

# Patterns of genetic diversity in the polymorphic ground snake (*Sonora semiannulata*)

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**Abstract** We evaluated the genetic diversity of a snake species with color polymorphism to understand the evolutionary processes that drive genetic structure across a large geographic region. Specifically, we analyzed genetic structure of the highly polymorphic ground snake, *Sonora semiannulata*, (1) among populations, (2) among color morphs (3) at regional and local spatial scales, using an amplified fragment length polymorphism dataset and multiple population genetic analyses, including  $F_{ST}$ -based and clustering analytical techniques. Based upon these methods, we found that there was moderate to low genetic structure among populations. However, this diversity was not associated with geographic locality at either spatial scale. Similarly, we found no evidence for genetic divergence among color morphs at either spatial scale. These results suggest that despite dramatic color polymorphism, this phenotypic diversity is not a major driver of genetic diversity within or among populations of ground snakes. We suggest that there are two mechanisms that could explain existing genetic diversity in ground snakes: recent range expansion from a genetically diverse founder

population and current or recent gene flow among populations. Our findings have further implications for the types of color polymorphism that may generate genetic diversity in snakes.

**Keywords** Population genetic structure · Genetic diversity · Range expansion · Color polymorphism

## Introduction

The genetic diversity of populations can have crucial implication for key evolutionary processes such as adaptation and speciation (Hartl and Clark 2007; Hughes et al. 2008). Genetic diversity within populations is linked to selection and fitness dynamics (Hughes et al. 2008), while gene flow between populations can counteract local adaptation (Lenormand 2002; Slatkin 1985, 1987; Reed and Frankham 2003). Similarly, restrictions in gene flow or genetic diversity can be important for reproductive isolation and part of incipient speciation (Nosil 2008; Slatkin 1987; Lande 1980). Thus, understanding the factors that shape population genetic structure can illuminate how populations adapt to local environments and how patterns of gene flow generate genetic diversity or homogeneity across the landscape.

Geography can impact patterns of genetic diversity and gene flow (Manel et al. 2003; Frankham 1997; Cox et al. 2012b; Miller et al. 2014) and be mediated by organismal biology such as breeding behavior (Bouzat and Johnson 2004; Johnson et al. 2003; McMillan et al. 1999), sex-biased dispersal (Fontenot et al. 2011; Lyrholm et al. 1999), vagility (Bohonak 1999) and susceptibility to anthropogenic disturbance (Goosens et al. 2006; Epps et al. 2005). In particular, color polymorphism is an organismal

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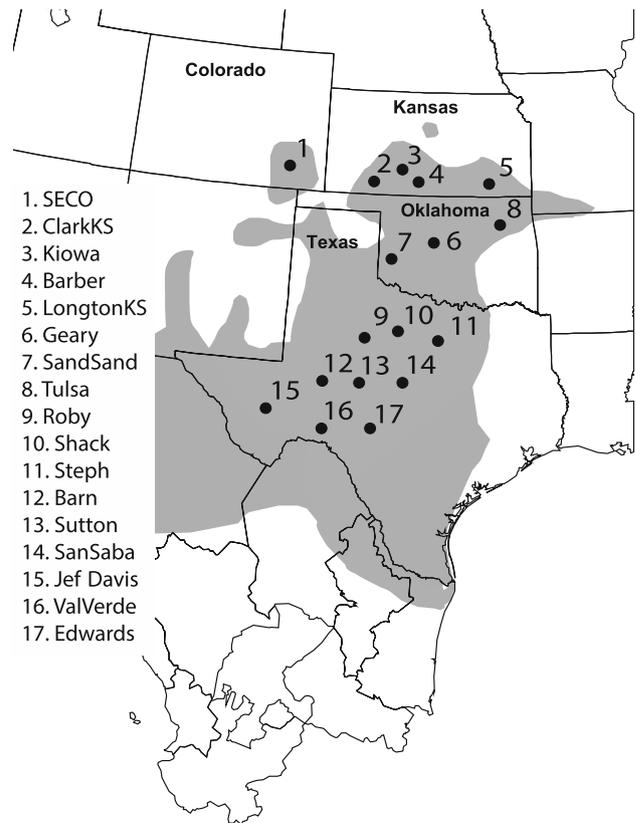


**Fig. 1** The four morphs of ground snakes; uniform (top right), red-striped (bottom right), both red and black (bottom left) and black-banded (top left). (Color figure online)

trait that has been linked to the development of genetic structure among populations (Corl et al. 2010; Gray and McKinnon 2006; Hugall and Stuart-Fox 2012).

Color polymorphism can promote genetic structure both among populations and among morphs within populations. First, color polymorphism can cause reproductive isolation among populations with different color morph compositions (Corl et al. 2010; Sinervo and Svensson 2002). Loss of alternate morphs in different populations can lead to prezygotic isolation between populations (i.e., if those morphs have alternate mating strategies). Alternately, postzygotic isolation may occur between populations with different morph compositions, due to mechanisms such as Dobzhansky–Muller incompatibilities or the reappearance of low-fitness morphs as the result of introgression into a population where they have been previously extirpated by selection. Second, morphs within populations can become reproductively isolated through other mechanisms such as assortative mating (Elmer et al. 2009; Avise et al. 1992; McMillan et al. 1999), disruptive selection (Smith 1962) or alternate adaptations (Sinervo et al. 2007; Pryke and Griffith 2006; West-Eberhard 1986).

We sought to characterize the genetic relationships among morphs and populations of the polymorphic ground snake, *Sonora semiannulata*. Ground snakes are small, arthropod-eating snakes that are distributed in arid to semiarid environments in south-central and western United States and northern Mexico (Fig. 2). Ground snake populations can have up to four color morphs: (1) longitudinal, dorsal red stripes, (2) dorsal black bands, (3) simultaneously red-striped with black bands, or (4) uniform gray or brown with no distinctive red or black markings of any kind (Cox et al. 2012a; Fig. 1; Ernst and Ernst 2003). Populations can possess a single morph or as many as all four morphs, in any combination (Cox and Davis Rabosky



**Fig. 2** Geographic range of ground snakes in central North America and the localities included in this study

2013). Their closest relatives are tricolored coral snake mimics in Mexico (also genus *Sonora*), and the red and black morph of *S. semiannulata* is considered a putative coral snake mimic (Cox et al. 2012a; Brodie and Brodie 2004; Savage and Slowinski 1992). Recent research has implicated both geographic and temporal variation in selection and negative frequency dependence as drivers of variation in the geographic distribution of color morphs in *S. semiannulata* (Cox and Davis Rabosky 2013). This type of selection could theoretically alter patterns of gene flow within or among color morphs or populations. For example, selection favoring alternate morphs in different populations could result in diminished gene flow among populations (if introgressed individuals have lower fitness). Similarly, selection that favors a single morph could result in assortative mating and create genetic structure among morphs of ground snakes.

We examined mechanisms underlying genetic diversity and structure among and within populations of ground snakes, focusing on populations in the Great Plains region (Fig. 2). Ground snakes are abundant in this area, which is a large proportion of their geographic range. This geographic area is ideal for testing how polymorphism

influences genetic structure, because there are no large geographic barriers which can complicate patterns of gene flow among populations with different morph frequencies. We extended our previous work on color polymorphism and genetic structure (Cox and Davis Rabosky 2013) by using an expanded geographic dataset (six additional localities) and additional analyses in novel tests of genetic structure among populations and morphs of ground snakes. We made three primary predictions about the relationship between genetic structure and color polymorphism in ground snakes. First, we predicted that isolation by distance or genetic clustering of geographically proximal populations is evidence that geography impacts genetic structure. Second, we predicted that in the absence of geographically based structure, genetic structure among populations with different morph frequencies is evidence that color polymorphism has altered gene flow among populations. Finally, we predicted that genetic structure among morphs within populations can be evidence for assortative mating among color morphs. Our results, considered in the context of published research, allow us to explore the general principles underlying the mechanisms that can influence genetic diversity among populations.

## Materials and methods

### Study system and geographic sampling

Ground snakes are distributed from Missouri to northern Mexico in the east to the Baja peninsula and Oregon in the west. However, we focused our sampling on snakes from the Great Plains region of the central United States because (1) this is the only part of their range where enough individuals can be reliably collected for population genetic analyses and (2) this area is largely homogeneous without major geographic barriers to disrupt or alter gene flow.

Snakes were collected by turning rocks in appropriate habitat from 2008 to 2010. We focused on collections from 17 different geographically restricted localities (Fig. 2; also see electronic supplementary material, available online). We preserved muscle, liver, or skin tissue in lysis buffer, 95 % ethanol or an RNA-preserving buffer. Although some specimens were sampled for tissues and released, most specimens were fixed in 10 % formalin and fluid-preserved in 70 % ethanol. Specimens were collected according to IACUC protocols (A.07.021 and A.08.025) and deposited in the University of Texas-Arlington Amphibian and Reptile Diversity Research Center and Sternberg Museum at Fort Hays State University. Additional tissue samples were obtained from the Sternberg Museum at Fort Hays State University.

### Molecular dataset

We generated a population genetic dataset for 248 ground snakes, using amplified fragment length polymorphisms (AFLPs) to determine the neutral genetic structure of ground snakes, following standard methods (Vos et al. 1995) using slightly modified primers (see electronic supplementary material, available online). We objectively scored AFLPs using the script AFLPscore (Whitlock et al. 2008) and processed AFLP loci for analysis using AFLP<sub>DATA</sub> (Ehrich 2006) in R v2.14.0 (R Development Core Team 2008). To avoid spurious results due to loci under strong selection, we screened our dataset for loci that were potentially under selection, using M<sub>CHEZA</sub> (Antao and Beaumont 2011; Beaumont and Balding 2004) and BAYESCAN (Foll and Gaggiotti 2008) to identify candidate loci. For both methods, we treated AFLPs as dominant markers and used suggested parameter settings and estimated outliers using 100,000 generations and a conservative false discovery rate (FDR = 0.001). We then excluded any loci that were identified as candidates for selection in either analysis, which resulted in a final dataset of 112 putatively neutral AFLP loci (see electronic supplementary material, available online).

### Population genetic analyses

#### *Genetic structure among populations*

We used AMOVA to test for genetic structure among populations by analyzing AFLPs as dominant markers (Excoffier et al. 1992; Huff et al. 1993; Peakall et al. 1995; Maguire et al. 2002), and assessed significance using 1,000 bootstrap pseudoreplicates. This method estimates  $\Phi_{PT}$  (Peakall et al. 1995; Maguire et al. 2002), which is the equivalent of  $F_{ST}$  for dominant markers (hereafter referred to  $F_{ST}$ ). Although our sample sizes for some populations were low, we found that iteratively removing populations with only two, three, four, and five individuals did not substantially change our estimate of  $F_{ST}$  (it varied by only 0.004), so we elected to retain all populations for final analyses. We then tested for genetic clustering of geographically proximal populations using both binary (Huff et al. 1993; Maguire et al. 2002) and Nei's genetic distance (Nei 1972, 1978). We also examined geographic patterns of genetic variation within populations using Nei's heterozygosity (Nei 1972, 1978) and Shannons I (Lewontin 1972; Shannon 1948). These analyses were conducted in GenAlEx (Peakall and Smouse 2006). We tested for isolation by distance using simple Mantel tests for correlation between geographic and genetic distance matrices in the program zt (Bonnet and van de Peer 2002). Some authors suggest that this test has low power and may only be able to detect strong patterns of isolation by distance (Legendre

and Fortin 2010; Balkenhol et al. 2009), and we use caution interpreting the results of this analysis.

#### *Population clustering analyses among populations*

We used Bayesian clustering analysis to explore patterns of genetic structure in ground snakes using the program STRUCTURE (Pritchard et al. 2000). For initial analyses, we experimented with different population models, number of replicates, and number of generations and found that most combinations of priors and parameters yielded consistent results. For our final analyses, we implemented a population model with genetic admixture and correlated allele frequencies, as this is a reasonable model for populations that likely are closely related (Falush et al. 2003; Pritchard et al. 2000), and analyzed 1,000,000 generations for a number of clusters (K) ranging from one to 17, with ten replicates for each K value. We then graphically examined the relationship between likelihood values and K to determine a range of likely population clusters, similar to Evanno et al. (2005). We also examined population clustering using discriminant analysis of principal components with the function DAPC (Jombart et al. 2010) in the R package adagenet (Jombart 2008). These results were very similar to Bayesian clustering results (see electronic supplementary material, available online), so we only present the results from Bayesian clustering analyses.

#### *Genetic structure among color morphs*

We analyzed genetic structure among color morphs at multiple genetic and geographic scales. The genetic architecture of color polymorphisms in ground snakes is unknown, although it is not associated with sequence variation in the *Mc1R* gene (Cox et al. 2013). For the following analyses, we simply scored color pattern by morph as uniform gray or brown (U), red-striped (S), black-banded (B), or red-striped and black-banded (M) as in Cox and Davis Rabosky 2013 (see electronic supplementary material, available online). Although the sample size was fewer than ten for six populations, we elected to include the populations with lower sample sizes for two reasons: (1) to gain a finer-scale understanding of how ground snakes are related across the landscape and (2) to increase the resolution of the geographic distribution of color morphs. At a regional scale, we separated our dataset into color morphs and calculated  $F_{ST}$  among these morphs ( $n = 244$  total for all morphs). Additionally, we calculated genetic distance and genetic variability within and among morphs (as in *Population analyses*). We focused on two local-scale geographic areas to examine the impact of spatial scale on inferences of genetic structure among populations and morphs. To this end, we restricted our analyses at the local spatial scale to areas with multiple geographically proximal localities with

high sample sizes. The two general areas that matched these criteria were three localities from southwestern Kansas ( $n = 49$ , Barber, ClarkKS, and Kiowa) and two from north Texas ( $n = 51$ , Shackelford and Stephens). Both between and within each of these local “populations”, we calculated  $F_{ST}$  based upon locality (as in *Population analyses*). We conducted Bayesian clustering analyses from  $K = 1$  to 4 with 10 replicates per K, as in *Bayesian clustering analyses*. We then tested for differential gene flow between morphs by calculating  $F_{ST}$  based upon color morph within both geographic areas. For this color pattern analysis, we excluded the only banded and red-striped snake (M) from the North Texas population to allow calculation of  $F_{ST}$ .

## Results

### Population genetic structure

#### *Locality-based $F_{ST}$ , genetic distance, and within-population genetic variation*

We found that although populations differed extensively in the frequency and presence of color morphs (Table 1), they did not show clear patterns of geographic structure using  $F_{ST}$  and genetic-distance based analyses. Populations displayed some genetic structure overall ( $F_{ST} = 0.074$ ,  $P < 0.01$ ; Table 2). However, genetic distances between populations (Nei’s unbiased genetic distance: 0.009–0.096; Binary genetic distance: 11.00–27.09) did not suggest that populations were clustered into geographic groups (see electronic supplementary material, available online). Similarly, within-population genetic variation (Nei’s unbiased heterozygosity: 0.054–0.233; Shannon’s I: 0.059–0.341) differed among populations but without a clear geographic pattern (see electronic supplementary material, available online). Estimates of  $F_{ST}$  using both local populations (southwestern Kansas and north Texas) were generally similar in value to those based on the regional analysis, but did not differ significantly from zero (Table 2). Interestingly,  $F_{ST}$  estimates between local populations were similar in value to estimates of  $F_{ST}$  among (sub)populations within the local populations. Genetic distance (Nei’s unbiased genetic distance: 0.02–0.031; Binary genetic distance: 12.73–15.90) and within-population genetic variation (Nei’s unbiased heterozygosity: 0.123–0.190; Shannon’s I: 0.181–0.283) were also similar between southwestern Kansas and north Texas (see electronic supplementary material, available online). The absence of geographic clustering of genotypes was consistent with the lack of evidence for isolation by distance at the local (Mantel Test,  $R = 0.03$   $P > 0.52$ ) and even the regional spatial scale (Mantel Test,  $R = 0.01$   $P > 0.37$ ).

**Table 1** Frequency of each color morph of ground snakes in each locality

Population	Locality	Uniform	Red-striped	Banded	Mimetic
Barber	Barber Co., KS	1	5	1	5
Barn	Crockett Co., TX	38	1	2	0
ClarkKS	Clark Co., KS	5	11	0	0
Edwards	Edwards Co., TX	5	0	0	0
Geary	Blaine Co., OK	0	10	0	8
Kiowa	Kiowa Co., KS	7	13	1	0
LongtonKS	Elk Co., KS	7	2	0	0
Roby	Fisher Co., KS	6	11	0	0
SandSand	Beckham Co., OK	5	10	0	0
SanSaba	San Saba Co., TX	1	0	1	1
SECO	Otero Co., CO	0	0	1	22
Shack	Shackelford Co., TX	21	3	6	1
Steph	Stephens Co., TX	17	2	2	0
Sutton	Sutton Co., TX	2	0	0	0
Tulsa	Tulsa Co., OK	2	0	2	0
ValVerde	Val Verde Co., TX	2	4	0	0
West	Jeff Davis Co., TX	0	2	0	1

**Table 2**  $F_{ST}$  values among localities and color morphs using regional and local populations (southwestern Kansas and north Texas)

Category	$F_{ST}$
Regional-Scale	
Locality	0.074**
Color	0.012**
Local-Scale	
Between localities	0.053**
Among localities	
	N Texas 0.051
	SW Kansas 0.040
Color	
	N Texas -0.010
	SW Kansas -0.015

\*  $P < 0.05$ \*\*  $P < 0.01$ 

### Bayesian population clustering

We found that likelihood increased with increasing number of genetic clusters ( $K$ ), but began to plateau at  $K = 3$  (see electronic supplementary material, available online). These clusters did not generally correspond to any locality or geographic region (Fig. 3), with the exception of potential genetic isolation of the population in southeastern Colorado (SECO). For our local-scale analyses, we found that likelihood increased with the number of clusters from one to two, but then remained stable or declined with greater numbers of clusters (see electronic supplementary material, available online). These clusters did not delineate localities

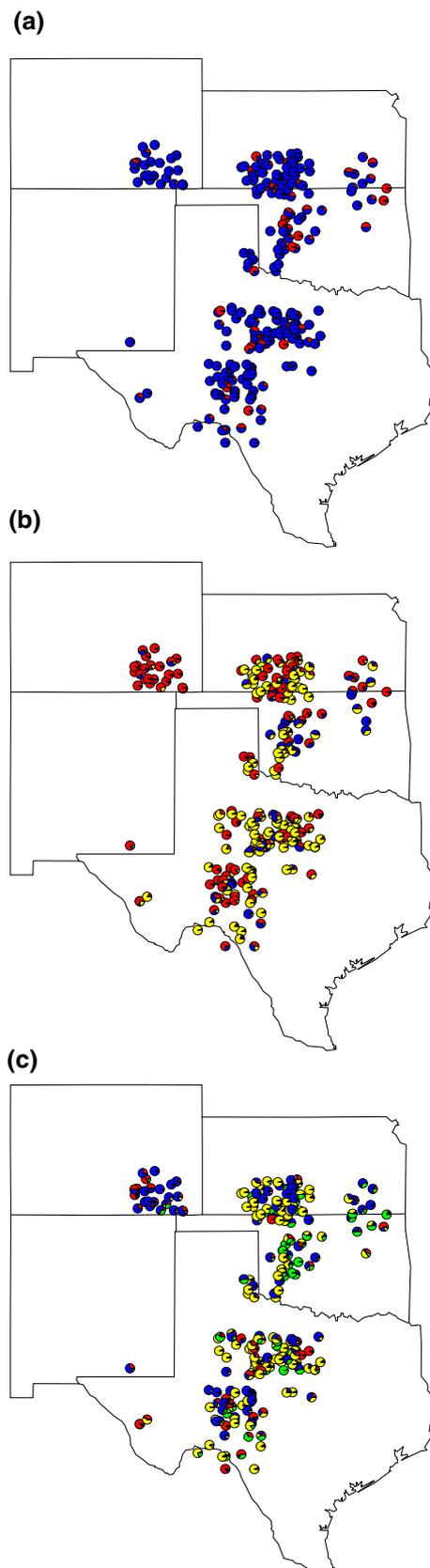
within Texas or Kansas local populations (Fig. 4), suggesting there is no correspondence between geography and genetic variation in ground snakes at this local scale.

### Genetic structure among color morphs

Genetic subdivision among color morphs was very low but significant at the regional scale (Table 2). Genetic distance among morphs (Nei's unbiased genetic distance: 0.008–0.018; Binary genetic distance: 13.00–16.99) and within-morph genetic variability (Nei's unbiased heterozygosity: 0.149–0.227; Shannon's  $I$ : 0.221–0.350) was low and similar among morphs at the regional scale. At a local scale, we found no evidence for genetic structure among color morphs at a local scale, with very low  $F_{ST}$ 's (–0.010 and –0.015 for north Texas and southwestern Kansas, respectively) that were not significantly different from zero ( $P > 0.63$ ) for either population (Table 2). Population genetic parameters among morphs were similar between spatial scales, with genetic distance among morphs (Nei's unbiased genetic distance: 0.013–0.092; Binary genetic distance: 11.00–16.23) and within-morph genetic variability (Nei's unbiased heterozygosity: 0.069–0.195; Shannon's  $I$ : 0.025–0.298) in both southwest Kansas and north Texas (see electronic supplementary material, available online).

### Discussion

Although we found some evidence of genetic structure in ground snakes (significant  $F_{ST}$ ,  $K > 1$ ), this structure was

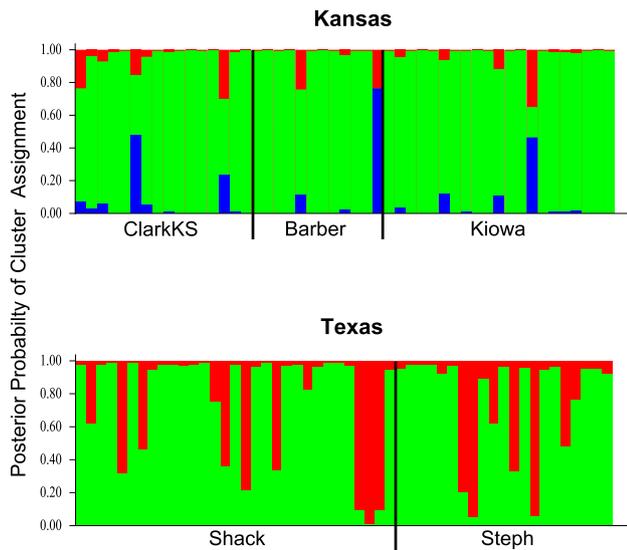


**Fig. 3** Distribution of genotypes across the landscape for the number of clusters ( $K$ ) from **a**  $K = 2$ , **b**  $K = 3$ , and **c**  $K = 4$  from Bayesian clustering analysis of genetic structure in ground snakes. Each individual in the study is represented by a *single pie chart*, and the percentage of each shade or color in the *pie chart* represents the probability of assignment to that genotypic cluster. Localities are the same as in Fig. 1, but the *pie charts* representing each individual have been randomly jittered to allow display of the majority of pie charts

not generally explained by distance or other geographic factors. However, we found that a single population (in southeastern Colorado) was somewhat genetically distinct and homogeneous in Bayesian clustering analyses (with  $K = 3$  or 4). This population is a geographic isolate (Fig. 2; Ernst and Ernst 2003), which may have caused genetic divergence from populations in the rest of the Great Plains. Additionally, color polymorphism does not seem to explain genetic structure, with no evidence of divergence between morphs within populations, or among populations with different morph composition. We do not interpret the very low but significant  $F_{ST}$  among color morphs at the regional level as evidence for genetic divergence among morphs, as this could easily be the signal of the genetic structure that we detected at the regional level. Indeed, morph composition and frequency varies among populations, and thus the population composition of each morph at the regional scale could be biased. Therefore, mechanisms other than geography or color polymorphism must be invoked to explain genetic structure in ground snakes in the Great Plains.

Geographic range expansion and current gene flow could explain both the low genetic distances between localities, relatively high within-locality genetic variation and the presence of sympatric genotypes across a geographic region (Ibrahim et al. 1996; Excoffier et al. 2009). Notably, other species have expanded their ranges in the Great Plains region and rates of gene flow are generally high (*Gastrophryne* narrowmouth frogs, Streicher et al. 2012; e.g., armadillos, Taulman and Robbins 1996; prairie grouses, Johnson 2008; *Aphonopelma* tarantulas, Hamilton et al. 2011; bufonid frogs, Fontenot et al. 2011), perhaps due to the lack of significant barriers to dispersal. Importantly, we note that the rest of the geographic range of ground snakes in western North America is much more topographically complex than the Great Plains. Future research on genetic structure in ground snakes will clarify whether the dispersal ability of ground snakes leads to similar shallow genetic structure across their geographic range, or if the dynamic geography of western North America has produced more intricate patterns of genetic relatedness across the landscape.

The low levels of genetic divergence or structure in *Sonora* across central North America may be a function of



**Fig. 4** Cluster assignment probabilities from Bayesian clustering analyses of ground snakes at the local scale with number of clusters ( $K$ ) set to  $K = 3$  (Kansas) or  $K = 2$  (Texas). Note that while individuals within each locality may be assigned with high probability to different clusters, the approximate genotypic composition of each locality is similar

the generally homogenous landscape of the Great Plains region, which lacks any major (or at least obvious) geographic barriers that might lead to vicariance (in fact, none of our localities included the most important biogeographic barrier of the central United States, the Edwards Plateau and the associated Balcones escarpment). Studies of other terrestrial vertebrates with similar distributions reveal that either (1) population genetic structure is minimal or absent (Streicher et al. 2012; Pyron and Burbrink 2009), (2) population structure is associated with organismal attributes such as lekking or male-biased dispersal (Bouzat and Johnson 2004; Fontenot et al. 2011), or (3) populations are structured genetically by the modest topographic features of this region (Hamilton et al. 2011; Neiswenter and Riddle 2010; Castoe et al. 2007). Ground snakes fit this pattern of genetic variation that is only minimally structured by geography.

#### Spatial scale and genetic structure

Our research emphasizes the importance of spatial scale for interpreting genetic structure. While genetic distance, genetic variation, and Bayesian clustering were not strongly impacted by scale, estimates of population subdivision were nearly halved and no longer significant at the local scale compared to the regional scale. Most importantly, analysis at the regional scale may suggest assortative mating based on coloration in ground snakes (e.g., low but significant  $F_{ST}$ ), while analysis at the local scale reveals

no genetic structure among color morphs. This finding is not surprising, as our results are consistent with research that has demonstrated scale-dependency in diverse fields such as macroecology (Brown and Nicoletto 1991; Cox et al. 2011), ecological interactions (Brodie et al. 2002), and both population genetics and phylogenetics (Runemark et al. 2010; Cavender-Bares et al. 2006). These results underscore the importance of explicitly analyzing spatial scale in population genetic research, especially for large spatial scales.

#### Color polymorphism and genetic structure

While selection acts upon the dramatic variation in color polymorphism across the geographic range in ground snakes (Cox and Davis Rabosky 2013), we did not find that polymorphism shapes genetic structure among populations in central North America. In other geographically widespread polymorphic systems with genetic structure, sexual selection is a primary driver of altered gene flow among populations due to reproductive incompatibilities (Corl et al. 2010; West-Eberhard 1986; Jiggins et al. 2001), and among morphs through assortative mating (e.g. Rift Lake cichlid fishes, Seehausen et al. 1999; *Hypoplectrus* reef fishes, Puebla et al. 2007; *Heliconius* butterflies, Chamberlain et al. 2009; dendrobatid poison frogs, Reynolds and Fitzpatrick 2007). Across these systems, the emergence of genetic structure is most prevalent in polymorphic systems with bright, conspicuous morphs where color pattern is used for sexual signaling (Corl et al. 2010; Seehausen et al. 2008), or both mimicry and sexual signaling (Wang and Summers 2010; Jiggins et al. 2001). However, sexual dichromatism is rare among snakes, and sexual selection on color in snakes is relatively rare (Shine and Madsen 1994). Indeed, the cellular histology of the eye indicates that most snakes either lack entirely or have only limited color vision (Walls 1942; Sillman et al. 1999). Ground snakes are semifossorial and nocturnal most of the year (Ernst and Ernst 2003; Tenant 1984; Degenhardt et al. 1996), which would further limit the opportunity for sexual selection to act upon color. Across all snakes, polymorphism is most commonly manifested as either cryptic morphs (e.g., *Thamnophis sirtalis* and *Nerodia sipedon*, King 1987; *Python brongersmai*, Shine et al. 1998; *Vipera berus*, Forsman 1995; King 1988; *Psammophis schokari*, Kark et al. 1997; *Elaphe quadrivirgata*, Tanaka 2007), or conspicuous morphs in coral snake mimicry complexes (Brodie and Brodie 2004; Cox et al. 2012a). In ground snakes, the dynamics of color polymorphism seems to be driven by frequency dependence in concert with spatial and temporal selection, perhaps linked to coral snake mimicry (Cox and Davis Rabosky 2013). This evidence suggests that genetic structure in polymorphic snake systems may be relatively

rare, and may also broadly alter expectations for genetic structure among other polymorphic systems where sexual selection does not play a major role in color pattern evolution.

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## References

- Antao T, Beaumont MA (2011) Mchaza: a workbench to detect selection using dominant markers. *Bioinformatics* 27:1717–1718
- Avise JC, Alisaukas RT, Nelson WS, Ankney CD (1992) Matriarchal population genetic structure in an avian species with female natal philopatry. *Evolution* 46:1084–1096
- Balkenhol N, Waits LP, Dezzani RJ (2009) Statistical approaches in landscape genetics: an evaluation of methods for linking landscape and genetic data. *Ecography* 32:818–830
- Beaumont MA, Balding DJ (2004) Identifying adaptive genetic divergence among populations from genome scans. *Mol Ecol* 13:969–980
- Bohonak AJ (1999) Dispersal, gene flow, and population structure. *Q Rev Biol* 74:21–45
- Bonnet E, van de Peer Y (2002) zt: a software tool for simple and partial Mantel tests. *J Stat Softw* 7:1–12
- Bouzat JL, Johnson K (2004) Genetic structure among closely spaced leks in a peripheral population of lesser prairie chickens. *Mol Ecol* 13:499–505
- Brodie ED III, Brodie ED Jr (2004) Venomous snake mimicry. In: Campbell JA, Lamar WW (eds) *The venomous reptiles of the Western hemisphere*, vol II. Comstock Publishing Associates, Ithaca p vii + 870 pp
- Brodie ED Jr, Ridenhour BJ, Brodie ED III (2002) The evolutionary response of predators to dangerous prey: hotspots and coldspots in the geographic mosaic of coevolution between garter snakes and newts. *Evolution* 56:2067–2082
- Brown JH, Nicoletto PF (1991) Spatial scaling of species composition: body masses of North American land mammals. *Am Nat* 138(6):1478–1512
- Castoe TA, Spencer CL, Parkinson CL (2007) Phylogeographic structure and historical demography of the western diamondback rattlesnake (*Crotalus atrox*): a perspective on North American desert biogeography. *Mol Phylogenet Evol* 42:193–212
- Cavender-Bares J, Keen A, Miles B (2006) Phylogenetic structure of Floridian plant communities depends on taxonomic and spatial scale. *Ecology* 87:S109–S122
- Chamberlain NL, Hill RI, Kapan DD, Gilbert LE, Kronforst MR (2009) Polymorphic butterfly reveals the missing link in ecological speciation. *Science* 326:847–850
- Corl A, Davis AR, Kuchta SR, Sinervo B (2010) Selective loss of polymorphic mating types is associated with rapid phenotypic evolution during morphic speciation. *Proc Natl Acad Sci USA* 107:4254–4259
- Cox CL, Davis Rabosky ARD (2013) Spatial and temporal drivers of phenotypic diversity in polymorphic snakes. *Am Nat* 182:E40–E57
- Cox CL, Boback SM, Guyer C (2011) Spatial dynamics of body size frequency distributions for North American squamates. *Evol Biol* 38:453–464
- Cox CL, Rabosky ARD, Reyes-Velasco J, Ponce-Campos P, Smith EN, Flores-Villela O, Campbell JA (2012a) Molecular systematics of the genus *Sonora* (Squamata: Colubridae) in central and western Mexico. *Syst Biodivers* 10:93–108
- Cox CL, Streicher JW, Sheehy CM, Campbell JA, Chippindale PT (2012b) Patterns of genetic differentiation among populations of *Smilisca fodiens*. *Herpetologica* 68:226–235
- Cox CL, Rabosky ARD, Chippindale PT (2013) Sequence variation in the *Mc1R* gene for a group of polymorphic snakes. *Gene* 513:282–286
- Degenhardt WG, Painter CW, Price AH (1996) *Amphibians and reptiles of New Mexico*, vol xiv + 431. University of New Mexico Press, New Mexico
- Ehrich D (2006) AFLPdat: a collection of R functions for convenient handling of AFLP data. *Mol Ecol Notes* 6:603–604
- Elmer KR, Lehtonen TK, Meyer A (2009) Color assortative mating contributes to sympatric divergence of neotropical cichlid fish. *Evolution* 63:2750–2757
- Epps CW, Pallsbøll PJ, Wehausen JD, Roderick GK, Ramey RR, McCullough DR (2005) Highways block gene flow and cause a rapid decline in genetic diversity of bighorn sheep. *Ecol Lett* 8:1029–1038
- Ernst CH, Ernst EM (2003) *Snakes of the United States and Canada*. Smithsonian Books, Washington
- Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Mol Ecol* 14:2611–2620
- Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction sites. *Genetics* 131:479–491
- Excoffier L, Foll M, Petit R (2009) Genetic consequences of range expansions. *Annu Rev Ecol Evol Syst* 40:481–501
- Falush D, Stephens M, Pritchard JK (2003) Inference of population structure using multilocus genotype data: linked loci and correlated gene frequencies. *Genetics* 164:1567–1587
- Foll M, Gaggiotti O (2008) A genome-scan method to identify selected loci appropriate for both dominant and codominant markers: a Bayesian perspective. *Genetics* 180:977–993
- Fontenot BE, Makowsky R, Chippindale PT (2011) Nuclear-mitochondrial discordance and gene flow in a recent radiation of toads. *Mol Phylogenet Evol* 59:66–80

- Forsman A (1995) Opposing fitness consequences of colour pattern in male and female snakes. *J Evol Biol* 8:53–70
- Frankham R (1997) Do island populations have less genetic variation than mainland populations? *Heredity* 78:311–327
- Goossens B, Chikhi L, Ancrenaz M, Lackman-Ancrenaz I, Andau P, Bruford MW (2006) Genetic signature of anthropogenic population collapse in orang-utans. *PLoS Biol* 4:e25
- Gray SM, McKinnon JS (2006) Linking color polymorphism maintenance and speciation. *Trends Ecol Evol* 22(2):71–79
- Hamilton CA, Formanowicz DR, Bond JE (2011) Species delimitation and phylogeography of *Aphonopelma hentzi* (Araneae, Mygalomorphae, Theraphosidae): cryptic diversity in North American tarantulas. *PLoS One* 6:e26207
- Hartl DL, Clark AG (2007) Principles of population genetics, 4th edn. Sinauer Associates, Sunderland
- Huff DR, Peakall R, Smouse PE (1993) RAPD variation within and among natural populations of outcrossing buffalograss *Buchloe dactyloides* (Nutt) Engelm. *Theor Appl Genet* 86:927–934
- Hugall AF, Stuart-Fox D (2012) Accelerated speciation in colour polymorphic birds. *Nature* 485:631–634
- Hughes AR, Inouye BD, Johnson MTJ, Underwood N, Vellend M (2008) Ecological consequences of genetic diversity. *Ecol Lett* 11:609–623
- Ibrahim KI, Nichols RA, Hewitt GM (1996) Spatial patterns of genetic variation generated by different forms of dispersal during range expansion. *Heredity* 77:282–291
- Jiggins CD, Naisbit RE, Coe RL, Mallet J (2001) Reproductive isolation caused by colour pattern mimicry. *Nature* 411:302–305
- Johnson JA (2008) Recent range expansion and divergence among North American prairie grouse. *J Hered* 99:165–173
- Johnson JA, Toepfer JE, Dunn PO (2003) Contrasting patterns of mitochondrial and microsatellite population structure in fragmented populations of greater prairie chickens. *Mol Ecol* 12:3335–3347
- Jombart T (2008) adagenet: a R package for the multivariate analysis of genetic markers. *Bioinformatics* 24:1403–1405
- Jombart T, Devillard S, Balloux F (2010) Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. *BMC Genet* 11:94
- Kark S, Warburg I, Werner YL (1997) Polymorphism in the snake *Psammophis schokari* on both sides of the desert edge. *J Arid Environ* 37:513–527
- King RB (1987) Color pattern polymorphism in the Lake Erie water snake, *Nerodia sipedon insularum*. *Evolution* 41:241–255
- King RB (1988) Polymorphic populations of the garter snake *Thamnophis sirtalis* near Lake Erie. *Herpetologica* 44:451–458
- Lande R (1980) Genetic variation and phenotypic evolution during allopatric speciation. *Am Nat* 116:463–479
- Legendre P, Fortin M-J (2010) Comparison of the Mantel test and alternative approaches for detecting complex multivariate relationships in the spatial analysis of genetic data. *Mol Ecol Res* 10:831–844
- Lenormand T (2002) Gene flow and the limits to natural selection. *Trends Ecol Evol* 17:183–189
- Lewontin RC (1972) The apportionment of human diversity. *Evol Biol* 6:381–398
- Lyrholm T, Leimar O, Johannesson B, Gyllenstein U (1999) Sex-biased dispersal in sperm whales: contrasting mitochondrial and nuclear genetic structure of global populations. *Proc R Soc B* 266:347–354
- Maguire TL, Peakall R, Saenger P (2002) Comparative analysis of genetic diversity in the mangrove species *Avicennia marina* (Forsk.) Vierh. (Avicenniaceae) detected by AFLPs and SSRs. *Theor Appl Genet* 104:388–398
- Manel S, Schwartz MK, Luikart G, Taberlet P (2003) Landscape genetics: combining landscape ecology and population genetics. *Trends Ecol Evol* 18:189–197
- McMillan WO, Weigt LA, Palumbi SR (1999) Color pattern evolution, assortative mating, and genetic differentiation in brightly colored butterflyfishes (Chaetodontidae). *Evolution* 53:247–260
- Miller AL, Makowsky RA, Formanowicz DR, Prendini L, Cox CL (2014) Cryptic genetic diversity and complex phylogeography of the boral North American scorpion, *Paruroctonus boreus* (Vaejovidae). *Mol Phylogenet Evol* 71:298–307
- Nei M (1972) Genetic distance between populations. *Am Nat* 106:283–392
- Nei M (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* 89:583–590
- Neiswenter SA, Riddle BR (2010) Diversification of the *Perognathus plavus* species in emerging arid grasslands of western North America. *J Mammal* 9:348–362
- Nosil P (2008) Speciation with gene flow could be common. *Mol Ecol* 17:2103–2106
- Peakall R, Smouse PE (2006) GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Mol Ecol Notes* 6:288–295
- Peakall R, Smouse PE, Huff DR (1995) Evolutionary implication of allozyme and RAPD variation in diploid populations of dioecious buffalograss *Buchloe dactyloides*. *Mol Ecol* 4:135–147
- Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics* 155(2): 945–959
- Pryke S, Griffith SC (2006) Red dominates black: agonistic signalling among head morphs in the colour polymorphic Gouldian finches. *Proc R Soc B* 273:949–957
- Puebla O, Bermingham E, Guichard F, Whiteman E (2007) Colour pattern as a single trait driving speciation in *Hypoplectrus* coral reef fishes? *Proc R Soc B* 274:1265–1271
- Pyron RA, Burbrink FT (2009) Lineage diversification in a widespread species: roles for niche divergence and conservatism in the common kingsnake. *Mol Ecol* 18:3443–3457
- R Development Core Team (2008) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna
- Reed DH, Frankham R (2003) Correlation between fitness and genetic diversity. *Conserv Biol* 17:230–237
- Reynolds RG, Fitzpatrick BM (2007) Assortative mating in poison-dart frogs based on an ecologically important trait. *Evolution* 61:2253–2259
- Runemark A, Hansson B, Pafilis P, Valakos ED, Svensson EI (2010) Island biology and morphological divergence of the Skyros wall lizard *Podarcis gaigeae*: a combined role for local selection and genetic drift on color morph frequency divergence? *BMC Evol Biol* 10:269
- Savage JM, Slowinski JB (1992) The colouration of venomous coral snakes (family Elapidae) and their mimics (families Aniliidae and Colubridae). *Biol J Linn Soc* 45:235–254
- Seehausen O, van Alphen JJM, Lande R (1999) Color polymorphism and sex ratio distortion in a cichlid fish as an incipient stage in sympatric speciation by sexual selection. *Ecol Lett* 2:367–378
- Seehausen O, Terai Y, Magalhaes IS, Carleton KL, Mrosso HDJ, Miyagi R, van der Sluijs I, Schneider MV, Maan ME, Tachida H, Imai H, Okada N (2008) Speciation through sensory drive in cichlid fish. *Nature* 455:620–626
- Shannon CE (1948) A mathematic theory of communication. *Bell Syst Tech J* 27:379–423
- Shine R, Madsen T (1994) Sexual dichromatism in snakes of the genus *Vipera*: a review and a new evolutionary hypothesis. *J Herpetol* 28:114–117
- Shine R, Ambariyanto, Harlow PS, Mumpumi (1998) Ecological divergence among sympatric colour morphs in blood pythons. *Oecologia* 116:113–119

- Sillman AJ, Carver JK, Loew ER (1999) The photoreceptors and visual pigments in the retina of a boid snake, the ball python (*Python regius*). *J Exp Biol* 202:1931–1938
- Sinervo B, Svensson E (2002) Correlational selection and the evolution of genomic architecture. *Heredity* 89:329–338
- Sinervo B, Heulin B, Surget-Groba Y, Clobert J, Miles DB, Corl A, Chaine A, Davis A (2007) Models of density-dependent genic selection and a new rock-paper-scissors social system. *Am Nat* 170:663–680
- Slatkin M (1985) Gene flow in natural populations. *Annu Rev Ecol Syst* 16:393–430
- Slatkin M (1987) Gene flow and the geographic structure of natural populations. *Science* 236:787–792
- Smith JM (1962) Disruptive selection, polymorphism and sympatric speciation. *Nature* 195:50–62
- Streicher JW, Cox CL, Campbell JA, Smith EN, de Sa RO (2012) Rapid range expansion in the Great Plains narrow-mouthed toad (*Gastrophryne olivacea*) and a revised taxonomy for North American microhylids. *Mol Phylogenet Evol* 64:645–653
- Tanaka K (2007) Thermal biology of a colour dimorphic snake, *Elaphe quadrivirgata*, in a montane forest: do melanistic snakes enjoy thermal advantages? *Biol J Linn Soc* 92:309–322
- Taulman JF, Robbins LW (1996) Recent range expansion and distributional limits of the nine-banded armadillo (*Dasypus novemcinctus*) in the United States. *J Biogeogr* 23:635–648
- Tenant A (1984) The snakes of Texas. Texas Monthly Press, Texas
- Vos P, Hogers R, Bleeker M, Reijans M, Lee TVD, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M (1995) AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Res* 23(21):4407–4414
- Walls GL (1942) The vertebrate eye and its adaptive radiation. Cranbrook Press, Bloomfield Hills
- Wang IJ, Summers K (2010) Genetic structure is correlated with phenotypic divergence rather than geographic isolation in the highly polymorphic strawberry poison-dart frog. *Mol Ecol* 19:447–458
- West-Eberhard MJ (1986) Alternative adaptations, speciation, and phylogeny. *Proc Natl Acad Sci USA* 83:1388–1392
- Whitlock R, Hipperson H, Mannerelli H, Butlin RK, Burke T (2008) An objective, rapid and reproducible method for scoring AFLP peak-height data that minimizes genotyping error. *Mol Ecol Res* 8:725–735