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Evidence of Hybridization between *Elaphe bairdi* and *Elaphe obsoleta lindheimeri* Including Comparative Population Genetics Inferred from Microsatellites and Mitochondrial DNA

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ABSTRACT.—Two species of Ratsnake (*Elaphe bairdi* and *Elaphe obsoleta*) inhabit different environments in Texas but come into contact with one another on the southwestern edge of the Edwards Plateau. Morphological intergrades have been described by previous studies, yet no genetic evidence of hybridization has ever been reported. We tested for evidence of hybridization and population structure using geographic data, mitochondrial DNA, and microsatellite markers. Cytochrome-*b* fragments were analyzed for 23 *E. bairdi* and 33 *Elaphe obsoleta lindheimeri* from Texas using maximum parsimony and Bayesian-based approaches. All individuals were subjected to Bayesian assignment probability tests based on six microsatellite loci. Overall, sequence divergence was low within *E. o. lindheimeri* (0.1–0.5%), and higher within *E. bairdi* (0.1–2.16%). Significant structuring was recovered from mitochondrial haplotypes but not from microsatellite genotypes. Phenotypic intergrades exhibited intermediate or incongruent species assignments based on their nuclear and mitochondrial genomes, suggesting admixture. However, gene flow between *E. bairdi* and *E. o. lindheimeri* remains strictly constrained to the zone of sympatry, and these separate lineages remain intact and independently evolving.

Baird's Ratsnake (*Elaphe bairdi*) and the Texas Ratsnake (*Elaphe obsoleta lindheimeri*) occur parapatrically along the southwestern edge of the Edwards Plateau in central Texas. During a survey of *E. bairdi*, Olson (1977) postulated that morphologically intermediate specimens were the result of hybridization between the two distinct species. Lawson and Lieb (1990) used morphology and allozymes to determine if intermediate specimens were the result of hybridization, but their data did not provide enough resolution to confirm admixture. Here, we seek to re-evaluate the possibility of hybridization within the contact zone using contemporary molecular tools to assess whether gene flow occurs between the two species and, if so, how frequently.

The use of molecular markers has become almost ubiquitous when inferring hybridization, gene flow, species relationships, and phylogeographic patterns (Zamudio et al., 1997; Burbrink, 2001; Gibbs et al., 2006). A considerable volume of work has been dedicated to addressing relationships among members of the family Colubridae (Utiger et al., 2002; Lawson et al., 2005; Burbrink and Lawson, 2007), and molecular data have contributed to several taxonomic revisions within this large group. Other classifications have been proposed for *E. bairdi* and *E. o. lindheimeri*, namely *Pantherophis bairdi* and *Pantherophis obsoletus* (Burbrink, 2001; Utiger et al., 2002) and *Scotophis bairdi* and *Scotophis obsoletus* (Collins and Taggart, 2008). We consider the nomenclature unresolved and will continue to refer to the species examined here by their classically accepted taxon names, *E. bairdi* and *E. o. lindheimeri* (Werler and Dixon, 2000). *Elaphe obsoleta lindheimeri* represents the western lineage of the *E. obsoleta* complex, which ranges from Louisiana to the western edge of the Edwards Plateau in south central Texas (Werler and Dixon, 2000). It inhabits areas with large, mature hardwood and pine forests where annual rainfall can vary between 40–120 cm and average daily temperatures range between 10–30°C. Within their range, elevation increases steadily from sea level near the Gulf of Mexico to 1,000 m above sea level on the Edwards Plateau. Texas Ratsnakes are semi-arboreal and prefer mature hardwood trees with cavities, which are used for shelter. The reasons for this behavior may

include predator avoidance, predation, and thermoregulation (Pierce et al., 2008). Studies of the ecology, dispersal, and gene flow of Ratsnakes have generally focused on *E. o. obsoleta* (Prior et al., 1997; Blouin-Demers and Weatherhead, 2002; Blouin-Demers et al., 2007), but little beyond taxonomy is known about the western lineage.

The range of Baird's Ratsnake extends from the western edge of the Edwards Plateau into parts of the Chihuahuan Desert in Texas and northern Mexico where the climate is more arid than central and eastern Texas. Temperatures do not vary significantly from those in the rest of Texas, yet average rainfall is less than 40 cm per year, resulting in a landscape dominated by shrubby vegetation. Changes in elevation are more dramatic in west Texas (500–1,500 m above sea level) owing to steep canyons and valleys, cliffs, and small mountain ranges. Few data regarding genetic variation, and nothing about the ecology or movement pattern, have been published on *E. bairdi* (Olson, 1977; Lawson and Lieb, 1990). This lack of biological information is partly due to their restricted range within rocky habitats and to their fossorial behavior, which limits human encounters in an already sparsely populated region (Lawson and Lieb, 1990). Sympatry between both species occurs within a narrow region on the southwestern edge of the Edwards Plateau where both rocky and forested habitats occur.

In this study, we sampled the mitochondrial and nuclear genomes to test for hybridization where *E. bairdi* and *E. o. lindheimeri* occur sympatrically. Cross-species amplification of microsatellite primers has proven useful in studying the population genetics of several other snake taxa (King, 2009). Therefore, we first tested previously published microsatellite primers (Blouin-Demers and Gibbs, 2003) for cross-species amplification, as well as designed, and tested novel mitochondrial primers for use in phylogenetic analyses. Second, we tested whether morphologically indistinct individuals genetically clustered with either species or intermediately. Finally, these same data were used to describe and compare the population genetics of each taxon.

MATERIALS AND METHODS

Sampling and Markers.—Whole blood, tail clips, or liver tissue was collected from wild-caught or museum-accessioned

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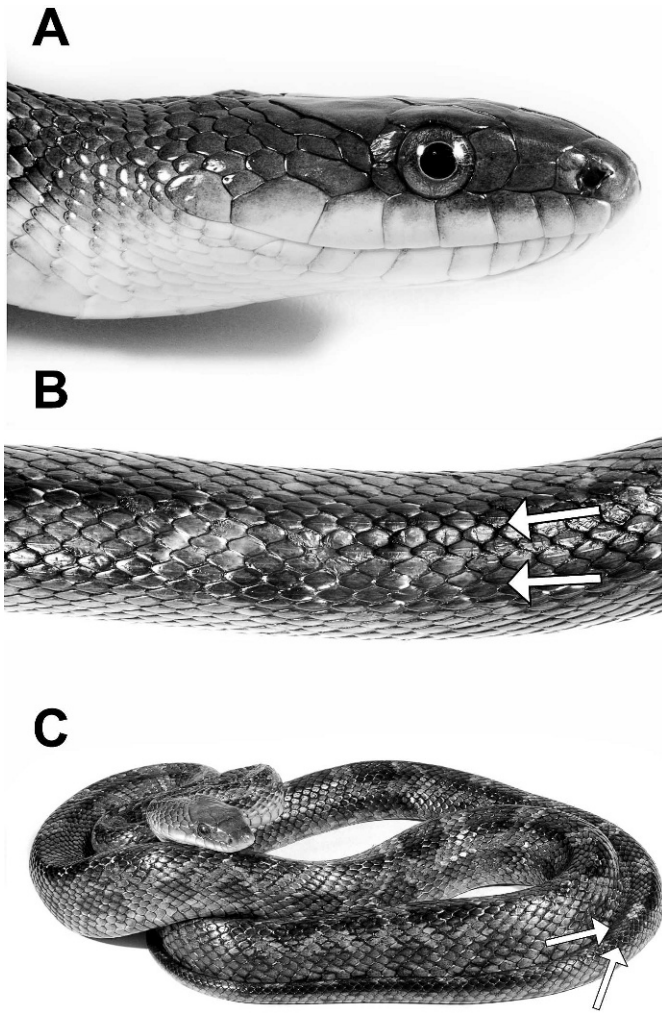


FIG. 1. An example of a specimen (RDZ264) exhibiting an integrated pattern. (A) Lateral labial view. (B) Arrows point to parallel stripes characteristic of adult *E. bairdi*. (C) Blotches are characteristic of *E. o. lindheimeri*; again, pronounced stripes are marked by arrows.

representatives of *E. o. lindheimeri* or *E. bairdi* from Texas. Individuals were identified to *E. o. lindheimeri* by standards presented in Burbrink (2001) or to *E. bairdi* by characters reported by Olson (1977). Individuals that exhibited characteristics of both species were considered anomalous (Fig. 1). Whole blood was collected via the caudal sinus using a 28½-gauge needle and a 1 cc syringe and was stored in cell lysis buffer (Longmire et al., 1997). Tail-clip or liver tissue was taken from deceased specimens and stored in 95% ethanol. Blood and tissue samples were stored at -20°C prior to DNA isolation. The PureGene® isolation kit (Gentra Systems, Minneapolis, MN) was used to isolate total genomic DNA (tDNA), which was electrophoresed on a 1.5% agarose gel and visualized with ethidium bromide under ultraviolet light.

Using the program Primer3 (Rozen and Skaletsky, 2000), oligonucleotide primers were designed based on mitochondrial DNA (mtDNA) data from *Elaphe slowinskii* (NC_009769). These novel primers (Table 1) were designed to amplify and sequence approximately 900 base pairs (bp) of the cytochrome-*b* (*cyt-b*) gene. Polymerase chain reaction (PCR) amplifications were performed in 12.5- μ l volumes using 0.2 μ l of tDNA (50 ng/ μ l), 8.98 μ l double-deionized H₂O (ddH₂O), 2.5 mL buffer (0.3 M TRIS, 0.0175 M MgCl₂, and 0.075 M [NH₄]₂SO₄), 0.5 μ l of 2.5 mM dNTPs, 0.13 μ l (10 mM) of forward primer, 0.13 μ l (10 mM) of reverse primer, and

TABLE 1. Primers used for both amplification and sequencing of *cyt-b* fragments.

Primer	Sequence
Elacytb01F	5'-GCCACAACACTTAAAAACCAAAAA-3'
Elacytb01R	5'-TTGATGCTCTAGGGGTGGAC-3'
Elacytbint02F	5'-CTCGCCTTTTCATCCATTGT-3'
Elacytbint02R	5'-AGATCGTATATGTGAGGTGTGTGT-3'
Ebair01R	5'-CGTTTTTATTAGGAAAAGAC-3'
Ebair02R	5'-TGGGTTGCTAGATCCTTCTG-3'
Ebair02F	5'-GATCCAACCTAACCCGATT-3'

0.0625 μ l (0.31 U) of Promega Taq polymerase (Promega Corp., Madison, WI). Thermocycling conditions for all primers consisted of an initial denaturation step of 2 min at 94°C, then 33 cycles of 30 sec at 94°C, 1 min at 58°C, 45 sec at 72°C, and a final extension of 7 min at 72°C. PCR products were prepared for cycle sequencing by adding 2 μ l of ExoSAP-IT® (USB Corporation, Cleveland, Ohio) per 8 μ l of product and incubated in a thermocycler at 37°C for 35 min and then at 80°C for 15 min. Cycle sequencing reactions were performed using Big Dye® v3.1 dye terminator (Applied Biosystems, Inc., Foster City, CA). Hydrated G-50 Sephadex™ (0.5 g of Sephadex/700 μ l ddH₂O) was incubated at room temperature for 30 min and centrifuged at 3,000 rpm for 2 min to construct Sephadex columns. Whole cycle sequence yields were then passed through the column to purify the products. Dried cycle-sequenced products were denatured in deionized formamide and electrophoresed on an ABI Prism® 3100-Avant genetic analyzer (Applied Biosystems). Chromatograms were trimmed and edited using Sequencher® 4.1.4 (Gene Codes Corp., Ann Arbor, MI), aligned using ClustalX (Thompson et al., 1997) and BioEdit 5.0.6 (Hall, 1999), and verified by eye. Newly generated sequences were accessioned into GenBank (GU073394-GU073447).

Six polymorphic microsatellite loci designed for *E. obsoleta* (Blouin-Demers and Gibbs, 2003) were used to build an allelic matrix for *E. o. lindheimeri* and *E. bairdi*. To further test for cross-species amplification, thirteen *Elaphe emoryi* were also screened at each locus. We modified each forward primer by adding an M13 sequence (CACGACGTTGTAAAACGAC) to the 5' end. A three-primer PCR method was used by adding fluorescent, dye-labeled M13 primers to the PCR reactions; this process labels amplicons for detection on a genetic analyzer (Schuelke, 2000). PCR conditions for all microsatellite reactions were the same for *cyt-b* reactions except that the volume of the forward primers was 0.07 μ l (10 mM), half the volume of the M13 forward primer, and the annealing temperature was set at 54°C (see above). Fragments were run on a CEQ™ 8000 genetic analyzer (Beckman Coulter, Inc., Fullerton, CA) and compared to a 400 bp size standard.

Phylogenetic Analyses.—Phylogenetic analyses of partial *cyt-b* sequences were performed using maximum parsimony (MP) and Bayesian inference (BI). A heuristic search was used to build a topology under the MP optimality criterion in PAUP*4.0b10 (Swofford, 2002). Starting trees were acquired by random stepwise addition (100 replicates) using the tree-bisection-reconnection (TBR) algorithm to swap branches. Node support was assessed by bootstrap resampling for 1,000 replicates. The best-fit model of evolution for the sequence data was estimated by MrModeltest (Nylander, 2004). A BI tree was constructed using MrBayes v3.1.2 (Huelsenbeck and Ronquist, 2001) under the model returned by MrModeltest. Four Markov chains were run for 4.0×10^6 iterations and the first 1.0×10^6 were discarded as burn-in. Additionally, a median joining haplotype network was constructed using Network 4.5.0.1 (Bandelt et al., 1999).

Population-based Analyses.—Cervus 3.0 (Marshall et al., 1998) was used to estimate basic measures of microsatellite variation for each species group. Genepop 3.4 (Raymond and Rousset,

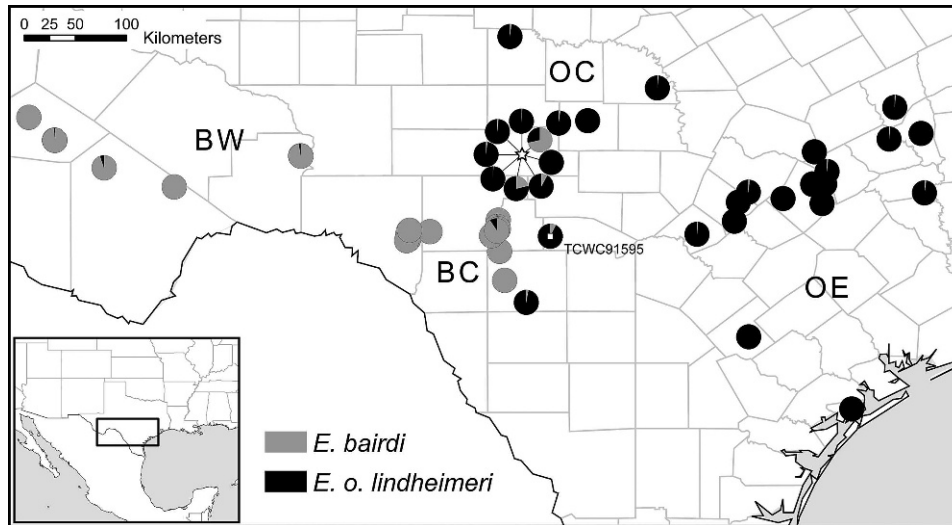


FIG. 2. A map of sampling localities and populations (BW = western *E. bairdi*, BC = central *E. bairdi*, OC = central *E. o. lindheimeri*, OE = eastern *E. o. lindheimeri*). Pie charts represent each individual's proportion of assignment to either species (see Fig. 6). Several individuals from Junction, Texas, denoted by a star, show higher levels of admixture.

1995) was used to test for Hardy-Weinberg equilibrium (Guo and Thompson, 1992) and linkage disequilibrium among pairs of loci. An exploratory neighbor-joining tree including all sampled genotypes of *E. bairdi* and *E. o. lindheimeri* was constructed in Populations v1.2.28 (Langella, 2002) from a matrix of Dc distances (Cavalli-Sforza and Edwards, 1967).

All members of *E. bairdi* and *E. o. lindheimeri*, except for TCWC91595 (see results), were pooled into subpopulations (BW = western *E. bairdi*, BC = central *E. bairdi*, OC = central *E. o. lindheimeri*, OE = eastern *E. o. lindheimeri*) based on relative ecoregion proximity (i.e., Chihuahuan Desert in the west, Edwards Plateau in central Texas, and the eastern coastal plains) (Fig. 2). Arlequin 3.1 (Excoffier et al., 2005) was utilized to assess variability within and among geographic populations of *E. bairdi* and *E. o. lindheimeri*, based on microsatellite and mitochondrial data, via an analysis of molecular variance (AMOVA). Pairwise F_{ST} values were also estimated for populations within and among species. A Mantel test was employed to test for a correlation between geographic and genetic distance in the program Alleles In Space (Miller, 2005). The significance of the correlation was determined after 100,000 permutations.

STRUCTURE (Pritchard et al., 2000) was used to test for population substructure using genotypic data. The optimum number of subpopulations (K) for both species was inferred by performing four independent runs of 1.0×10^6 generations, after an initial burn-in of 1.0×10^5 generations, for predefined values of K ranging from one to five. The average of four independent runs is presented for each value of K . An ad hoc statistic (ΔK) was used to infer the most appropriate K (Evanno et al., 2005).

Detection of Hybrids.—STRUCTURE was also used to detect levels of admixture by plotting Bayesian assignment probabilities for each individual ($q^{(i)}$) when K was constrained to two. Vähä and Primmer (2006) demonstrated that setting different threshold values of $q^{(i)}$ to designate pure parental species can affect the accuracy and efficiency of detecting hybrids. In a simulation study, STRUCTURE could efficiently ($\geq 95\%$) detect simulated F_1 hybrids using 12 loci with an overall F_{ST} of 0.21 between parental groups (Vähä and Primmer, 2006). We performed a STRUCTURE analysis (data not shown) using pure parental genotypes ($q^{(i)} > 0.98$), and simulated hybrid genotypes (F_1 , F_2 , and F_1 backcrosses to either species) generated in HYBRIDLAB (Neilsen et al., 2001) to assess the efficiency, accuracy, and overall performance of our data and the analytical parameters for detecting hybridization. Hybrids

were also identified by an incongruence between genotypic and haplotypic species assignments.

RESULTS

Phylogenetic Analyses.—An alignment of 834 bp of *cyt-b* was obtained after chromatograms were edited and trimmed. All sequences had an open reading frame without any internal stop codons, indels, or gaps; thus, we are confident that mitochondrial DNA was amplified and not nuclear pseudogenes. MrModeltest indicated the best model of nucleotide substitution was GTR+G for BI. Each species fell distinctly into their respective clades with strong support (Fig. 3A). In 33 sampled *E. o. lindheimeri*, ten sites were found to be polymorphic and ten unique haplotypes (o1–o10) were recovered, the majority of which clustered into two haplogroups. Within the 23 *E. bairdi*, 26 sites were found to be variable and 12 unique haplotypes (b1–b12) were identified (Fig. 3B). Average percent divergence between both species was 7.23% (6.60–7.67%). Average sequence divergence between *E. o. lindheimeri* populations was 0.21% (0.1–0.5%) and was 1.23% (0.1–2.16%) between *E. bairdi* populations. Haplotype frequencies within populations are represented in Figure 4.

Microsatellite Analyses.—Measures of microsatellite variation, including observed and expected heterozygosities of each species, are shown in Table 2. The average number of alleles per locus was 5.17 and 5.67 in *E. bairdi* and *E. o. lindheimeri*, respectively. All loci were successfully amplified in *E. emoryi* and averaged 9.30 alleles per locus. Hardy-Weinberg (HW) exact probability tests indicated that Eob μ 1 in *E. bairdi* and Eob μ 3 in both species were not in HW equilibrium. Following a Bonferroni correction, no pairwise tests for linkage disequilibrium were significant. The exploratory neighbor-joining tree based on Dc distances returned two species clades (Fig. 5). TCWC91595 carried an *E. bairdi* haplotype but fell within the *E. o. lindheimeri* genotypic clade and RDZ264 clustered intermediately between the two species groups.

Population-based Analyses.—Interspecific and intraspecific pairwise F_{ST} estimates are shown in Table 3. Only interspecific F_{ST} values were found to be significant for microsatellites while all mtDNA F_{ST} estimates were statistically significant. Based on microsatellites, the highest degree of pairwise population differentiation was found between populations BC and OE ($F_{ST} = 0.32$) and likewise for mtDNA data ($F_{ST} = 0.97$). A high

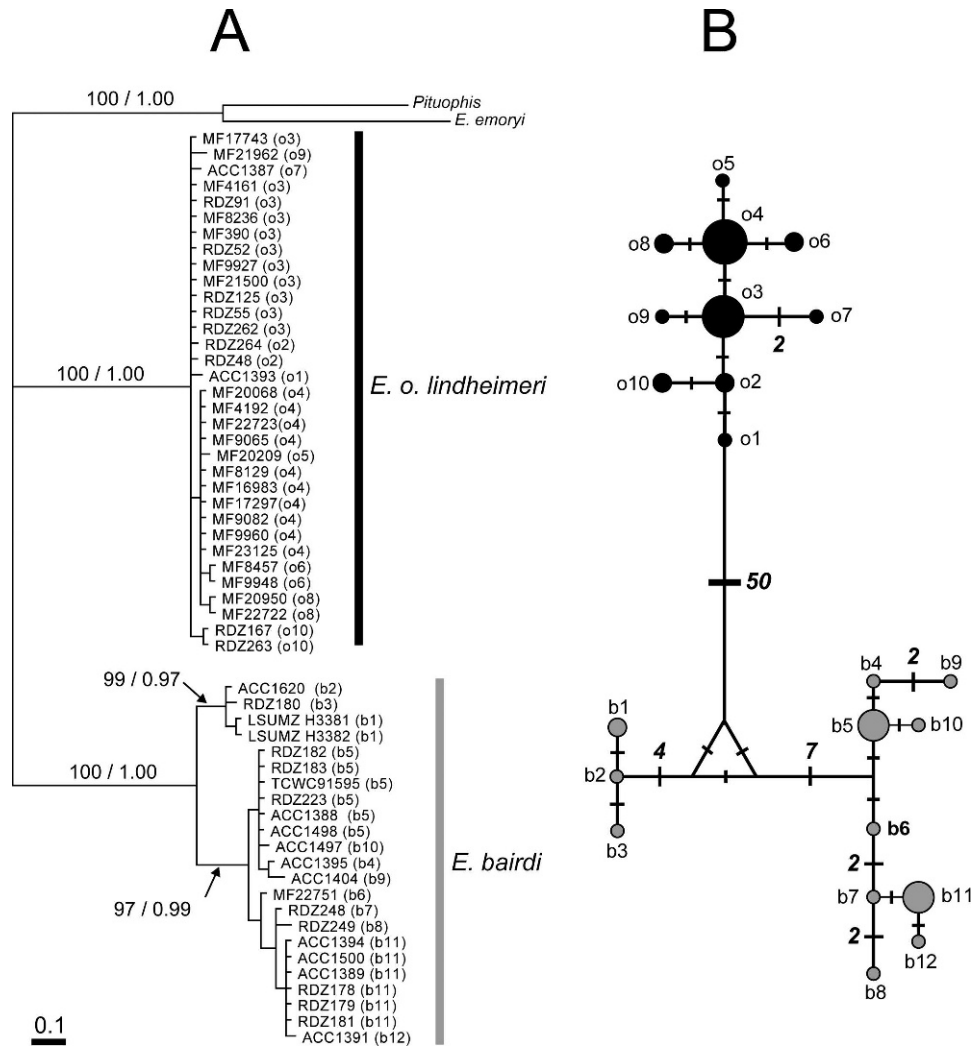


FIG. 3. (A) Cyt-*b* phylogenetic tree of all individuals used in the study with support values (MP/BI). Corresponding haplotypes are in parentheses next to the sample name. (B) Median joining haplotype network using the same sequence data. Each circle represents one haplotype and the size of the circle represents the frequency of haplotypes. Changes between haplotypes are connected by lines, where one cross-bar represents one base pair mutation, unless otherwise stated. Each distinct haplotype is labeled by species (*b* = *E. bairdi* and *o* = *E. o. lindheimeri*). In both analyses, *E. bairdi* (gray) individuals partition into two groups and *E. o. lindheimeri* (black) form one group separate and distinct from *E. bairdi*.

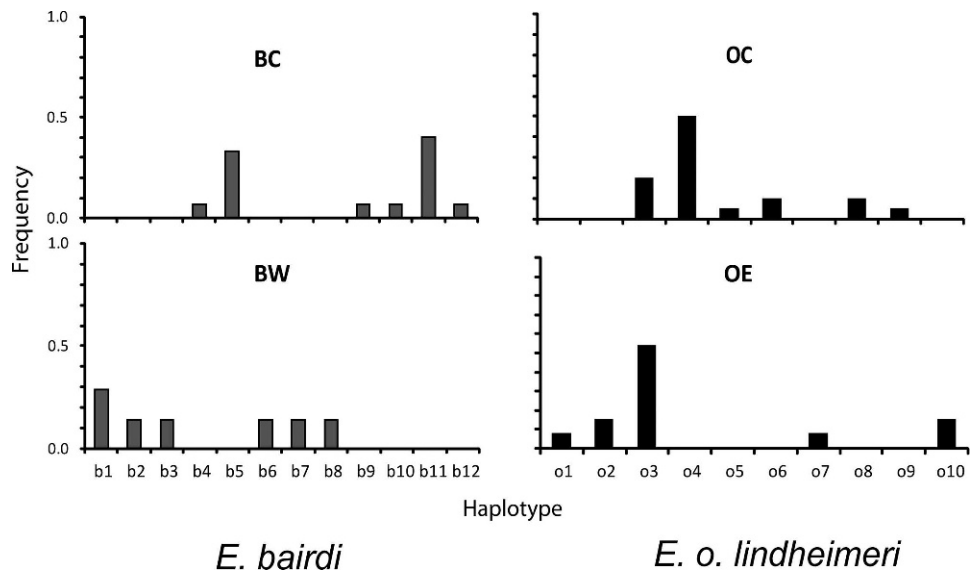


FIG. 4. Bar graphs of haplotype frequency for each sampled population of *Elaphe* from Texas (see Fig. 3B).

TABLE 2. Microsatellite loci designed for *E. o. obsoleta* that amplified and exhibited polymorphism in three species of Ratsnake from Texas, United States. The number of individuals sampled per locus (N), number of alleles per locus (A), observed (H_O) and expected (H_E) heterozygosity, and polymorphic information content (PIC) are listed for each species.

Locus	<i>E. bairdi</i>					<i>E. o. lindheimeri</i>					<i>E. emoryi</i>				
	N	A	H_O	H_E	PIC	N	A	H_O	H_E	PIC	N	A	H_O	H_E	PIC
Eob μ 1	23	9	0.43	0.68	0.63	33	7	0.77	0.78	0.73	13	8	0.85	0.85	0.80
Eob μ 3	22	7	0.45	0.77	0.72	33	5	0.19	0.58	0.51	13	10	0.54	0.87	0.82
Eob μ 4	22	2	0.05	0.05	0.05	33	11	0.74	0.79	0.75	13	14	0.62	0.95	0.90
Eob μ 13	23	1	0.00	0.00	0.00	33	2	0.45	0.39	0.31	13	7	0.69	0.69	0.64
Eob μ 358	22	3	0.35	0.42	0.35	31	1	0.00	0.00	0.00	13	2	0.25	0.34	0.28
Eob μ 366	23	9	0.62	0.86	0.82	33	8	0.68	0.68	0.77	13	15	0.85	0.95	0.91
Mean		5.17	0.32	0.46	0.43		5.67	0.47	0.54	0.51		9.30	0.63	0.78	0.72

degree of overall population differentiation was found in *E. bairdi* (mtDNA, $F_{ST} = 0.44$), although an AMOVA using microsatellite data was not significant. Based on mtDNA, *E. bairdi* exhibited greater variation among populations than did *E. o. lindheimeri* (42.9% vs. 37.3%) (Table 4). The Mantel test revealed a significant positive correlation between genetic distance (mtDNA) and geographic distance for both *E. bairdi* ($r = 0.80$, $P < 0.01$) and *E. o. lindheimeri* ($r = 0.35$, $P < 0.01$), consistent with an isolation by distance model. *Elaphe obsoleta lindheimeri* also showed a slight correlation ($r = 0.12$, $P = 0.01$) between geographic distance and genetic distance based on microsatellites. Measures of ΔK ($\Delta K_{K=2} = 2362$, $\Delta K_{K=3} = 251$, $\Delta K_{K=3} = 3$) estimated using log likelihoods from the four independent runs suggested that two genetic demes were present when all *E. bairdi* and *E. o. lindheimeri* genotypes were pooled. The two genetic demes corresponded to the mitochondrial species assignments (Fig. 6). STRUCTURE analyses revealed no significant structuring within species (data not shown).

Detection of Hybrids.—In our simulated data, we set a threshold $q^{(i)}$ value of 0.95 for each species. Our efficiency and accuracy at detecting hybrids was 0.88 and 0.81, respectively, with an overall performance measure of 0.71. In the experimental dataset we detected five admixed individuals (TCWC91595,

RDZ262, RDZ264, RDZ223, and ACC1497) when the $q^{(i)}$ threshold was set to 0.95, $K = 2$, six microsatellite loci were sampled, and parental species had a pairwise F_{ST} of 0.31. Among these individuals, TCWC91595, RDZ264, and RDZ223 were considered morphologically anomalous.

DISCUSSION

Our results from analyses of mitochondrial and nuclear data from *E. bairdi* and *E. o. lindheimeri* further support the presence of two distinct evolutionary lineages. Our chosen markers were sensitive enough to identify fixed nuclear differences and admixture where the two species overlap. To better estimate how frequently admixture occurs will require more-detailed surveys in areas of sympatry; however, we have presented a method to accurately detect hybridization between these two closely related taxa. Analyses of microsatellite data revealed little population subdivision among geographic sites within each species although, using *cyt-b* sequences, we were able to detect phylogeographic patterns suggesting isolation by distance.

Genetic Variation.—The number of microsatellite loci sampled, and the observed heterozygosity among other colubrids, ranges from 1–16 and 0.28–0.80, respectively (King, 2009). Our measures fell within these ranges, although observed heterozygosity in *E. bairdi* ($H_O = 0.32$) was lower than most other colubrid species to date (King, 2009). Interestingly, we sampled fewer *E. bairdi* than *E. o. lindheimeri*, but more genetic variation and a stronger correlation between geographic and genetic distance were detected from the mtDNA of *E. bairdi*. Two well-supported mitochondrial clades were partitioned into eastern and western lineages of *E. bairdi*, which contributed to stronger measures of population differentiation and isolation by distance. Population variation within a species is correlated to dispersal capabilities (Bohonak, 1999). Our data seem to suggest that *E. bairdi* has limited dispersal in comparison to *E. o. lindheimeri*, which could occur for a number of reasons. For instance, the geographic terrain representing *E. bairdi* habitat is composed of high cliffs, canyons, and rocky outcrops with an arid climate and less available water. It is possible that difficult terrain may be preventing widespread dispersal within the species or that limited resources may dissuade movement outside a small home range, causing an increase in haplotype

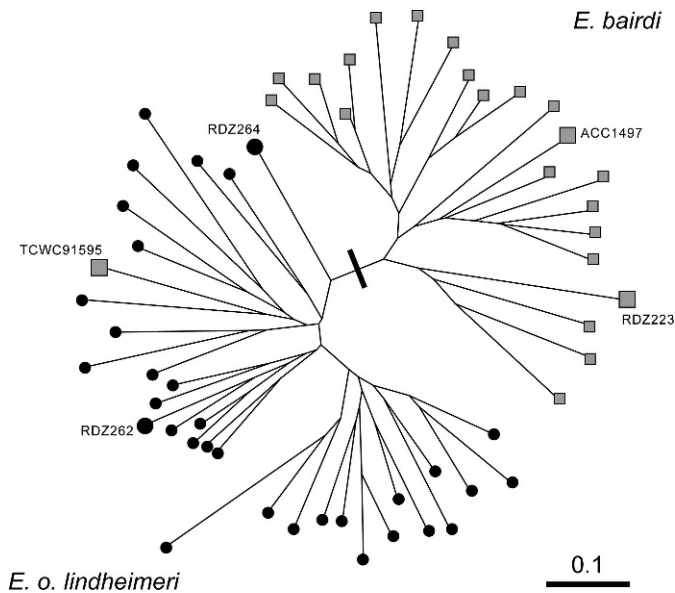


FIG. 5. A distance-based tree (Dc) generated using microsatellite genotypes. Gray squares denote individuals that carried *E. bairdi* haplotypes (*cyt-b*) and individuals with black circles carried *E. o. lindheimeri* haplotypes. Individuals of particular interest are labeled with larger icons; TCWC91595 had incongruent mtDNA and microsatellite genetic assignments. RDZ262, RDZ223, ACC1497, and RDZ264 were identified as admixed in a model-based analysis of the same genotypes (see Fig. 6).

TABLE 3. Pairwise F_{ST} values for geographic groups within *E. bairdi* (BW and BC) and *E. o. lindheimeri* (OC and OE) based on mtDNA (upper diagonal) and microsatellite (lower diagonal) data (see Fig. 2). Asterisks indicate significance values ($P < 0.01$).

	BW	BC	OC	OE
BW	–	0.44*	0.92*	0.94*
BC	0.02	–	0.95*	0.97*
OC	0.25*	0.28*	–	0.35*
OE	0.30*	0.32*	0.02	–

TABLE 4. Results for an AMOVA based on mitochondrial and microsatellite data for two species of *Elaphe* from Texas, United States. df = degrees of freedom. Asterisks indicate significance values ($P < 0.01$).

	<i>E. bairdi</i>		<i>E. o. lindheimeri</i>	
	df	Variation %	df	Variation %
mtDNA				
Among groups	1	42.9*	1	37.3*
Within groups	19	57.1*	29	62.7*
Microsatellites				
Among groups	1	2.8	1	3.2
Within groups	40	97.2	60	96.8

variation. Female philopatry can also lead to a similar scenario, but previous studies have reported no significant differences between the dispersal capabilities of males and females in a phylogenetically similar species (Blouin-Demers and Weatherhead, 2002; Blouin-Demers et al., 2007). Nonetheless, *E. bairdi* inhabits a demanding habitat where radio telemetry and field observations, in conjunction with landscape genetics, could provide a better understanding of their behavior.

Studies using microsatellites and RAPDs have both demonstrated hierarchical patterns of population structure among *E. o. obsoleta* hibernacula (Prior et al., 1997; Loughheed et al., 1999). In our study, the genotypic pairwise population F_{ST} and the output from STRUCTURE suggest there is little, if any, underlying population subdivision within either species. However, this result could be an artifact of limited sampling, given the results from the mitochondrial data, which suggest there is significant population subdivision within both species as well as significant correlations between geographic and genetic distance.

Hybridization.—Microsatellites and Bayesian assignment tests have proven effective in detecting hybridization events in several reptiles including marine turtles (Lara-Ruiz et al., 2006), crocodiles (Rodriguez et al., 2008; Weaver et al., 2008), lizards (Leaché and Cole, 2007), and other members among the *Elaphe* species complex (Gibbs et al., 2006). Lawson and Lieb

(1990) were unable to find fixed allozyme allele differences between *E. bairdi* and *E. o. lindheimeri* and, thus, could not make any strong conclusions regarding hybridization. On the contrary, we were able to resolve and define species groups using microsatellite alleles and detected admixture in 8.9% of the sampled population. No individuals with $q^{(i)}$ values less than 0.95 were found outside of the contact zone (see Fig. 2), and most admixed individuals were collected near Junction, Texas.

Based on microsatellite data, RDZ262 and ACC1497 exhibited some evidence of genetic admixture according to the Bayesian analysis but not when using Dc distances. Stronger evidence for hybridization was found in anomalous individuals RDZ223, TCWC91595, and RDZ264. For instance, RDZ223 had the lowest assignment probability in the data set ($q^{(i)} = 0.71$ to *E. bairdi*) and RDZ264 had the next-lowest assignment probability ($q^{(i)} = 0.79$ to *E. o. lindheimeri*) and was intermediately positioned between species clades in the Dc distance phylogeny. TCWC91595 displayed a high assignment probability to *E. o. lindheimeri* ($q^{(i)} = 0.92$) but carried a haplotype typical of *E. bairdi*. We interpreted the incongruence between two genetic data sets and an admixed nuclear genome as strong evidence for hybridization.

When hybridization is bimodal, hybrids occur where parent species exist sympatrically but the retention of hybrid genotypes does not occur outside of the overlapping zone (Fitzpatrick et al., 2008). The hybrids we detected exhibited varying degrees of admixture, implying that F_1 hybrids are not sterile and several generations of backcrossing have occurred in the zone of sympatry. In Junction, Texas, we found individuals assigned to *E. o. lindheimeri* with a high probability ($q^{(i)} > 0.95$) along with hybrids, which suggests hybridization events in this area are likely to be infrequent. Gene flow in *E. o. obsoleta* from eastern Ontario has been shown to diminish over a geographic range of 15–50 km, which contributes to an isolation by distance pattern (Loughheed et al., 1999). Therefore, because each species retains their own distinct lineage in and out of the contact zone, we propose hybridization is a localized event and that the effects of hybridization cause little disturbance in the gene pools of either species.

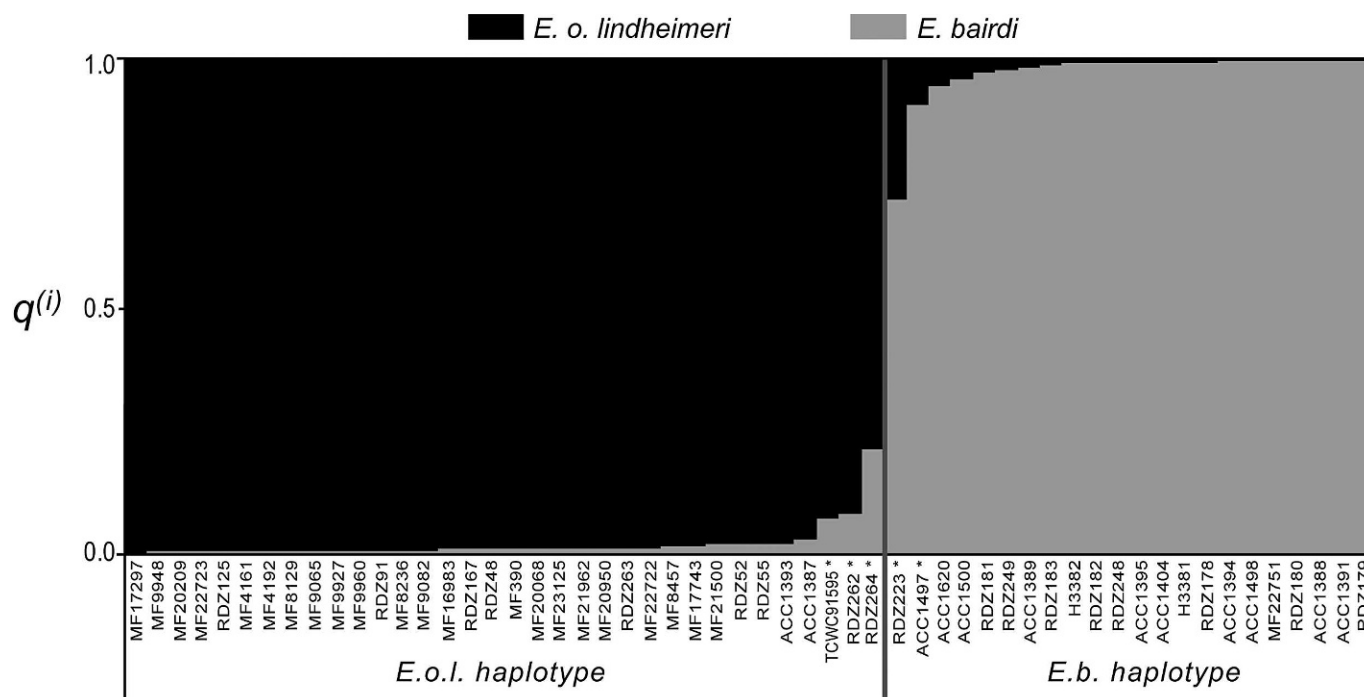


FIG. 6. Averaged assignment probabilities to either *E. o. lindheimeri* or *E. bairdi* from STRUCTURE when $K = 2$; individuals are grouped by their mitochondrial haplotype (*cyt-b*). Admixed individuals are designated with asterisks after their sample label.

In summary, our work furthers the phylogenetic and population genetics understanding of the genus *Elaphe* and addresses the lack of published genetic information for *E. bairdi*. We found the markers developed by Blouin-Demers and Gibbs (2003) were polymorphic in *E. bairdi*, *E. o. lindheimeri*, and *E. emoryi* and will be useful for subsequent evolutionary inferences in New World *Elaphe*. Finally, these markers, in conjunction with mtDNA sequences, successfully detected hybrids between two independent evolutionary lineages.

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