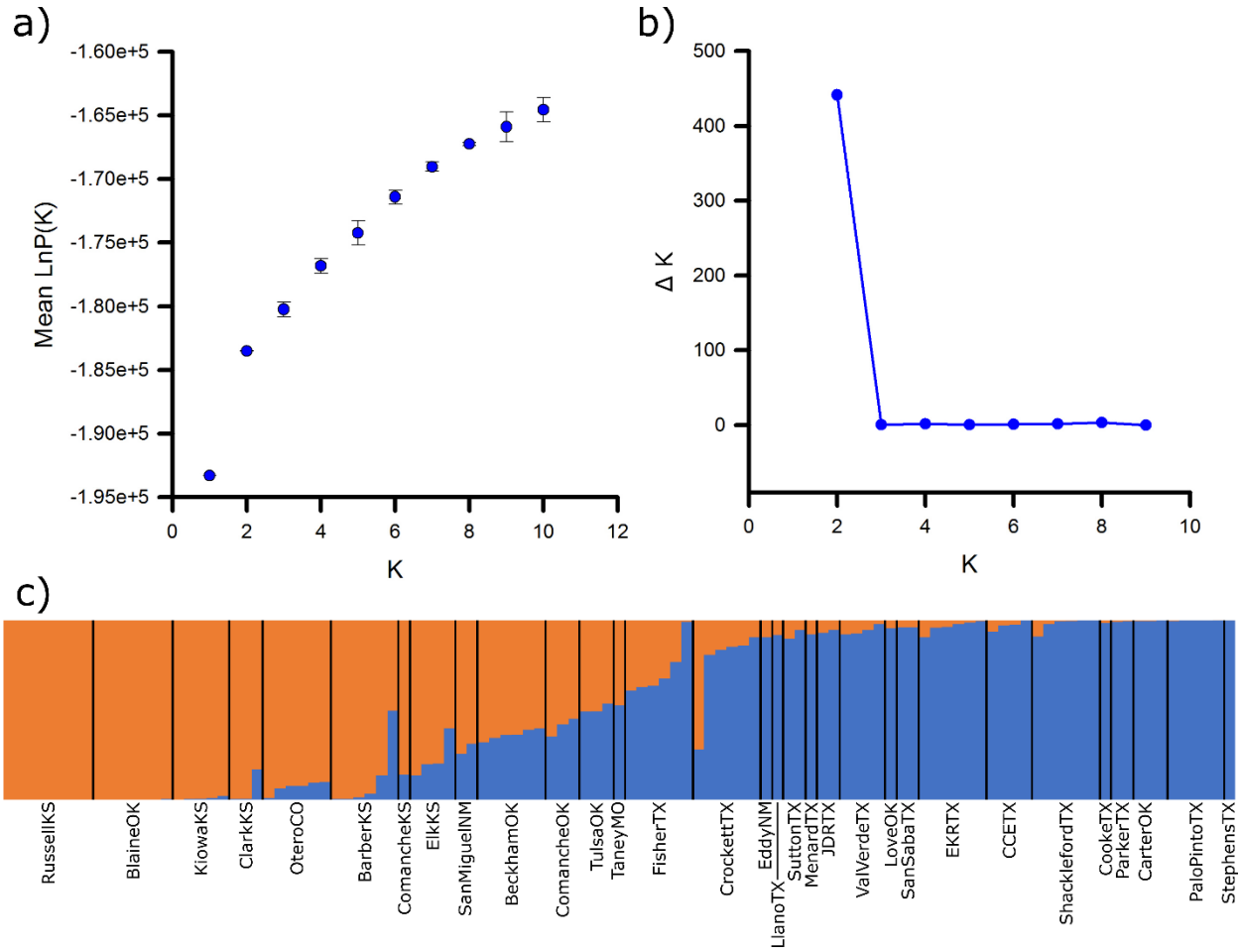

Figure 2.1: Results of analyses using STRUCTURE and estimating the most probable value of genetic clusters (K). During multiple iterations of multiple models exploring possible values of K, the log likelihood of each independent run was calculated. (a) The average log likelihood for 12 potential values of K. (b) The estimation of ΔK, calculated using the methods of Evanno et al. (2005). The K value with the highest ΔK is the most likely number of genetic clusters given the data. (c) A STRUCTURE plot based on the results of the most likely run with a K value of two.

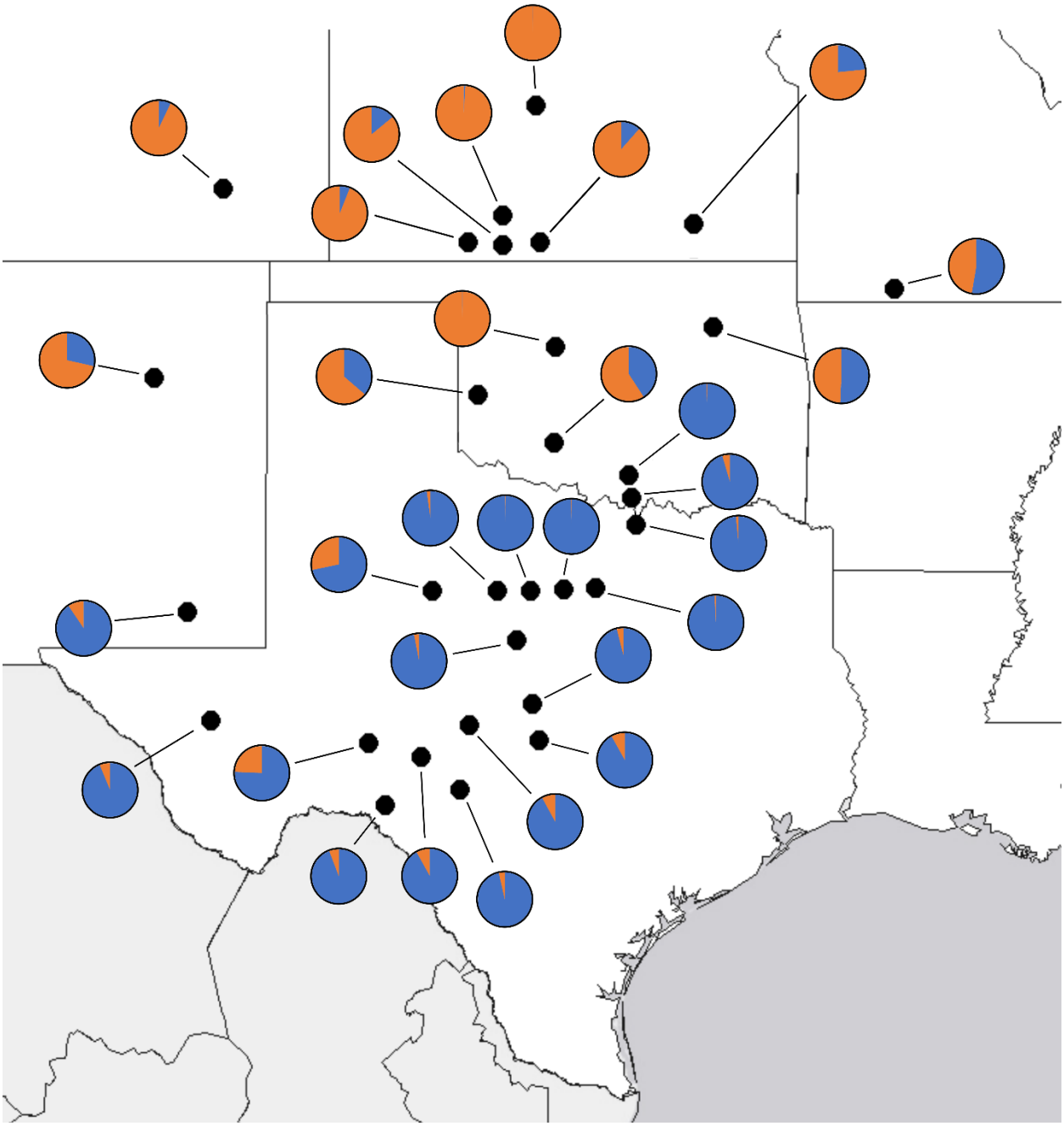

Figure 2.2: Distribution of genotypes across the landscape, based on analyses in *STRUCTURE*. Each pie chart represents the average proportions of genotypes assigned to a genetic cluster within a population.

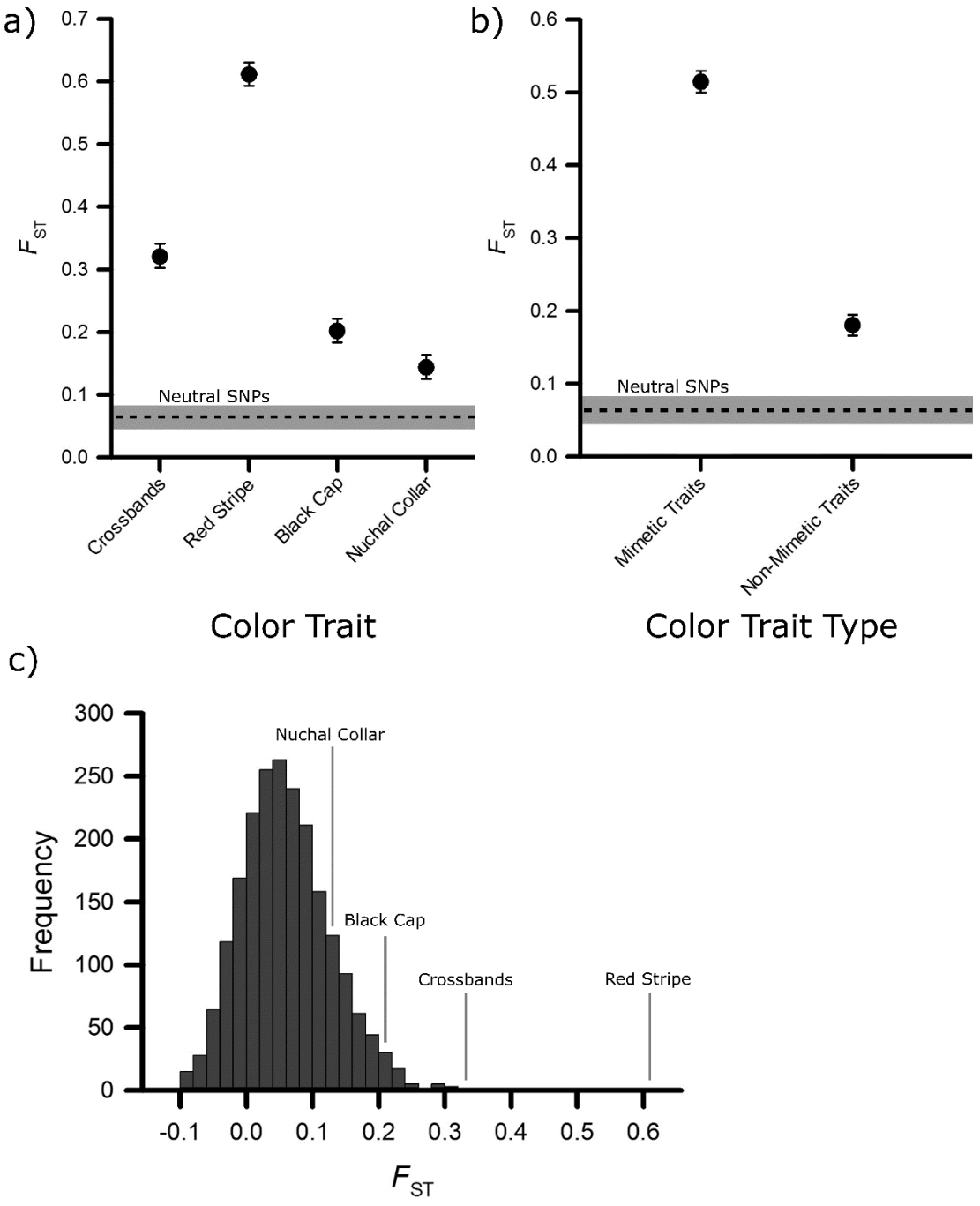

Figure 2.3: Among-population F_{ST} values for color patterns with 95% confidence intervals, presented with (a) color traits separated or (b) grouped into their respective type (mimetic or non-mimetic). The dashed line indicates the mean F_{ST} value for neutral SNPs, and the gray bar represents the 95% confidence interval. Note that in both panels, mimetic and non-mimetic color traits had significantly higher F_{ST} values than did the neutral genetic markers. When (c) F_{ST} was calculated for each locus individually, color trait F_{ST} values are higher on average than most SNP F_{ST} values (frequencies shown in dark gray).

GENERAL CONCLUSIONS

Color polymorphism in mimicry systems has received a fair amount of attention in the literature, and we now have a theoretical framework for why this phenomenon may arise and persist (Davis Rabosky *et al.* 2016; Jiggins *et al.* 2001; Joron and Mallet 1998; Maan and Cummings 2011; Noonan and Comeault 2009; Ohsaki 1995; Plowright and Owen 1980; Wang and Shaffer 2008). However, a major gap in knowledge concerns non-mimetic polymorphism in mimicry systems; no previous study has formally addressed how or why color morphism of non-mimetic traits may persist in a mimicry system, or whether the evolution of this type of polymorphism is influenced by selection on mimetic color polymorphism. In this study, I answered such questions using the ground snake (*Sonora semiannulata*), which possesses both mimetic and non-mimetic color polymorphism. Using 2,140 specimens from natural history collections, I took a geographic approach and a population genetic approach (with ddRADseq SNP data) and found support for genetic linkage of multiple types of color polymorphism, such that diversifying selection on mimetic traits drives color polymorphism and diversity of non-mimetic traits.

My work contributes to a deeper understanding of how selection on coral snake-mimicking phenotypes can drive broad patterns of phenotypic diversity. Previous work has demonstrated that mimicry-based selection can generate phenotypic diversity of mimetic species (Davis Rabosky *et al.* 2016; Jiggins *et al.* 2001; Joron and Mallet 1998; Maan and Cummings 2011; Noonan and Comeault 2009; Ohsaki 1995; Plowright and Owen 1980; Wang and Shaffer 2008). My findings suggest that selection on mimetic traits not only impacts the diversity of mimetic traits, but also genetically-linked non-mimetic traits. I believe that my findings present a novel mechanism underlying how mimicry can maintain color pattern diversity. While beyond

the scope of this present research, future studies that determine color pattern loci and patterns of linkage among color loci can clarify the genetic underpinnings of mimetic trait and non-mimetic trait linkage. Comparatively, research that measures rates of phenotypic diversification for mimetic and non-mimetic traits can determine how selection on mimetic traits influences rates of evolution for non-mimetic traits in a macroevolutionary context. Such research will highlight the role of mimicry in driving patterns of phenotypic diversity across the tree of life.

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APPENDICES

APPENDIX I: *List of museum collections that provided samples*

Table A1: A list of all museum collections that provided ground snake specimens, tissues, and/or photographs for use in this study.

Institution
Arizona State University
California Academy of Sciences
Museum of Southwestern Biology at the University of New Mexico
Museum of Vertebrate Zoology at the University of California
New Mexico State University
Sam Noble Museum at the University of Oklahoma
San Diego Natural History Museum
Sternberg Museum of Natural History at Fort Hayes State University
University of Arizona
University of Kansas
University of Texas
University of Texas at Arlington
University of Texas at Austin
University of Texas at El Paso

APPENDIX II: *Spatial analysis results using alternative scoring for the nuchal collar*

Table A2: The results of all spatial analyses involving the nuchal collar when individuals with bands are scored as possessing the nuchal collar. All tests were conducted in the same manner as Chapter 1, using the same individuals and populations. Significance is indicated by a *. Abbreviations: RS = Red stripe; BC = Black cap; NC = Nuchal collar; Mim = Mimetic traits; NonMim = Non-mimetic traits; H = Shannon index of diversity.

Test	Variable 1	Variable 2	Test statistic	*P*-value	Result from Ch. 1 tests
Contingency analysis	RS frequency	NC frequency	$\chi^2 = 153.765$	< 0.0001*	Significant
Contingency analysis	BC frequency	NC frequency	$\chi^2 = 254.682$	< 0.0001*	Significant
Correlation	H Mim	H NonMim	Spearman $\rho = 0.3445$	0.0235*	Significant
Correlation	Latitude	% NC	Spearman $\rho = 0.5248$	0.0002*	Significant
Correlation	Longitude	% NC	Spearman $\rho = -0.0059$	0.9688	Not significant
Correlation	Latitude	H NonMim	Spearman $\rho = 0.0062$	0.9675	Not significant
Correlation	Longitude	H NonMim	Spearman $\rho = -0.0640$	0.6727	Not significant
Mann-Whitney-Wilcoxon	Coral snake sympatry/allopatry	% NC	$Z = -1.88145$	0.0599	Not significant
Mann-Whitney-Wilcoxon	Coral snake sympatry/allopatry	H NonMim	$Z = 0.61274$	0.5400	Not significant
Partial Mantel test	Mim distance	NonMim distance	$r = 0.644866$	< 0.0001*	Significant
Partial Mantel test	RS distance	NC distance	$r = 0.548117$	< 0.0001*	Not significant
Partial Mantel test	BC distance	NC distance	$r = 0.596808$	< 0.0001*	Not significant

APPENDIX III: *Population genetic analysis results using alternative scoring for the nuchal collar*

Table A3: The results of all population genetic analyses involving the nuchal collar when individuals with bands are scored as possessing the nuchal collar. All tests were conducted in the same manner as Chapter 2, using the same individuals and populations. Significance is indicated by a *. Abbreviations: NC = Nuchal collar; NonMim = Non-mimetic Traits; I = Shannon's information index.

Test	Variable 1	Variable 2	Test Statistic	*P*-value	Result from Ch. 2 tests
Partial Mantel test	Genetic distance	NC distance	$r = 0.118946$	0.1497	Not significant
Partial Mantel test	Genetic distance	NonMim distance	$r = 0.112760$	0.1128	Not significant
Correlation	Genetic diversity (I)	NC diversity (I)	Spearman $\rho = -0.1829$	0.4034	Not significant
Correlation	Genetic diversity (I)	NonMim diversity (I)	Spearman $\rho = -0.1043$	0.6358	Not significant
F_{ST}	NC		$F_{ST} = 0.258$		$F_{ST} = 0.143$
F_{ST}	NonMim		$F_{ST} = 0.227$		$F_{ST} = 0.180$