The spatial ecology of Mojave rattlesnakes (Crotalus scutulatus), prairie rattlesnakes (Crotalus viridis), and their hybrids in southwestern New Mexico

Article *in* Biological Journal of the Linnean Society · March 2024

DOI: 10.1093/biolinnean/blae037

CITATION
1

READS
262

5 authors, including:

Dylan Wallace Maag
San Diego State University
9 PUBLICATIONS **26** CITATIONS
SEE PROFILE

Todd Castoe
University of Texas at Arlington
450 PUBLICATIONS **10,975** CITATIONS
SEE PROFILE

Gordon W Schuett
Georgia State University
255 PUBLICATIONS **4,143** CITATIONS
SEE PROFILE

Rulon W Clark
San Diego State University
121 PUBLICATIONS **2,184** CITATIONS
SEE PROFILE

Biological Journal of the Linnean Society, 2024, **XX**, 1–15
https://doi.org/10.1093/biolinnean/blae037
Advance access publication 25 March 2024
Original Article

OXFORD

Original Article

The spatial ecology of Mojave rattlesnakes (*Crotalus scutulatus*), prairie rattlesnakes (*Crotalus viridis*), and their hybrids in southwestern New Mexico

Dylan W. Maag[1,2,3,*], Yannick Z. Francioli[4], Todd A. Castoe[4], Gordon W. Schuett[5,6] and Rulon W. Clark[1,6]

[1]Department of Biology, San Diego State University, San Diego, CA, USA
[2]Department of Evolution, Ecology, and Organismal Biology, University of California, Riverside, Riverside, CA, USA
[3]Department of Life, Earth, and Environmental Sciences, West Texas A&M University, Canyon, TX, USA
[4]Department of Biology, University of Texas at Arlington, Arlington, TX, USA
[5]Department of Biology | Neuroscience Institute, Georgia State University, Atlanta, GA, USA
[6]Chiricahua Desert Museum, Rodeo, NM, USA

[*]Corresponding author. Department of Biology, San Diego State University, San Diego, CA, USA. E-mail: dmaag@wtamu.edu or dr.dylan.maag@gmail.com

ABSTRACT

Hybridization between species provides unique opportunities to understand evolutionary processes that are linked to reproductive isolation and, ultimately, speciation. However, the extrinsic factors that limit hybridization are poorly understood for most animal systems. Although the spatial ecology of individuals in natural habitats is fundamental to shaping reproductive success and survival, analyses of the spatial ecology of hybrids and their parental groups are rarely reported. Here, we used radiotelemetry to monitor wild rattlesnakes across an interspecific hybrid zone (*Crotalus scutulatus* and *Crotalus viridis*) and measured movement parameters and space use (utilization distributions) of individuals to evaluate the hypothesis that hybridization resulted in transgressive or atypical movement patterns. Unexpectedly, of the spatial metrics we investigated, we found that hybrids were very similar to parental individuals. Nonetheless, hybrids did show increased patchiness of core utilization distributions, but this result is likely to be driven by increased habitat patchiness in the hybrid zone. Overall, we did not find evidence for overt extrinsic barriers to hybridization associated with spatial ecology; thus, we suggest that the close evolutionary history between the two parental species and their ecological and behavioural similarities are likely to increase the probability of hybridization events in this unique region of New Mexico.

Keywords: pitvipers; hybridization; radiotelemetry; telemetry; space use; movements; home range

INTRODUCTION

Although the concept of species is central to our understanding of biological organization, it has been notoriously difficult for biologists to reach consensus on theory and operational standards for what constitutes a species (de Queiroz 2007). Many examples have been documented where individuals from clearly divergent and well-defined species will still breed and produce viable offspring in some portion of their range. Hybridization between divergent lineages has been a topic of interest in the field of evolution since the process of natural selection was first recognized (Darwin 1859). Regions where interspecific hybridization occur have been used increasingly as case studies to understand the general processes associated with reproductive isolation, introgression, and speciation (Harrison and Larson 2014). Understanding behavioural variation of individuals with mixed ancestry (i.e. hybrids) is key to determining why particular hybrid zones exist and to predicting how they might shift in response to future changes in climate, habitat availability, and other natural or man-made disturbances (Rosenthal 2013, Chunco 2014).

Most behavioural studies of hybridization, particularly in amphibians and reptiles, focus on taxa consensus with significant prezygotic isolating barriers (e.g. Brown 1971, Doherty and Gerhardt 1984, Abt and Reyer 1993, Malmos *et al.* 2001, Smadja

Received 10 October 2023; revised 10 January 2024; accepted 26 February 2024

et al. 2004, Pfennig 2007, Maroja *et al.* 2009, Melo *et al.* 2009, Kozak and Boughman 2015, Feller *et al.* 2016). Many of these species have sexually selected traits that are expressed differentially across the hybrid zone and that strongly affect mate choice and reproduction. However, hybridization also occurs between species that appear to have weak prezygotic isolating mechanisms, and in many of these systems hybridization events remain constrained to a narrow geographical area, with limited introgression of alleles across the zone, indicating that postzygotic isolating mechanisms are likely to be impacting the survival or reproduction of individuals with hybrid genotypes.

Historically, research on traits contributing to postzygotic isolation of lineages has been heavily focused on hybrid inviability (e.g. Moore 1951, Nurnberger *et al.* 1995, Fitzpatrick 2004), physiological sterility (e.g. Volpe 1960, Peterson *et al.* 2005, Jančúchová-Lásková *et al.* 2015), or other intrinsic traits that decrease the fitness of hybrid individuals (reviewed by Coyne and Orr 2004). Thus, we still have a limited understanding of how extrinsic barriers (which stem from how hybrids interact with their environment, conspecifics, and heterospecific individuals) shape hybridization dynamics. In part, this bias might stem from the logistical problems involved with measuring traits in hybrids that contribute to extrinsic barriers, because such traits must be examined *in situ* to understand how they correspond to survival and reproductive success. Quantifying spatial ecology, foraging ecology, reproductive behaviour, and predation in nature all require that individuals are monitored intensely for prolonged time periods, especially in long-lived vertebrates (Reinert 1992, Bushar *et al.* 1998, Brown 2008, Durbian *et al.* 2008, Clark 2016). For many species, this usually requires some combination of radio/GPS tracking, long-term mark–recapture sampling, and detailed behavioural observations of individuals, all of which involve significant investments of time and resources. Overcoming these logistical hurdles is necessary to evaluate the potential extrinsic barriers to hybridization.

Given that hybridization involves unique admixtures of parental genotypes (Barton 2001, Mallet 2007, Rieseberg *et al.* 2007), hybrids often express more novel or extreme (transgressive) phenotypes when compared with parental populations (Rieseberg *et al.* 1999, Stelkens *et al.* 2009, Harrison and Larson 2014). Depending on how trait expression is influenced by the environment, these transgressive or abnormal phenotypes can limit further backcrossing into parental populations, hence contributing to the isolation of lineages involved in hybridization. Because mating (and thus backcrossing) in most animal species involves synchronization of key behaviours in space and time, individual-level variation in movement and space use could be a major driver of that variation in resource use and reproduction that ultimately would shape patterns of hybridization across the zone of admixture. Findings to date on the spatial ecology of hybrids vary depending on the study system in question. For example, a study of wolves (*Canis lupus* and *Canis lycaon*), coyotes (*Canis latrans*), and their hybrids revealed that social groups of the different canid types had spatially segregated home ranges (Benson and Patterson 2013). Although a similar pattern of spatial segregation was reported in two species of woodrats (*Neotoma bryanti* and *Neotoma lepida*) and their hybrids, segregation was sex specific, with females exhibiting more separation by genetic group than males (Shurtliff *et al.* 2013). Hybrid groups also can become increasingly isolated owing to increases in variability of movements. Hybrid Swanson's thrushes (*Catharus ustulatus ustulatus* and *Catharus ustulatus swainsoni*), for example, had increased variability in migratory pathways and locations of overwintering sites compared with individuals of either of the parental populations. These intermediate travel routes and overwintering sites were seemingly less optimal than those used by parental individuals, thus resulting in decreased fitness of the hybrids (an extrinsic barrier; Delmore and Irwin 2014). Conversely, if hybrid and parental individuals exhibit overlap in use of spatial and temporal resources, subsequent gene flow and hybridization between the two parental species might be enhanced. Austin *et al.* (2019) found that hybrids between Balearic shearwaters (*Puffinus mauretanicus*) and Yelkouan shearwaters (*Puffinus yelkouan*) shared foraging areas with Yelkouan shearwaters during the breeding season, which presumably led to further backcrossing between them.

These handful of case studies indicate that a detailed understanding of the spatial ecology of individuals across a hybrid zone provides important and unique insights into traits that might influence extrinsic barriers to further hybridization. Despite this, relevant data are lacking for many prospective experimental systems, particularly in cryptic or secretive species that can be difficult to observe in nature. Species for which researchers have developed detailed methodological approaches for quantifying movement patterns and space use would be particularly important for addressing this shortcoming.

A robust methodological and comparative literature on spatial ecology has developed over the previous decades for North American pitvipers (Serpentes: Crotalinae) (e.g. Reinert and Zappalorti 1988, Dreslik 2005, Waldron *et al.* 2006, Cardwell 2008, Greenberg and McClintock 2008, Hayes *et al.* 2008, Roth 2009, Smith *et al.* 2009, Hoss *et al.* 2010, DeGregorio *et al.* 2011, Davis *et al.* 2015, Maag *et al.* 2022). Pitvipers are also unique among vertebrates in that many taxa exhibit fairly high levels of hybridization and interspecific gene flow, with few obvious pre-mating barriers (Zancolli *et al.* 2016, Schield *et al.* 2018, 2019, Myers 2021, Nikolakis *et al.* 2022, Roldán-Padrón *et al.* 2022). Generally, crotaline snakes are sit-and-wait ambush hunters of small mammals and lizards that move infrequently over relatively short distances (reviewed by Nowak *et al.* 2008). These movements are typically between ambush sites that offer shelter (Cardwell 2013, Gardiner *et al.* 2015, Maag *et al.* 2022) and hunting opportunities (reviewed by Maag and Clark 2022). Movements during their active season (i.e. when not overwintering) are often partitioned by multiple days of inactivity (DeSantis *et al.* 2020). Thus, the home range sizes of these snakes tend to be relatively small, yet variable. Differences between the sexes are common, with males typically exhibiting larger home ranges owing to their larger body size and long-distance movements made during breeding seasons (Duvall *et al.* 1992, Duvall and Schuett 1997, Cardwell 2008, DeGregorio *et al.* 2011, Bailey *et al.* 2012). However, non-pregnant females are known to exhibit similar patterns of movement and space use to males in certain populations (e.g. Reinert and Zappalorti 1988, King and Duvall 1990, Prival *et al.* 2002, Kingsbury *et al.* 2003, Nowak 2005, Hamilton 2009, Tozetti *et al.* 2009, Shipley *et al.* 2013, Smith 2013, Patten *et al.* 2016, Dreslik *et al.* 2017). Pregnant females typically move short

distances (Fitch and Shirer 1971, Hamilton 2009, DeGregorio *et al.* 2011, Wastell and MacKessy 2011, Shipley *et al.* 2013, Maag *et al.* 2022), although movement varies from species to species (e.g. Schuett *et al.* 2013), and they prioritize sites for optimal thermoregulation of fetuses (Johnson 1995, Crane and Greene 2008). The increased movement of adult males during the breeding season is a reproductive strategy to locate receptive females (Duvall *et al.* 1992, Aldridge 1993, Holycross 1995, Duvall and Schuett 1997, Schuett *et al.* 2002). Both males and females are active on the surface only during non-winter months (late spring to late autumn, depending on the species and population) and typically return to a winter shelter (either communally or individually) during colder months.

Both Mojave rattlesnakes (*Crotalus scutulatus*) and prairie rattlesnakes (*Crotalus viridis*) generally follow the typical pattern of pitviper spatial ecology, with a few exceptions. Although *Crotalus viridis* have been studied more intensively than *Crotalus scutulatus*, most of this work has been focused on northern and high-elevation populations of *Crotalus viridis*, which might exhibit very different patterns of seasonal migration (King and Duvall 1990, Jorgenson *et al.* 2008, Chiszar *et al.* 2014, Martin *et al.* 2017). Northern populations of *Crotalus viridis* exhibit variable home range sizes (0.19–31.4 ha; Macartney *et al.* 1988, Shipley *et al.* 2013) and are known for their long (≤20 km; Jorgenson *et al.* 2008) migrations to and from winter shelters (Duvall *et al.* 1990, King and Duvall 1990, Chiszar *et al.* 2014). The much more limited data on *Crotalus scutulatus* indicate that they are likely to have similar home range sizes (2–52.6 ha; Cardwell 2008), but do not exhibit long migrations to and from winter shelters. Both species are known to mate primarily in late summer (Aldridge 1993, Schuett *et al.* 2002), with an increase in male movements typically coinciding with the late summer monsoonal period in the southwest.

Crotaline snakes have long been known to hybridize between species, and several examples of naturally occurring hybrid rattlesnakes have been documented (Bailey 1942, Campbell *et al.* 1989, Meik *et al.* 2008, Montgomery *et al.* 2013). Although signatures of interspecific hybridization have been found in *Crotalus scutulatus* (Schield *et al.* 2018) and *Crotalus viridis* + *Crotalus oreganus* complexes (Schield *et al.* 2019, Nikolakis *et al.* 2022), and extrinsic isolation mechanisms are likely in the *Crotalus viridis* + *Crotalus oreganus* hybrid zone (Nikolakis *et al.* 2022), no previous studies have yet quantified the basic spatial ecology of individuals across these zones. Differences in the extent or frequency of individual movements could have fundamental implications for resource use, reproductive behaviour, and ultimately, reproductive success. Infrequent or short-distance movements could be insufficient for locating suitable refugia or prey, and more limited movements or abnormal timing of movements during the breeding season would negatively impact male reproductive success by limiting their potential mating encounters. Additionally, unsuitable patterns of movement could leave snakes vulnerable to their own predators. Rattlesnakes are known to be predated by multiple species of carnivorous mammals and birds (Klauber 1956); however, a recent study found that they are very rarely preyed upon when hunting in ambush, and thus must be most vulnerable to predation when exposed and moving through the landscape or engaging in reproductive behaviour (Maag and Clark 2022).

Here, we used established telemetry methods (Reinert 1992) to quantify the spatial ecology of individuals across the *Crotalus scutulatus* and *Crotalus viridis* hybrid zone in southwestern New Mexico, USA (Zancolli *et al.* 2016, Maag *et al.* 2023). Specifically, we quantified daily movement distances (DMDs), movement frequencies (FMs), and the pattern of space use during the active season in relationship to seasonal breeding activity and analysed these spatial behavioural patterns in relationship to hybrid ancestry. We hypothesized that hybrid individuals would display transgressive or abnormal patterns of movements and spatial distribution in comparison to individuals of parental species. We thus predicted that hybrids would show shorter and less frequent movements, with smaller utilization distributions (UDs) or more aseasonal mate-searching movements than parental snakes, potentially contributing to extrinsic barriers to further hybridization (Zancolli *et al.* 2016). Hybrid males with these traits would be expected to be less successful at finding females, further contributing to an extrinsic barrier limiting the geographical extent of the hybrid zone.

MATERIALS AND METHODS

Study sites

The hybrid zone is located within the Cochise filter barrier, a transitional region between the Chihuahuan and Sonoran deserts in the southwestern USA frequently implicated in lineage divergence, with climatic and vegetation community shifts induced by glacial cycling (Van Devender *et al.* 1984). Because there is not a major physical barrier separating the two deserts, the Cochise filter barrier is a region of frequent gene flow and hybridization between genetic groups of organisms (Castoe *et al.* 2007, Pyron and Burbrink 2010).

The Cochise filter barrier hybrid zone between *Crotalus scutulatus* and *Crotalus viridis* occupies a valley between two mountain ranges in the extreme southwest of New Mexico, USA (Fig. 1). The valley contains isolated homesteads with various amounts of active pasture/agricultural land. Although a forthcoming manuscript will present detailed habitat analyses in the context of microhabitat preferences of snakes, we provide general macrohabitat details here, as they relate broadly to patterns of space use we analyse. Hybrid snakes in this region are found in a narrow band (~12 km) of transitional/mosaic habitat in the centre of the valley, with parental populations located on either side of the bordering mountain ranges (Zancolli *et al.* 2016, Maag *et al.* 2023). On the southwestern side of the hybrid zone, the *Crotalus scutulatus* site (31.891703°N, 109.034757°W) is characterized as a lowland scrub desert macrohabitat consisting of large continuous stands of mesquite (*Prosopis glandulosa*) and patches of tuft grasses. *Crotalus viridis* occurs northeast of the hybrid zone (*Crotalus viridis*; 32.259056°N, 108.844943°W). This site is dominated by short-grass prairie habitat type, with similar plant species to the *Crotalus scutulatus* habitat except that the mesquite is less common. Within the hybrid zone (32.152532°N, 108.914127°W), in the middle of the valley, the macrohabitat transitions from a creosote (*Larrea tridentata*)-dominated lowland desert to an arid short-grass prairie, similar to the *Crotalus viridis* habitat. However, scattered throughout the hybrid zone are large patches of barren soil that are devoid of almost all vegetation, mammal burrows, and woodrat (*Neotoma* spp.) middens

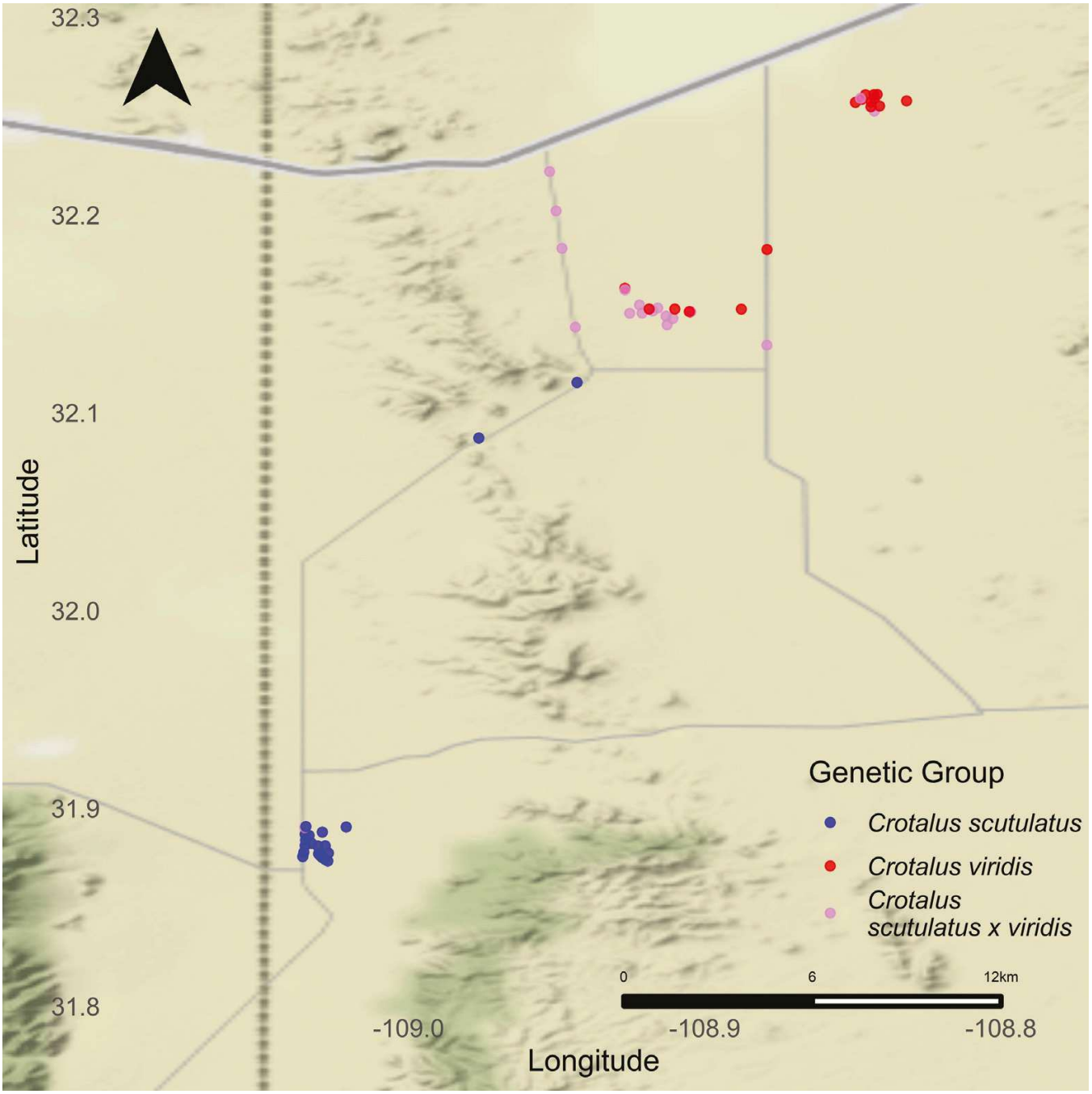

Figure 1. Genetic group and capture location for all snakes implanted with transmitters ($N = 56$). We classified any snake with a hybrid index between 5% and 95% as a hybrid. Coloured points indicate the genetic group based on their hybrid index (*Crotalus scutulatus*, HI = 21; *Crotalus viridis*, HI = 17; *Crotalus scutulatus* × *viridis*, HI = 18).

(large nests built from various materials). Across all three active seasons of data collection, 2019–2021, the average temperature was 28.0°C and ranged from 4.67 to 48.5°C. Total accumulated rainfall ranged from 1507 to 1780 cm (https://www.wunderground.com/, station PF01), which occurred mostly during the late summer monsoonal wet season.

Snake sampling and surgical procedures

We collected and sampled all rattlesnakes encountered via road and visual encounter surveys within and adjacent to the hybrid zone. Upon capture, we recorded GPS coordinates (precision, ±5 m) and assigned a putative status (*Crotalus scutulatus* × *viridis*, *Crotalus viridis*, or *Crotalus scutulatus*) to each individual based on species-typical morphological features (tail banding pattern, head scalation, and facial coloration). Captured snakes were transported back to a field station at the Chiricahua Desert Museum in Rodeo, NM for processing and released at the point of capture after processing, with the exception of some individuals deposited in the museum collection of the University of Texas Arlington. Every snake was processed by assaying behavioural types (Maag 2023), marking with a passive integrated transponder (PIT) tag, sampling tissue and venom, measuring a

suite of morphometric traits, and photographing coloration and scalation features.

A subset of captured snakes was implanted with very-high frequency (VHF) radio transmitters (Wildlife Materials SOPI-2380) in order that we could quantify movement and spatial ecology. We anaesthetized these snakes with isoflurane and followed a standard surgical procedure (Reinert and Cundall 1982) to implant miniature VHF radio transmitters into their body cavities. Radio transmitters weighed <5% of the body mass of the snake, and we released snakes at their point of capture after a 24–72 h recovery period. During recovery, snakes were housed in individual containers at a temperature range of 22–26°C and provided with water *ad libitum*. All procedures were approved by the San Diego State University Institutional Animal Care and Use Committee (22-07-008C). Animals were collected via a New Mexico Department of Game and Fish Scientific Collection permit (authorization number 3605).

Radio-tracking procedures

We used a hand-held Yagi antenna attached to a VHF receiver to track radio signals and locate snakes twice daily. Individuals were typically located once before sundown and once after sundown, 3–6 nights each week, from mid-May to late August or early September (depending on the year). Although this period encompassed the majority of the active season of our snakes, we were unable to track snakes regularly during early and late months of the active season, because snakes at our site typically overwintered from mid-November to late-March. Upon locating a snake, we recorded Universal Transverse Mercator coordinates with a hand-held GPS (MotionX-GPS app v.24.4; precision, ±5 m) and noted the body position and behaviour of the snake when it was visible [moving, within a retreat site, hunting/eating, or resting (found on the surface, not moving but not in a stereotyped ambush posture; Reinert *et al.* 2011: fig. 4)]. We used GPS coordinates and straight-line distances between successive points to quantify movement patterns and estimate spatial UDs. A home range (the more typical term applied to spatial range quantification for snakes) is a form of a UD, and the terms are sometimes used synonymously, but we use UD because we were not able to track snakes for their entire active season. To account for any lingering effects of the capture and surgery process, we retained GPS locations for analysis only after the snake had moved away from its release site and was actively seen performing surface activity (hunting, moving, or reproductive behaviours), typically a period of 3–7 days after release.

In arid ecosystems, heavy rains can drive changes in ecological communities (Ostfeld and Keesing 2000). Because these sites exhibit strong seasonal patterns of precipitation that coincide with the seasonal late-summer mating period, we also examined changes in spatial behaviour across the wet and dry seasons. We determined the onset of the wet season each year using precipitation data logged by the weather stations in closest proximity to our three field sites (KNMANIMA5, KNMRODEO1, and KNMLORDS12; https://www.wunderground.com/; Supporting Information, Table S1). Although mating behaviours occurred too infrequently across all sites for any quantitative analysis, we describe qualitatively all behaviours related to male–female courtship or mating and male–male combat associated with intrasexual selection (Supporting Information, Table S2).

Genetic assignments of individuals to parental species or hybrids

To assign snakes as parental or hybrid individuals, we analysed reduced-representation genomic data obtained from double-digest RAD sequencing and whole-genome sequencing. All sequenced samples were mapped to the *Crotalus viridis* reference genome (Schield *et al.* 2019). For individuals that had their whole genome sequenced, whole genome data were downsampled to include only loci that overlapped with double-digest RAD sequencing loci. Hybrid index (HI) scores for individuals were inferred across individuals using ADMIXTURE with $K = 2$ (K denotes the number of distinct populations assumed in the ADMIXTURE model). We classified any snake with an HI between 5% and 95% as a hybrid (Fig. 1). For further details, see Maag *et al.* (2023).

Statistical analyses

For each radio-tagged individual, we calculated standard movement statistics of mean distance moved per day (DMD) and frequency of movement (FM) (Reinert 1992). Some individuals were tracked for more than one consecutive year. In these cases, we used paired Wilcoxon signed rank tests to determine whether spatial metrics differed significantly between the two years, and if not, combined data across years for DMD and FM. For snakes with sufficient tracking samples (more than five unique GPS positions and tracked for a period of ≥30 days), we estimated their spatial UDs using 100% minimum convex polygons (MCPs), in addition to 95%, 75%, and 50% kernel density estimators (KDEs). For KDEs, we used h-ref and ad hoc (Kie 2013) to select the smoothing parameter (h) and 95%, 75%, and 50% Brownian-bridge KDEs (bbKDEs). We used the bbKDE as the primary estimator for comparisons among snakes because this method considers the past movements of individuals when estimating the shape and size of the UD. Thus, bbKDE UDs are more likely to include corridors of habitat that connect frequently used centres. Furthermore, bbKDE assumes that locations are not independent of each other and incorporates the time interval between locations when generating the UD (Horne *et al.* 2007). This method was especially relevant to our sampling design because the time between tracking events can vary for extraneous reasons (temporary signal loss, inclement weather, etc.). We also calculated the patchiness of the UDs by counting the amount of discontinuous 50% bbKDE area for each snake.

We used either a linear model or generalized linear model framework to assess the relationship between the ancestry of an individual and its spatial ecology. Genetic group (*Crotalus scutulatus*, *Crotalus viridis*, or *Crotalus scutulatus* × *viridis*, as determined by its hybrid index) was used as an independent variable for all models. For each dependent variable (individual mean DMD, FM, UD size, and UD patchiness), we created three models with the following fixed factors: genetic group, genetic group + snout–vent length (SVL), and genetic group × SVL. We included SVL because the size or age of snakes could influence their movement. We used Akaike's information criterion corrected for small sample size to select which of the three models fitted the data best. Owing to the limited number of

non-pregnant females in the *Crotalus scutulatus* × *viridis* ($N = 3$), *Crotalus viridis* ($N = 1$), and *Crotalus scutulatus* ($N = 5$) populations, we used only males for these analyses, but we have reported the data from females, for comparative purposes, in the Supporting Information (Table S3). Gamma distributions were used when the data could not be transformed to fit a Gaussian distribution. Pairwise comparisons were made with the Tukey adjustment.

To assess differences in variance of the data between groups, we used Bartlett's test of homogeneity of variances when the data fitted a normal distribution or could be transformed to fit one, and Levene's test of homogeneity of variances when the data could not. When the initial test of homogeneity of variances was statistically significant, meaning that the variances were not equal between the groups, to determine which pairs of groups were driving the pattern we performed three additional tests to address all the pairwise comparisons and adjusted the *P*-values with the Holm adjustment.

All statistical analyses were done in R (v.3.6.3, 2021) using the following packages: tidyverse (Wickham *et al.* 2019), Hmisc (Harrell Jr, with contributions from Charles Dupont and many others 2021), gridExtra (Auguie 2017), MuMIn (Barton 2020), adehabitatHR (Calenge 2006), ggplot2 (Wickham 2016), and emmeans (Lenth 2021). Values are reported as the mean ± SEM.

RESULTS

Subjects

Overall, 56 snakes (47 males and 9 females) were implanted with radio transmitters, and these individuals were part of a larger sample of individuals for which we had estimated the hybrid index (fig. 2; Maag *et al.* 2023). Of this group, 21 individuals were classified as *Crotalus scutulatus*, 17 *Crotalus viridis*, and 18 hybrids (Supporting Information, Tables S4–S6). However, we were unable to obtain sufficient spatial data for analysis on four hybrids and one *Crotalus viridis* individual. One additional hybrid became pregnant in 2021, hence only her 2020 data are reported. The remaining 51 snakes were tracked on average for 65.8 ± 5.94 days (*Crotalus scutulatus*, 48.0 ± 5.38 days; *Crotalus viridis*, 100 ± 12.8 days; hybrids, 53.4 ± 7.66 days), yielding on average 55.3 ± 4.64 spatial points per snake (*Crotalus scutulatus*, 40.1 ± 4.64; *Crotalus viridis*, 77.4 ± 9.32; hybrids, 52.6 ± 8.22).

Movements

We were able to measure movement parameters for 42 male snakes (*Crotalus scutulatus*, 16; *Crotalus viridis*, 15; *Crotalus scutulatus* × *viridis*, 11). Both DMD and FM conformed to normality after FM underwent a natural logarithmic transformation. For DMD, we report the results of the model with genetic group as the only independent variable (the most informative model set included genetic group and genetic group + SVL models). For FM, the genetic group model was most informative. Males of the three groups did not differ significantly in DMD ($F = .601$; d.f. = 2, 39; $P = .553$; Fig. 2A). Averaged across the groups, males moved 67.2 ± 4.88 m/day (*Crotalus scutulatus*, 74.1 ± 8.08 m/day; *Crotalus viridis*, 63.4 ± 6.53 m/day; hybrid, 62.5 ± 11.7 m/day). Male rattlesnakes did differ in their FM ($F = 3.63$; d.f. = 2, 39; $P = .036$; Fig. 2B). *Crotalus scutulatus* moved more frequently (one move every 1.56 ± .071 days or every 37.3 ± 1.71 h) than *Crotalus viridis* (one move every 1.87 ± .098 days or 44.8 ± 2.36 h; *t*-ratio = −2.59, $P = .035$). Hybrids were intermediate, moving every 1.63 ± .135 days (or 39.1 ± 3.23 h), no more or less often than *Crotalus scutulatus* (*t*-ratio = −0.491, $P = .876$) or *Crotalus viridis* (*t*-ratio = 1.86, $P = .164$). Variation in DMD and FM was not different between the three groups ($K^2 = 2.12$ and .579; d.f. = 2, 39 and 2, 39; $P = .346$ and .749, respectively; Fig. 2).

Spatial utilization

Thirty-three male rattlesnakes (*Crotalus scutulatus*, 12; *Crotalus viridis*, 14; hybrids, 7) had sufficient samples for estimation of UD. The MCP and KDE UDs resulted in similar patterns between the parental and hybrid groups, thus only bbKDEs were used in further analyses of UD area. The MCP and the other KDE averages and SEM for the genetic groups are reported in the Supporting Information (Table S7). However, five individuals had insufficient data for bbKDE analysis (two *Crotalus scutulatus* and three *Crotalus viridis*). Additionally, we found that snakes tracked for two consecutive years had similar UD sizes across years regardless of whether we used the MCPs or bbKDEs ($V = 19$ and 18; $N = 10$ and 8; Wilcoxon signed rank test $P = .43$ and 1.00, respectively). Thus, we averaged the respective UD size estimations between the years for those snakes.

The UDs estimated by bbKDE conformed to normality after a natural logarithmic transformation. We report the results from the model containing genetic group as the only predictor variable (the most informative model set included genetic group and genetic group + SVL). Male UD size did not differ significantly between the groups ($F = 2.309$; d.f. = 2, 25; $P = .120$). Male rattlesnakes had an average UD area of 59.1 ± 10.1 ha (*Crotalus scutulatus*, 38.8 ± 6.06 ha; *Crotalus viridis*, 85.5 ± 21.1 ha; hybrid, 46.4 ± 16.9 ha). Additionally, variation in UD area was not different between the three genetic groups ($K^2 = 5.71$; d.f. = 2, 25; $P = .058$; Fig. 3).

The model with genetic group as the sole predictor variable was most informative for patchiness of the core UDs. The patchiness of the core UDs was significantly different between the groups ($\chi^2 = 32.3$; d.f. = 2, 29; $P < .001$). Patchiness of the core UDs was greater in hybrids than in either parental population (*Crotalus viridis*: *z*-ratio = 4.46, $P < .001$; *Crotalus scutulatus*: *z*-ratio = 5.14, $P < .001$), and *Crotalus scutulatus* and *Crotalus viridis* both had similarly continuous core UDs (*z*-ratio = 1.05, $P = .544$). Additionally, we found differences in the variation of the patchiness between the groups ($F = 3.47$; d.f. = 2, 29; $P = .045$; Fig. 4). However, post hoc multiple comparisons did not indicate that there were significant differences between the genetic groups (*Crotalus scutulatus* vs. *Crotalus viridis*: $F = 1.15$; d.f. = 1; adjusted $P = .296$; *Crotalus scutulatus* vs. hybrids: $F = 6.81$; d.f. = 1; adjusted $P = .055$; *Crotalus viridis* vs. hybrids: $F = 2.70$; d.f. = 1; adjusted $P = .236$). Examples of a typical UD for each of the groups are depicted in Figure 5.

Seasonal shifts and mating behaviours

Twenty-three male rattlesnakes (*Crotalus scutulatus*, 3; *Crotalus viridis*, 14; hybrids, 6) were tracked in both the dry and wet seasons within the same year. Six of these snakes were tracked in both seasons in 2020 and 2021. Both DMD and FM were found to be different between the years during the dry season (paired

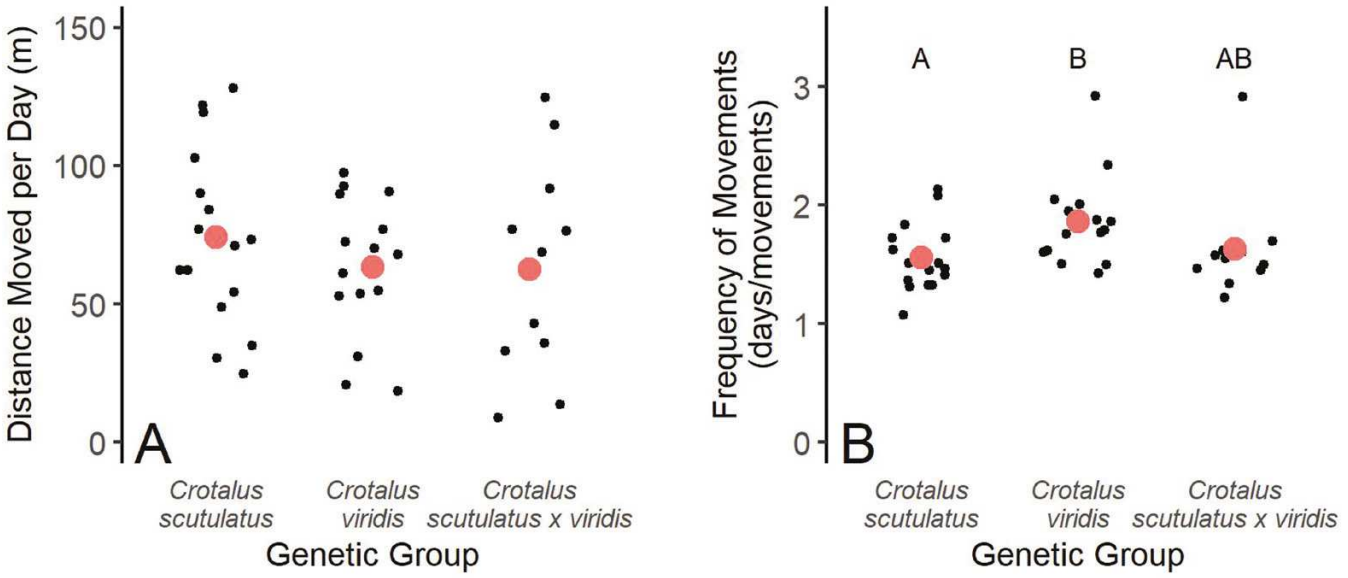

Figure 2. Distance moved per day (DMD; A) and number of days between movements (FM; B) calculated for each male snake radio tracked. Each black dot represents the overall average for an individual snake, and the red dots represent the averages for the genetic groups. Dots are jittered horizontally to help differentiate individuals from each other. No differences were found in the means of DMD (F = .601; d.f. = 2, 39; P = .553). Male rattlesnakes did differ in their FM (F = 3.63; d.f. = 2, 39; P = .036). *Crotalus scutulatus* moved more frequently, one move every 1.56 ± .071 days (or every 37.3 ± 1.71 h), than *Crotalus viridis*, one move every 1.87 ± .098 days (or 44.8 ± 2.36 h; t-ratio = −2.59, P = .035). Hybrids moved neither more nor less often than *Crotalus scutulatus* (t-ratio = −0.491, P = .876) or *Crotalus viridis* (t-ratio = 1.86, P = .164), moving every 1.63 ± .135 days (or 39.1 ± 3.23 h). The variances of these spatial metrics were the same between the three groups (DMD: K^2 = 2.12; d.f. = 2, 39; P = .346; FM: K^2 = .579; d.f. = 2, 39; P = .749). The values of DMD and FM were calculated assuming straight-line movements of the snakes.

Wilcoxon signed rank tests: V = 0 and 21; N = 6 and 6; P = .031 and .031, respectively) but not during the wet season (paired Wilcoxon signed rank tests: V = 5 and 14; N = 6 and 6; P = .313 and .563, respectfully). Thus, we randomly selected one of the years of each of these six snakes to keep for the analyses. Given that some snakes decreased their DMD and/or FM from the dry to the wet season, resulting in negative values, we scaled all the data by adding a constant to every value and subtracting the smallest negative number of each dataset from each value.

The models with genetic group as the only independent factor were most informative for both ΔDMD and ΔFM, but the overall change in DMD was only a strong trend (χ^2 = 5.73; d.f. = 2, 20; P = .057; Fig. 6A). Male snakes increased DMD in the wet season by 60.1 ± 11.5 m (*Crotalus scutulatus*, 29.1 ± 3.83 m; *Crotalus viridis*, 78.1 ± 16.5 m; hybrid, 33.5 ± 12.8 m). Additionally, we found no differences between the groups in FM in the dry and wet seasons (χ^2 = 2.02; d.f. = 2, 20; P = .364; Fig. 6B). All male snakes increased movement rate in the wet season (average number of days per move decreased by .272 ± .191 days; increased in *Crotalus scutulatus* by .173 ± .198 days; decreased in *Crotalus viridis* by .185 ± .231 days; decreased in hybrids by .699 ± .474 days). Lastly, we found no differences in dry and wet season variance in DMD (F = 2.96; d.f. = 2, 20; P = .075; we used Levene's test because data were non-normal after transformation) and FM (K^2 = 2.63; d.f. = 2; P = .268). There was not sufficient tracking data across seasons to investigate seasonal shifts in UD size between groups. Although mating behaviours were documented only rarely (as is common within these taxa, given their generally secretive behaviour), we observed male–female sexual behaviour during the breeding season across all three areas, with 11 instances of attendance behaviour, five instances of cohabitation, and eight instances of coitus (for descriptions, see Supporting Information, Table S2).

DISCUSSION

Our analysis of the spatial ecology of parental and hybrid rattlesnakes revealed that individuals with hybrid ancestry were broadly similar to individuals from both parental species and exhibited patterns of movement frequency, space use, and seasonal variation in movement patterns that were typical of other North American rattlesnakes and other pitvipers. We were not able to detect any clear-cut transgressive or abnormal movement patterns associated with hybridization. Although we did find that hybrid snakes had patchier UDs (Fig. 4), spatial behaviours in pitvipers and many other animal species are associated typically with resource acquisition, and a heterogeneous distribution of resources across the landscape could result in more patchy UDs. Pitvipers often exhibit preferences for particular habitat features, and the type of habitat can drive patterns of space use (e.g. Reinert 1984, Cardwell 2013, Maag *et al.* 2022, Tetzlaff *et al.* 2023). The *Crotalus scutulatus* and *Crotalus viridis* hybrid zone spans an area of transition between lowland scrub desert and short-grass prairie. Both parental habitats appear more homogeneous than the transitional habitat found in the middle of the hybrid zone, which contains intermittent barren patches of soil with sparse vegetation cover and almost no mammal burrows or middens (D. W. Maag, pers. obs.). Such areas are likely to be largely devoid of prey and habitat structures that offer protection from predators and extreme weather. Therefore, snakes within

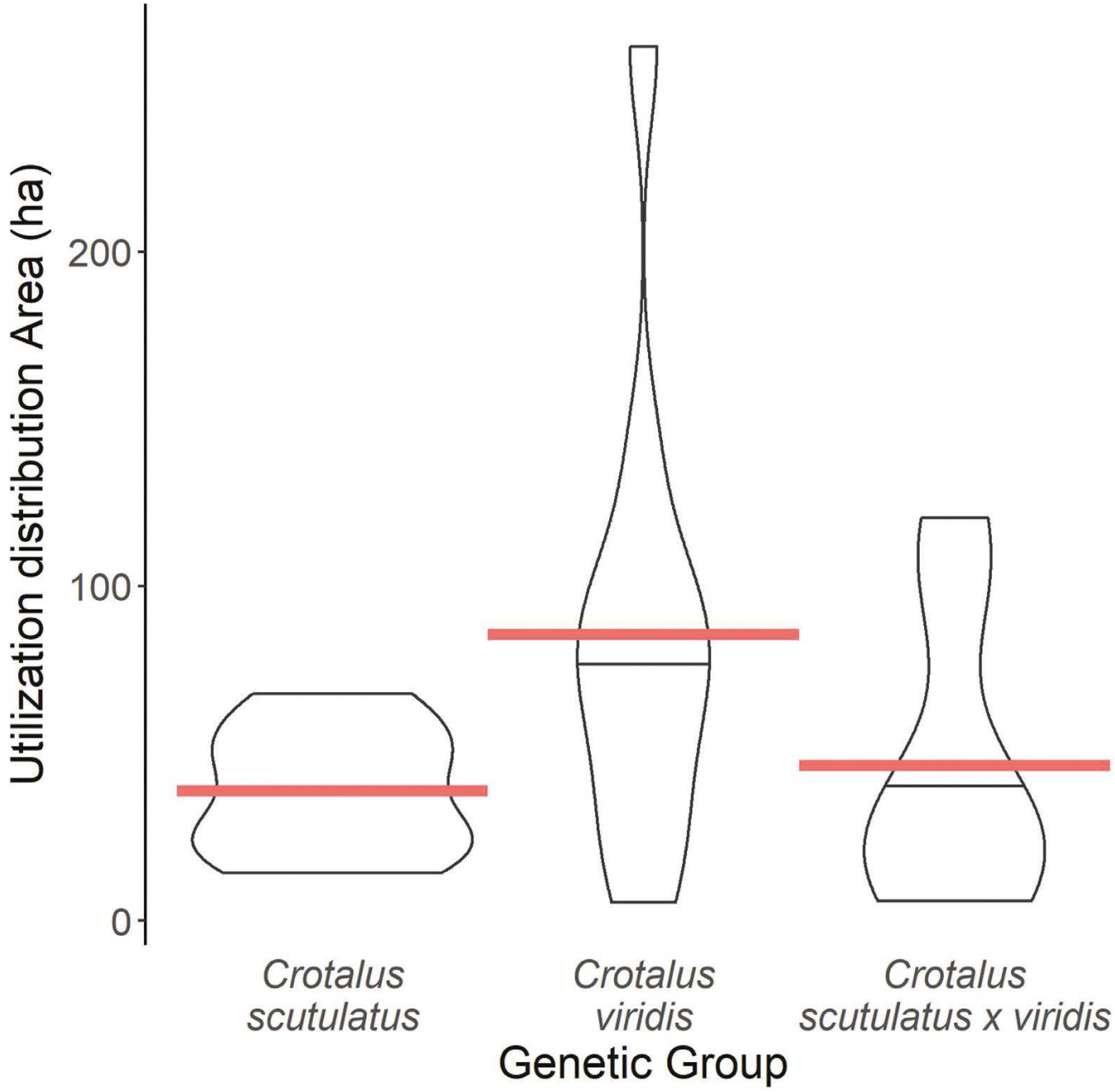

Figure 3. Violin plots of 95% utilization distributions (UDs) estimated by Brownian-bridge kernel-density estimators (bbKDE)s from radio-tracked male snakes during their active season, from May to September. The size of UDs did not differ significantly across groups ($F = 2.31$; d.f. = 2, 25; $P = .120$). Variance was also not statistically different between the groups ($K^2 = 5.71$; d.f. = 2, 25; $P = .058$). The red line indicates mean values. Sample sizes: *Crotalus scutulatus*, 10; *Crotalus viridis*, 11; and *Crotalus scutulatus* × *viridis*, 7.

the hybrid zone are likely to be restricted to using habitat corridors associated with these barren patches, which is possibly driving the significant increase in UD patchiness (subsequent analyses will examine the microhabitat preferences of individuals across this hybrid zone quantitatively). It is also possible that the increased patchiness found in the UDs of hybrid rattlesnakes is an indirect result of fragmented habitat driving a heterogeneous distribution of females. Male rattlesnakes exhibit long-distance mate searching during the mating season(s) to locate receptive females (Duvall *et al.* 1992, Duvall and Schuett 1997), and subsequently, show extended periods of attendance (pair-bonding) and courtship (Schuett and Gillingham 1988, Duvall *et al.* 1992, Smith and Schuett 2015, Schuett *et al.* 2016a, b, DeSantis *et al.* 2019) before coitus. Thus, if the females were less uniform in their distribution in the central areas of the hybrid zone, this would be reflected in the patchiness of male UDs, which could potentially increase the incidence of costly and time-consuming male–male combat for access to females.

Seasonal precipitation patterns were a dominant feature of the environment throughout the habitats used by the hybrids and

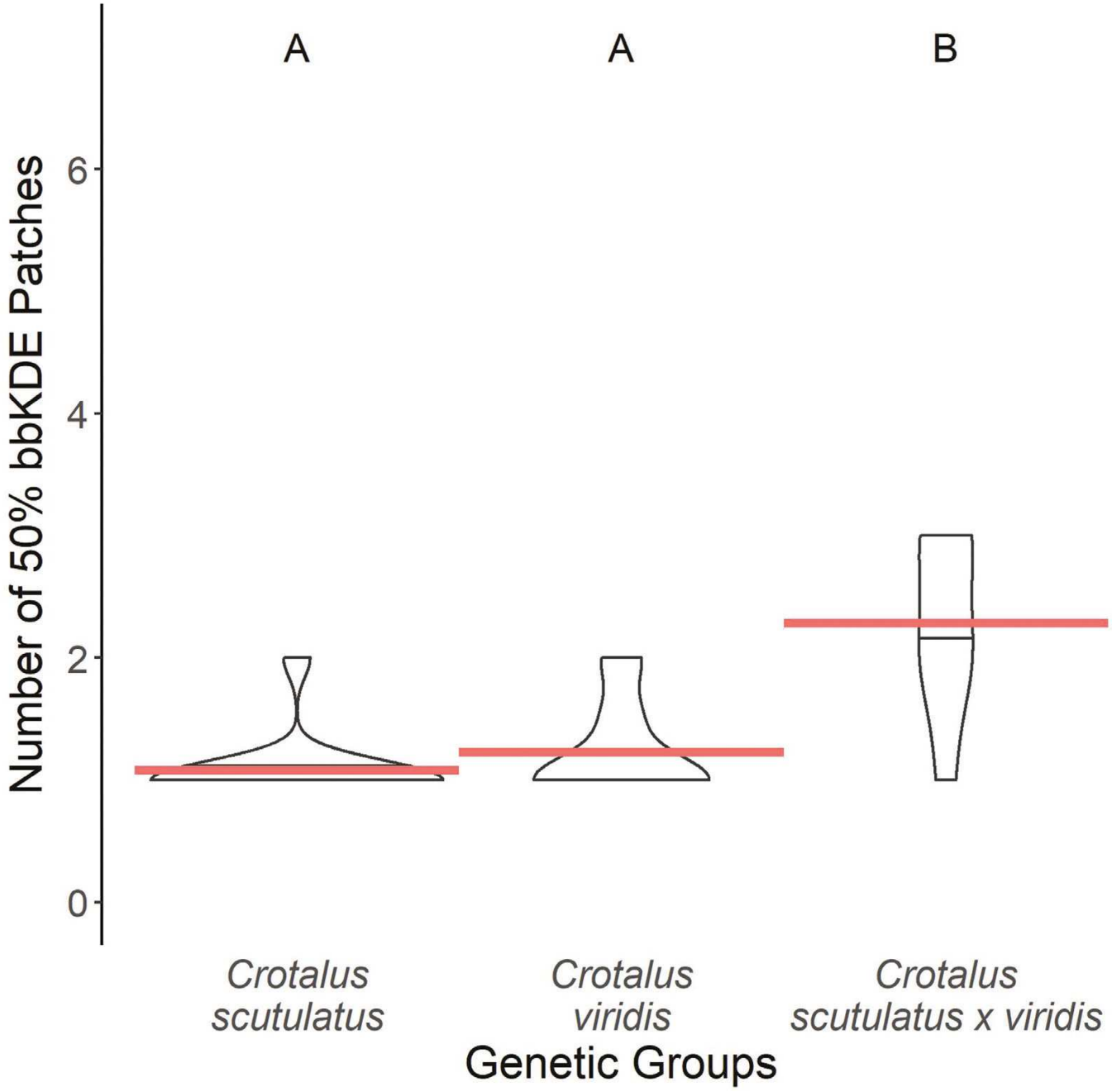

Figure 4. Violin plots of the patchiness of core utilization distributions (UDs) from radio-tracked snakes during their active season, from May to September. Patchiness was determined by the number of disconnected 50% Brownian-bridge kernel-density estimator (bbKDE) isopleths. Patchiness of the core UDs was different between the groups (χ^2 = 32.3; d.f. = 2, 29; P < .001). Tukey's post hoc tests found that connectivity in the core UDs of the parental groups was similar (z-ratio = 1.05; P = .544), whereas hybrids had more disconnected core UDs (*Crotalus scutulatus* vs. *Crotalus scutulatus* × *viridis*: z-ratio = 5.14; P < .001; *Crotalus viridis* vs. *Crotalus scutulatus* × *viridis*: z-ratio = 4.46; P < .001). A difference in the variation of the patchiness was also found for the groups (F = 3.47; d.f. = 2, 29; P = .045). However, multiple comparisons did not find any significant differences between the three genetic groups in their variation of the patchiness (*Crotalus scutulatus* vs. *Crotalus viridis*: F = 1.15; d.f. = 1; adjusted P = .296; *Crotalus scutulatus* vs. hybrids: F = 6.81; d.f. = 1; adjusted P = .055; *Crotalus viridis* vs. hybrids: F = 2.70; d.f. = 1; adjusted P = .236). Red lines indicate mean values, and letters above the box plots indicate statistically significant groupings. Sample sizes: *Crotalus scutulatus*, 12; *Crotalus viridis*, 13; and *Crotalus scutulatus* × *viridis*, 7.

parentals. *Crotalus viridis* increased their DMD around twice as much (78.1 ± 16.5 m) as either *Crotalus scutulatus* or hybrids (29.1 ± 3.83 and 33.5 ± 12.8 m, respectively). Given that the majority of *Crotalus viridis* tracked during both the dry and wet seasons resided at the *Crotalus viridis* site (60%), the relatively larger change in movement could be attributable to the more exaggerated habitat change (flooding and annual plant growth) we observed at this site in comparison to the *Crotalus scutulatus* site and hybrid zone (D. W. Maag, pers. obs.). The parental groups have similar mating seasons overlapping the dry-to-wet

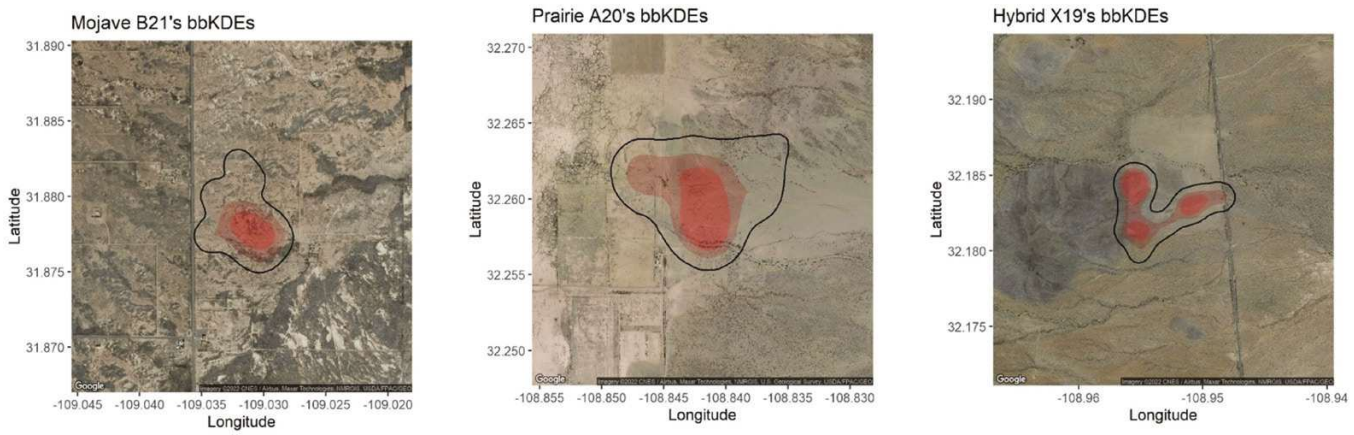

Figure 5. Representative utilization distributions (UDs) of an individual *Crotalus scutulatus* (left), *Crotalus viridis* (middle), and *Crotalus scutulatus* × *viridis* (right) estimated by Brownian-bridge kernel-density estimators (bbKDEs). The black line is the border of the 95% bbKDE, the light red shading is the 75% bbKDE, and the dark red shading indicates the 50% bbKDE.

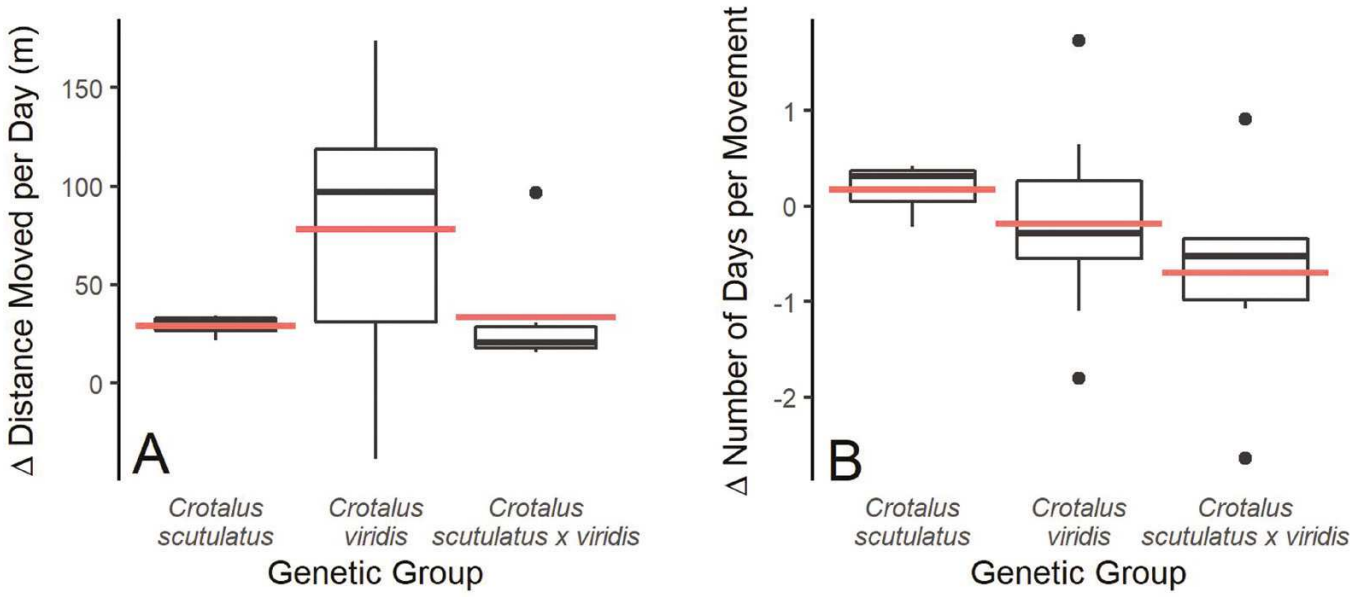

Figure 6. Box plots of the change in distance moved per day (DMD) and number of days between movements (FM) between the dry and wet seasons for males within the same year. Genetic group was not a significant factor affecting the change in DMD (χ^2 = 5.73; d.f. = 2, 20; P = .57) or FM (χ^2 = 2.02; d.f. = 2, 20; P = .364). Male snakes increased their average DMD by 60.1 ± 11.5 m/day and decreased their FM (i.e. increased their movement frequency) by .272 ± .191 days per move going from the dry to wet season. Variance in DMD and FM was also not significantly different between groups (DMD: F = 2.96; d.f. = 2, 20; P = .075; FM: K^2 = 2.63; d.f. = 2; P = .268). The values of DMD and FM were calculated assuming straight-line movements of the snakes. Red lines indicate group means, black lines indicate group medians, the bottom and top of the boxes indicate group first and third quartiles, and the ends of the whiskers indicate the largest (top whisker) or smallest (bottom whisker) values within the 1.5 interquartile range from the third and first quartile, respectively. Sample sizes: *Crotalus scutulatus*, 3; *Crotalus viridis*, 14; and *Crotalus scutulatus* × *viridis*, 5.

season transition [*Crotalus viridis*, from mid-summer to autumn (Aldridge 1993, Holycross 1993); and *Crotalus scutulatus*, from June to October (Jacob *et al.* 1987, Goldberg and Rosen 2000, Schuett *et al.* 2002, Cardwell 2008)]. Both these characteristics point to the apparent similarity between the reproductive strategies and behaviours of these species, which might be a key factor predisposing them to hybridization.

Outside of the breeding season, movements by snakes were associated with ambush hunting behaviour, with snakes of all three groups exhibiting the typical pitviper pattern of infrequent (i.e. once every 2–3 days on average) movement to a new site, wherein the individual would resume ambush hunting for several days (Nowak *et al.* 2008, Clark 2016). We did find a statistical difference in the frequency of movements between the parental groups, with *Crotalus scutulatus* moving every 1.56 days and *Crotalus viridis* every 1.87 days. Hybrids were not statistically different from either of the parental groups, moving every 1.63 days. Individuals within groups also were highly variable in movement frequency (Fig. 2), and it is likely that the minor differences in movement are attributable more to variation in

habitat features or other conditions between the sites, rather than intrinsic differences between groups.

Differences in hunting behaviour, more broadly speaking, can drastically affect the spatial behaviour of animals (e.g. Secor 1995, Montgomery 2005). Pitvipers often use ambush hunting as their primary predation strategy (reviewed by Nowak *et al.* 2008, Hanscom *et al.* 2023), with infrequent movements and extended periods of inactivity (DeSantis *et al.* 2020). However, there are some notable exceptions. For example, some populations of copperheads (*Agkistrodon contortrix*) deploy a more active foraging strategy ('mobile ambushing') with longer periods of sustained movement (Montgomery 2005, Hendricks 2019). In this case, the change in movement for copperheads is driven directly by a shift in prey type, with actively foraging snakes almost exclusively consuming emerging cicadas (Hendricks 2019). Prey type and prey availability are broadly similar across the *Crotalus scutulatus* and *Crotalus viridis* hybrid zone (Maag *et al.* 2023), possibly also contributing to the similarity in their basic spatial ecology.

The limited sample size of adult female rattlesnakes in this study precluded statistical analysis to make robust comparisons of spatial ecology between the groups. Nonetheless, non-pregnant females moved far shorter distances per day over the active season when compared with males (females, 23.0 ± 5.80 m; males, 67.2 ± 4.88 m). Non-pregnant females also moved less frequently than males (females, 2.25 ± .243 days per move; males, 1.69 ± .059 days per move) and had smaller UDs (95% bbKDEs for females, 9.09 ± 4.22 ha; males, 59.1 ± 10.1 ha). This pattern is consistent across a number of different species of pitvipers and is thought to be driven by male-specific mating movements (e.g. Cardwell 2008, DeGregorio *et al.* 2011, Bailey *et al.* 2012). There are, however, exceptions in this trend that are likely to be species dependent (Schuett *et al.* 2013).

Although behavioural traits, such as habitat preference, can shape use of space, patterns of space use and movement frequencies also directly impact the risk of predation. Like many species, rattlesnake largely appear to rely on crypsis to avoid their own predators, and past studies have found that rattlesnakes appear to be more vulnerable to predation when moving between sites rather than when sitting in ambush coils (Maag and Clark 2022). Although hybrid snakes have patchier UDs, they do not move more frequently or longer distances, indicating that they are unlikely to spend more time vulnerable to predators than parental individuals. It is possible that the transitional habitat they move through is more dangerous, because it might lack cover and involve a higher level of exposure to potential predators, which is a possibility that we are seeking to assess by collecting more data about the microhabitat variation between sites.

Not only did we find that male hybrids had similar spatial behaviours to the parental species, but we also found that the parental groups themselves were broadly similar in spatial ecology (Fig. 3). Estimates of home ranges of *Crotalus viridis* males range between .3 and 31.4 ha (Shipley *et al.* 2013), and the more limited data for *Crotalus scutulatus* indicates a somewhat larger home range of 14.6–52.6 ha (Cardwell 2008). Although our estimates of MCP size (Supporting Information, Table S6) for *Crotalus scutulatus* are in line with previous studies, our average MCP estimate for *Crotalus viridis* (36.6 ha) is larger than what is typically reported for northern populations of this species, despite the fact that we were not able to include spatial data for the entire active season, possibly reflecting significant intraspecific variation in spatial behaviours of this genetic group. However, given that individuals were tracked only during their active season, we were not able to assess any potential differences between the groups in timing of hibernation, emergence from hibernacula, or seasonal migratory movements to and from hibernacula. Although *Crotalus viridis* does display migratory movements in northern populations (King and Duvall 1990, Jorgenson *et al.* 2008, Shipley *et al.* 2013, Chiszar *et al.* 2014), there are no previous studies examining this behaviour in southern portions of the range, and there is no evidence thus far that *Crotalus scutulatus* performs such migratory movements. Future studies could examine the potential for hybridization to disrupt the timing or extent of seasonal migratory movements exhibited by *Crotalus viridis* (assuming such behaviour is still typical for southern populations).

Additional metrics also indicate that intraspecific space use can vary substantially between sites. Male *Crotalus scutulatus* in our study showed longer DMDs than members from a population in California (74.1 m in this study vs. 38 m in the study by Cardwell 2008), and male *Crotalus viridis* move more often (1.87 days between moves in this study vs. 2.38–4.76 days in the study by Jorgenson *et al.* 2008). Habitat structure, predator communities, and prey resources vary substantially between different geographical locales occupied by these wide-ranging species, and future research comparing these factors between sites could allow us to determine how biotic factors influence these intraspecific differences in movement.

Although previous studies have demonstrated how space use and movement can shape hybridization dynamics (Benson and Patterson 2013, Delmore and Irwin 2014, Austin *et al.* 2019), we did not find evidence for transgressive or abnormal movement behaviour in the hybrid rattlesnakes studied here. However, our sampling design did not allow us to evaluate whether co-occurring parental and hybrid individuals were spatially segregated (as has been found in other systems), because we purposefully selected parental sites outside the hybrid zone to maximize the probability that we were comparing hybrids with individuals having minimally admixed genomes. Nevertheless, we did find overlap between groups, with some parental individuals (both *Crotalus scutulatus* and *Crotalus viridis*) being found within the hybrid zone (Fig. 1; Supporting Information, Tables S4–S6), making it possible that future studies could examine more closely the factors leading to spatial segregation.

A major and unavoidable shortcoming of our analysis was the limited sample of female rattlesnakes found in these areas at a time suitable for radio-transmitter implantation, despite extensive search effort. Future studies should attempt to obtain a larger sample size of females, perhaps by relying on techniques other than visual encounter and driving surveys. Although we were able to locate a small number of females by tracking males during the mating season (overall, we observed 11 male–female pairings involving *Crotalus viridis*, 11 involving *Crotalus scutulatus*, and 4 involving hybrids; see Supporting Information, Table S2), these observations occurred late in the active season (both species mate almost exclusively in the late autumn), and we avoided conducting surgical implantation of transmitters late in the active season owing to the increased risk of mortality

(Rudolph *et al.* 1998). Another possible approach to locating more females would be to visit field sites during the winter to identify overwintering sites of radio-tagged snakes and attempt to survey these habitats during the spring egress period, because many *Crotalus* species tend towards communal hibernation, even in more temperate climates (reviewed by Schuett *et al.* 2016b).

Not only would tracking a large sample of females create more opportunities to collect data on mating behaviour, but also the inclusion of females could be particularly important because females might be affected more detrimentally by hybridization. Critical extrinsic barriers to hybridization could be linked to aspects of female ecology or physiology that we were not able to quantify in detail. As in all snakes (except for boids and pythonids; Gamble *et al.* 2017), rattlesnakes follow ZW sex determination, in which the females are heterogametic, ZW (Emerson 2017). Genetic incompatibilities and negative impacts on individual fitness are expected to occur in greater frequency in heterogametic individuals (Haldane's rule; Coyne and Orr 2004), and laboratory studies of sex differences in physiology and energetics could be a fruitful future direction for understanding this hybrid zone.

CONCLUSION

Research into the behaviour and ecology of hybrid individuals expressed in natural conditions might be key in developing a general understanding of the processes that lead to reproductive isolation and speciation. However, teasing apart minor differences between two species that are ecologically and behaviourally similar can be difficult owing to the complexity of these phenotypes, especially in cryptic species that are difficult to observe directly. Our study is one of only a few to examine explicitly the implications of hybridization on a spatial and ecological scale in a natural interspecific hybrid zone. Our study also represents an important step in assembling the types of detailed datasets that could lead to a more general understanding of how extrinsic barriers might be limiting the spread and extent of hybridization in species without known strong pre-mating isolation barriers.

Important future directions for developing this hybrid zone further as a model for understanding how ecological trait expression creates extrinsic barriers to further hybridization would be to develop methods that would allow for increased sampling of adult females and to sample key behaviours related to seasonal migrations to and from overwintering sites. These investigations would then set the stage for directed empirical studies that could address specific hypotheses developed from field observations related to the physiology and behaviour of hybrids, ultimately leading to a more holistic understanding of factors that generally limit the extent and spread of hybridization in animal systems.

SUPPLEMENTARY DATA

Supplementary data is available at *Biological Journal of the Linnean Society* online.

ACKNOWLEDGEMENTS

We would like to thank the following field technicians for helping to acquire the field data for this research: Joshua Benavidez, Nicholas Dunham, Diana Ouk, Ricardo Galaz Gibert, Kaitlyn Wiese, Nathaniel Cornille, Emily Allen, Matthew Goetz, Elizabeth Boulanger, Megan Quinn, Lynn Jacobs, Alexander Megerle, Andressa Viol, and Jeffrey Ferrer. We thank Bob and Sheri Ashley and the staff of the Chiricahua Desert Museum for providing logistical support during the field collection seasons. We also thank Bill Cavaliere, the Kerr family, Everett and Leah Ashearst, Mike and Joan Tracey, Jim Walter, Mark Thomas, and Bob Ashley for granting us access to private properties. We thank Drs Polly Campbell, Timothy Higham, and Marshal Hedin for their insights and advice during the development, collection of data, and preparation of this manuscript. We thank two anonymous reviewers for comments that improved the final draft of the manuscript.

Conflict of interest: None declared.

FUNDING

This research was funded by grants to D.W.M. from the Society for the Study of Amphibians and Reptiles (Roger Conant Grant in Herpetology), the Chiricahua Desert Museum (Charles W. Painter Grant in Herpetology), the American Society of Ichthyologists and Herpetologists (Helen T. and Frederick M. Gaige Grant), the Southwestern Center for Herpetological Research (Klauber Summer Research Grant), the University of California, Riverside (Irwin M. Newell Graduate Research Fund; Graduate Research Award; Herman T. and Evelyn W. Spieth Memorial Graduate Award), and San Diego State University (Completion of Research and Creative Activity Fellowship). Additional support came from NSF IOS-1856404 to R.W.C.

DATA AVAILABILITY

Raw sequence reads for restriction site-associated DNA sequencing data are available under the NCBI SRA (sequence read archive) BioProjectID number: PRJNA1010815. The final VCF alignment file used for hybrid index analysis, individual movement metrics, UD sizes and patchiness, and the change in DMD and FM from the dry to wet season is available on Dryad: https://doi.org/10.5061/dryad.tht76hf59.

REFERENCES

Abt G, Reyer HU. Mate choice and fitness in a hybrid frog: *Rana esculenta* females prefer *Rana lessonae* males over their own. *Behavioral Ecology and Sociobiology* 1993;**32**:221–8.

Aldridge RD. Male reproductive anatomy and seasonal occurrence of mating and combat behavior of the rattlesnake *Crotalus v. viridis*. *Journal of Herpetology* 1993;**27**:481–4. https://doi.org/10.2307/1564845

Auguie B. *gridExtra: Miscellaneous Functions for 'Grid' Graphics*. R package version 2.3. 2017. https://CRAN.R-project.org/package=gridExtra

Austin RE, Wynn RB, Votier SC *et al.* Patterns of at-sea behaviour at a hybrid zone between two threatened seabirds. *Scientific Reports* 2019;**9**:14720. https://doi.org/10.1038/s41598-019-51188-8

Bailey RL, Campa H, Bissell KM *et al.* Resource selection by the Eastern Massasauga rattlesnake on managed land in southwestern Michigan. *Journal of Wildlife Management* 2012;**76**:414–21. https://doi.org/10.1002/jwmg.266

Bailey RM. An intergeneric hybrid rattlesnake. *The American Naturalist* 1942;**76**:376–85. https://doi.org/10.1086/281054

Barton K. 2020. *MuMIn: Multi-Model Inference. R package version 1.43.17. 2020*. https://CRAN.R-project.org/package=MuMIn

Barton NH. The role of hybridization in evolution. *Molecular Ecology* 2001;**10**:551–68. https://doi.org/10.1046/j.1365-294x.2001.01216.x

Benson JF, Patterson BR. Inter-specific territoriality in a *Canis* hybrid zone: spatial segregation between wolves, coyotes, and hybrids. *Oecologia* 2013;**173**:1539–50. https://doi.org/10.1007/s00442-013-2730-8

Brown LE. Natural hybridization and trend toward extinction in some relict Texas toad populations. *The Southwestern Naturalist* 1971;**16**:185–99. https://doi.org/10.2307/3670498

Brown WS. Sampling timber rattlesnakes (*Crotalus horridus*): phenology, growth, intimidation, survival, and a syndrome of undetermined origin in a northern population. In: Hayes WK, Beaman KR, Cardwell MD *et al.* (eds.), *The Biology of Rattlesnakes*. Loma Linda: Loma Linda University Press, 2008, 235–56.

Bushar LM, Reinert HK, Gelbert L. Genetic variation and gene flow within and between local populations of the timber rattlesnake, *Crotalus horridus*. *Copeia* 1998;**1998**:411–22. https://doi.org/10.2307/1447435

Calenge C. The package adehabitat for the R software: tool for the analysis of space and habitat use by animals. *Ecological Modelling* 2006;**197**:516–9. https://doi.org/10.1016/j.ecolmodel.2006.03.017

Campbell JA, Brodie ED Jr, Barker DG *et al.* An apparent natural hybrid rattlesnake and *Crotalus willardi* (Viperidae) from the Peloncillo Mountains of southwestern New Mexico. *Herpetologica* 1989;**45**:344–9.

Cardwell MD. The reproductive ecology of Mohave rattlesnakes. *Journal of Zoology* 2008;**274**:65–76. https://doi.org/10.1111/j.1469-7998.2007.00358.x

Cardwell MD. Behavioral changes by Mohave rattlesnakes (*Crotalus scutulatus*) in response to drought. Master of Science (MS) Thesis, California State University, Sacramento, 2013.

Castoe TA, Spencer CL, Parkinson CL. Phylogeographic structure and historical demography of the western diamondback rattlesnake (*Crotalus atrox*): a perspective on North American desert biogeography. *Molecular Phylogenetics and Evolution* 2007;**42**:193–212. https://doi.org/10.1016/j.ympev.2006.07.002

Chiszar D, Shipley BK, Smith HM *et al.* Straightness-of-path during and after vernal migration in prairie rattlesnakes, *Crotalus viridis*, in Eastern Colorado. *Herpetology Notes* 2014;**7**:425–36.

Chunco AJ. Hybridization in a warmer world. *Ecology and Evolution* 2014;**4**:2019–31. https://doi.org/10.1002/ece3.1052

Clark RW. The hunting and feeding behavior of wild rattlesnakes. In: Schuett GW, Feldner MJ, Smith CF *et al.* (eds.), *Rattlesnakes of Arizona*. Rodeo: Eco, 2016, 91–118.

Coyne JA, Orr HA. Postzygotic isolation. In: *Speciation*. Sunderland: Sinauer Associates, 2004, 247–81.

Crane AL, Greene BD. The effect of reproductive condition on thermoregulation in female *Agkistrodon piscivorus* near the northwestern range limit. *Herpetologica* 2008;**64**:156–67. https://doi.org/10.1655/07-021.1

Darwin C. *The Origin of Species by Means of Natural Selection*. London: Murray, 1859.

Davis MA, Douglas MR, Webb CT *et al.* Nowhere to go but up: impacts of climate change on demographics of a short-range endemic (*Crotalus willardi obscurus*) in the sky-islands of Southwestern North America. *PLoS One* 2015;**10**:e0131067. https://doi.org/10.1371/journal.pone.0131067

DeGregorio BA, Manning JV, Bieser N *et al.* The spatial ecology of the Eastern Massasauga (*Sistrurus c. catenatus*) in northern Michigan. *Herpetologica* 2011;**67**:71–9.

Delmore KE, Irwin DE. Hybrid songbirds employ intermediate routes in a migratory divide. *Ecology Letters* 2014;**17**:1211–8. https://doi.org/10.1111/ele.12326

de Queiroz K. Species concepts and species delimitation. *Systematic Biology* 2007;**56**:879–86. https://doi.org/10.1080/10635150701701083

DeSantis DL, Mata-Silva V, Johnson JD *et al.* Integrative framework for long-term activity monitoring of small and secretive animals: validation with a cryptic pitviper. *Frontiers in Ecology and Evolution* 2020;**8**:169.

DeSantis DL, Wagler AE, Mata-Silva V *et al.* Effects of human-made resource hotspots on seasonal spatial strategies by a desert pitviper. *Scientific Reports* 2019;**9**:16690. https://doi.org/10.1038/s41598-019-52957-1

Doherty JA, Gerhardt HC. Acoustic communication in hybrid treefrogs: sound production by males and selective phonotaxis by females. *Journal of Comparative Physiology A* 1984;**154**:319–30. https://doi.org/10.1007/bf00605231

Dreslik MJ. Ecology of the Eastern Massasauga (*Sistrurus catenatus catenatus*) from Carlyle Lake, Clinton County, Illinois. *Ph.D. Thesis*, University of Illinois at Urbana-Champaign, 2005.

Dreslik MJ, Shepard DB, Jellen BC *et al.* Movement and home range of the Eastern Massasauga (*Sistrurus catenatus*) at its southern range limit. In: Dreslik MJ, Hayes WK, Beaupre SJ *et al.* (eds.), *The Biology of Rattlesnakes II*. Rodeo: Eco, 2017, 168–78.

Durbian FE, King RS, Crabill T *et al.* Massasauga home range patterns in the midwest. *Journal of Wildlife Management* 2008;**72**:754–9.

Duvall D, Arnold SJ, Schuett GW. Pitviper mating systems: ecological potential, sexual selection and microevolution. In: Campbell JA, Brodie ED Jr. (eds.), *The Biology of the Pitvipers*. Tyler: Selva, 1992, 321–36.

Duvall D, Goode MJ, Hayes WK *et al.* Prairie rattlesnake vernal migration: field experimental analyses and survival value. *National Geographic Research* 1990;**6**:457–69.

Duvall D, Schuett GW. Straight-line movement and competitive mate searching in prairie rattlesnakes, *Crotalus viridis viridis*. *Animal Behaviour* 1997;**54**:329–34. https://doi.org/10.1006/anbe.1996.0418

Emerson JJ. Evolution: a paradigm shift in snake sex chromosome genetics. *Current Biology: CB* 2017;**27**:R800–3. https://doi.org/10.1016/j.cub.2017.06.079

Feller AF, Seehausen O, Lucek K *et al.* Habitat choice and female preference in a polymorphic stickleback population. *Evolutionary Ecology Research* 2016;**17**:419–35.

Fitch HS, Shirer HW. A radiotelemetric study of spatial relationships in some common snakes. *Copeia* 1971;**1971**:118–28. https://doi.org/10.2307/1441605

Fitzpatrick BM. Rates of evolution of hybrid inviability in birds and mammals. *Evolution* 2004;**58**:1865–70. https://doi.org/10.1111/j.0014-3820.2004.tb00471.x

Gamble T, Castoe TA, Nielsen SV *et al.* The discovery of XY sex chromosomes in a *Boa* and *Python*. *Current Biology: CB* 2017;**27**:2148–2153.e4. https://doi.org/10.1016/j.cub.2017.06.010

Gardiner LE, Somers CM, Parker DL *et al.* Microhabitat selection by prairie rattlesnakes (*Crotalus viridis*) at the northern extreme of their geographic range. *Journal of Herpetology* 2015;**49**:131–7. https://doi.org/10.1670/12-266

Goldberg SR, Rosen PC. Reproduction in the Mojave rattlesnake, *Crotalus scutulatus* (Serpentes: Viperidae). *Texas Journal of Science* 2000;**52**:101–9.

Greenberg DB, McClintock WJ. Remember the third dimension: terrain modeling improves estimates of snake home range size. *Copeia* 2008;**2008**:801–6. https://doi.org/10.1643/ce-07-119

Hamilton CM. Home range size and habitat selection of timber rattlesnakes (*Crotalus horridus*) in southwestern Wisconsin. MSc Thesis, College of Natural Resources, University of Wisconsin, 2009.

Hanscom RJ, Higham TE, Ryan D *et al.* Ambush hunting in snakes: behavior, function, and diversity. In: Penning DA (ed.), *Snakes: Morphology, Function, and Ecology*. Hauppauge: Nova Science, 2023, in press.

Harrell FE Jr, with contributions from Charles Dupont & many others. Hmisc: Harrell Miscellaneous. *R package version 4.5-0*. 2021. https://CRAN.R-project.org/package=Hmisc

Harrison RG, Larson EL. Hybridization, introgression, and the nature of species boundaries. *The Journal of Heredity* 2014;**105**:795–809. https://doi.org/10.1093/jhered/esu033

Hayes WK, Beaman KR, Cardwell MD *et al.* (eds). *The Biology of Rattlesnakes*. Loma Linda: Loma Linda University Press, 2008.

Hendricks JJ. Population demographics and active foraging behavior of copperheads (*Agkistrodon contortrix*) at a recreational site in eastern Kentucky. MSc Thesis, Faculty of the Graduate School of Eastern Kentucky University, 2019.

Holycross AT. Movements and natural history of the prairie rattlesnake (*Crotalus viridis viridis*) in the Sand Hills of Nebraska. MSc Thesis, University of Nebraska,1993.

Holycross AT. *Crotalus viridis* (western rattlesnake). Phenology. *Herpetological Review* 1995;**26**:37–8.

Horne JS, Garton EO, Krone SM *et al.* Analyzing animal movements using Brownian bridges. *Ecology* 2007;**88**:2354–63. https://doi.org/10.1890/06-0957.1

Hoss SK, Guyer C, Smith LL *et al.* Multiscale influences of landscape composition and configuration on the spatial ecology of Eastern diamond-backed rattlesnakes (*Crotalus adamanteus*). *Journal of Herpetology* 2010;**44**:110–23. https://doi.org/10.1670/08-277.1

Jacob JS, Williams SR, Reynolds RP. Reproductive activity of male *Crotalus atrox* and *C. scutulatus* (Reptilia: Viperidae) in Northeastern Chihuahua, Mexico. *The Southwestern Naturalist* 1987;**32**:273–6.

Jančúchová-Lásková J, Landová E, Frynta D. Are genetically distinct lizard species able to hybridize? A review. *Current Zoology* 2015;**61**:155–80. https://doi.org/10.1093/czoolo/61.1.155

Johnson G. Spatial ecology, habitat preference, and habitat management of the Eastern Massasauga, *Sistrurus c. catenatus* in a New York weakly-minerotrophic peatland. Ph.D. Thesis, College of Environmental Science and Forestry, State University of New York, Suracuse, 1995.

Jorgenson D, Cormack Gates C, Whiteside DP. Movements, migrations, and mechanisms: a review of radiotelemetry studies of prairie (*Crotalus viridis viridis*) and western (*Crotalus oreganus*) rattlesnakes. In: Hayes WK, Beaman KR, Cardwell MD *et al.* (eds.), *The Biology of Rattlesnakes*. Loma Linda: Loma Linda University Press, 2008, 303–316.

Kie JG. A rule-based *ad hoc* method for selecting a bandwidth in kernel home-range analyses. *Animal Biotelemetry* 2013;**1**:13. https://doi.org/10.1186/2050-3385-1-13

King MB, Duvall D. Prairie rattlesnake seasonal migrations: episodes of movement, vernal foraging and sex differences. *Animal Behaviour* 1990;**39**:924–35. https://doi.org/10.1016/s0003-3472(05)80957-1

Kingsbury BA, Marshall JC, Manning J. *Activity Patterns and Spatial Resource Selection of the Eastern Massasauga Rattlesnake in Northeastern Indiana*. Fort Wayne: Indiana-Purdue University, 2003.

Klauber LM. Enemies of rattlesnakes. In: *Rattlesnakes: Their Habits, Life Histories, and Influence on Mankind*. Berkley: University of California Press, 1956, 1064–115.

Kozak GM, Boughman JW. Predator experience overrides learned aversion to heterospecifics in stickleback species pairs. *Proceedings of the Royal Society B: Biological Sciences* 2015;**282**:20143066. https://doi.org/10.1098/rspb.2014.3066

Lenth RV. emmeans: estimated marginal means, aka least-squares means. *R package version 1.7.0.* 2021. https://CRAN.R-project.org/package=emmeans

Maag DW. How does behavior impact hybridization dynamics in systems lacking significant prezygotic isolating mechanisms? A case study of free-ranging rattlesnakes across a hybrid zone. Ph.D. Thesis, University of California, Riverside and San Diego State University, 2023.

Maag D, Clark R. Safety in coils: predation rates of ambush hunting rattlesnakes are extremely low. *Amphibia-Reptilia* 2022;**43**:425–30. https://doi.org/10.1163/15685381-bja10101

Maag DW, Francioli YZ, Shaw N *et al.* Hunting behavior and feeding ecology of Mojave rattlesnakes (*Crotalus scutulatus*), prairie rattlesnakes (*C. viridis*), and their hybrids in southwestern New Mexico. *Ecology and Evolution* 2023;**13**:e10683.

Maag DW, Maher SP, Greene BD. Spatial ecology and microhabitat selection of the pygmy rattlesnake (*Sistrurus miliarius*) in southwestern Missouri, USA. *Herpetological Conservation and Biology* 2022;**17**:316–30.

Macartney JM, Gregory PT, Larsen KW. A tabular survey of data on movements and home ranges of snakes. *Journal of Herpetology* 1988;**22**:61–73. https://doi.org/10.2307/1564357

Mallet J. Hybrid speciation. *Nature* 2007;**446**:279–83. https://doi.org/10.1038/nature05706

Malmos KB, Sullivan BK, Lamb T. Calling behavior and directional hybridization between two toads (*Bufo microscaphus* × *B. woodhousii*) in Arizona. *Evolution* 2001;**55**:626–30. https://doi.org/10.1111/j.0014-3820.2001.tb00795.x

Maroja LS, Andres JA, Walters JR *et al.* Multiple barriers to gene exchange in a field cricket hybrid zone. *Biological Journal of the Linnean Society* 2009;**97**:390–402.

Martin AE, Jørgenson D, Gates CC. Costs and benefits of straight versus tortuous migration paths for prairie rattlesnakes (*Crotalus viridis viridis*) in semi-natural and human-dominated landscapes. *Canadian Journal of Zoology* 2017;**95**:921–8.

Meik JM, Fontenot BE, Franklin CJ *et al.* Apparent natural hybridization between the rattlesnakes *Crotalus atrox* and *C. horridus*. *The Southwestern Naturalist* 2008;**53**:196–200. https://doi.org/10.1894/0038-4909(2008)53[196:anhbtr]2.0.co;2

Melo MC, Salazar C, Jiggins CD *et al.* Assortative mating preferences among hybrids offers a route to hybrid speciation. *Evolution* 2009;**63**:1660–5. https://doi.org/10.1111/j.1558-5646.2009.00633.x

Montgomery CE. Bioenergentic and ecological correlates of foraging mode in copperheads, *Agkistrodon contortrix*, and timber rattlesnakes (*Crotalus horridus*) in Northwest Arkansas. *Ph.D. Thesis*, University of Arkansas, 2005.

Montgomery WB, Schuett GW, Douglas MR *et al.* *Crotalus atrox* × *Crotalus horridus* (western diamond-back rattlesnake × timber rattlesnake). Natural hybrid. *Herpetological Review* 2013;**44**:689.

Moore BJA. Hybridization and embryonic temperature adaptation studies of *Rana temporaria* and *Rana sylvatica*. *Proceedings of the National Academy of Sciences of the United States of America* 1951;**37**:862–6.

Myers EA. Genome-wide data reveal extensive gene flow during the diversification of the western rattlesnakes (Viperidae: Crotalinae: *Crotalus*). *Molecular Phylogenetics and Evolution* 2021;**165**:107313. https://doi.org/10.1016/j.ympev.2021.107313

Nikolakis ZL, Schield DR, Westfall AK *et al.* Evidence that genomic incompatibilities and other multilocus processes impact hybrid fitness in a rattlesnake hybrid zone. *Evolution* 2022;**76**:2513–30. https://doi.org/10.1111/evo.14612

Nowak EM. Movement patterns and natural history of western diamond-backed rattlesnakes at Tuzigoot national monument, Arizona. In: van Riper C III, Mattson DJ (eds.), *The Colorado Plateau II*. Tucson: The University of Arizona Press, 2005, 253–74.

Nowak EM, Theimer TC, Schuett GW. Functional and numerical responses of predators: where do vipers fit in the traditional paradigms? *Biological Reviews of the Cambridge Philosophical Society* 2008;**83**:601–20. https://doi.org/10.1111/j.1469-185X.2008.00056.x

Nurnberger B, Barton N, MacCallum C *et al.* Natural selection on quantitative traits in the *Bombina* hybrid zone. *Evolution* 1995;**49**:1224–38. https://doi.org/10.1111/j.1558-5646.1995.tb04449.x

Ostfeld RS, Keesing F. Pulsed resources and community dynamics of consumers in terrestrial ecosystems. *Trends in Ecology & Evolution* 2000;**15**:232–7. https://doi.org/10.1016/s0169-5347(00)01862-0

Patten TJ, Fogell DD, Fawcett JD. Spatial ecology and habitat use of the western massasauga (*Sistrurus tergeminus*) in Nebraska. *Journal of North American Herpetology* 2016;**2016**:31–8. https://doi.org/10.17161/jnah.vi1.11922

Peterson MA, Monsen KJ, Pedersen H *et al.* Direct and indirect analysis of the fitness of *Chrysochus* (Coleoptera: Chrysomelidae) hybrids. *Biological Journal of the Linnean Society* 2005;**84**:273–86. https://doi.org/10.1111/j.1095-8312.2004.00429.x

Pfennig KS. Facultative mate choice drives adaptive hybridization. *Science* 2007;**318**:965–7. https://doi.org/10.1126/science.1146035

Prival DB, Goode MJ, Swann DE *et al.* Natural history of a northern population of twin-spotted rattlesnakes, *Crotalus pricei*. *Journal of Herpetology* 2002;**36**:598–607. https://doi.org/10.2307/1565929

Pyron RA, Burbrink FT. Hard and soft allopatry: physically and ecologically mediated modes of geographic speciation. *Journal of Biogeography* 2010;**37**:2005–15. https://doi.org/10.1111/j.1365-2699.2010.02336.x

R Core Team. *R: A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing, 2021.

Reinert HK. Habitat separation between sympatric snake populations. *Ecology* 1984;**65**:478–86. https://doi.org/10.2307/1941410

Reinert HK. Radiotelemetric field studies of pitvipers: data acquisition and analysis. In: Campbell JA, Brodie ED Jr (eds.), *The Biology of the Pitvipers*. Tyler: Selva, 1992, 185–198.

Reinert HK, Cundall D. An improved surgical implantation method for radio-tracking snakes. *Copeia* 1982;**1982**:702–5. https://doi.org/10.2307/1444674

Reinert HK, MacGregor GA, Esch M *et al*. Foraging ecology of timber rattlesnakes, *Crotalus horridus*. *Copeia* 2011;**2011**:430–42. https://doi.org/10.1643/ce-09-130

Reinert HK, Zappalorti RT. Timber rattlesnakes (*Crotalus horridus*) of the Pine Barrens: their movement patterns and habitat preference. *Copeia* 1988;**1988**:964–78. https://doi.org/10.2307/1445720

Rieseberg LH, Archer MA, Wayne RK. Transgressive segregation, adaptation and speciation. *Heredity* 1999;**83**:363–72. https://doi.org/10.1038/sj.hdy.6886170

Rieseberg LH, Kim SC, Randell RA *et al*. Hybridization and the colonization of novel habitats by annual sunflowers. *Genetica* 2007;**129**:149–65. https://doi.org/10.1007/s10709-006-9011-y

Roldán-Padrón O, Sandra Cruz-Pérez M, Luis Castro-Guillén J *et al*. Hybridization between *Crotalus aquilus* and *Crotalus polystictus* species: a comparison of their venom toxicity and enzymatic activities. *Biology* 2022;**11**:661.

Rosenthal GG. Individual mating decisions and hybridization. *Journal of Evolutionary Biology* 2013;**26**:252–5. https://doi.org/10.1111/jeb.12004

Roth ED. Spatial ecology of a cottonmouth (*Agkistrodon piscivorus*) population in east Texas. *Journal of Herpetology* 2009;**39**:308–12. https://doi.org/10.1670/134-04n

Rudolph DC, Burgdorf SJ, Schaefer RR *et al*. Snake mortality associated with late season radio-transmitter implantation. *Herpetological Review* 1998;**29**:155–6.

Schield DR, Adams RH, Card DC *et al*. Cryptic genetic diversity, population structure, and gene flow in the Mojave rattlesnake (*Crotalus scutulatus*). *Molecular Phylogenetics and Evolution* 2018;**127**:669–81. https://doi.org/10.1016/j.ympev.2018.06.013

Schield DR, Perry BW, Adams RH *et al*. Allopatric divergence and secondary contact with gene flow: a recurring theme in rattlesnake speciation. *Biological Journal of the Linnean Society* 2019;**128**:149–69. https://doi.org/10.1093/biolinnean/blz077

Schuett GW, Carlisle SL, Holycross AT *et al*. Mating system of male Mojave rattlesnakes (*Crotalus scutulatus*): seasonal timing of mating, agonistic behavior, spermatogenesis, sexual segment of the kidney, and plasma sex steroids. In: Schuett GW, Hoggren M, Douglas ME *et al*. (eds.), *Biology of the Vipers*. Eagle Mountain: Eagle Mountain, 2002, 515–32.

Schuett GW, Clark RW, Repp RA *et al*. Social behavior of rattlesnakes: a shifting paradigm. In: Schuett GW, Feldner MJ, Smith CF *et al*. (eds.), *Rattlesnakes of Arizona*. Rodeo: Eco, 2016b, 161–244.

Schuett GW, Gillingham JC. Courtship and mating of the copperhead, *Agkistrodon contortrix*. *Copeia* 1988;**1988**:374–81. https://doi.org/10.2307/1445877

Schuett GW, Repp RA, Amarello M *et al*. Unlike most vipers, female rattlesnakes (*Crotalus atrox*) continue to hunt and feed throughout pregnancy. *Journal of Zoology* 2013;**289**:101–10. https://doi.org/10.1111/j.1469-7998.2012.00969.x

Schuett GW, Repp RA, Spencer CL *et al*. Species accounts. *Crotalus atrox* (western diamond-backed rattlesnake). In: Schuett GW, Feldner MJ, Smith CF *et al*. (eds.), *Rattlesnakes of Arizona*. Rodeo: Eco, 2016a, 333–94.

Secor SM. Ecological aspects of foraging mode for the snakes *Crotalus cerastes* and *Masticophis flagellum*. *Herpetological Monographs* 1995;**9**:169–86. https://doi.org/10.2307/1467004

Shipley BK, Chiszar D, Fitzgerald KT *et al*. Spatial ecology of prairie rattlesnakes (*Crotalus viridis*) associated with black-tailed prairie dog (*Cynomys ludovicianus*) colonies in Colorado. *Herpetological Conservation and Biology* 2013;**8**:240–50.

Shurtliff QR, Murphy PJ, Matocq MD. Ecological segregation in a small mammal hybrid zone: habitat-specific mating opportunities and selection against hybrids restrict gene flow on a fine spatial scale. *Evolution* 2013;**68**:729–42. https://doi.org/10.1111/evo.12299

Smadja C, Catalan J, Ganem G. Strong premating divergence in a unimodal hybrid zone between two subspecies of the house mouse. *Journal of Evolutionary Biology* 2004;**17**:165–76. https://doi.org/10.1046/j.1420-9101.2003.00647.x

Smith CF, Schuett GW. Putative pair-bonding in *Agkistrodon contortrix* (copperhead). *Northeastern Naturalist* 2015;**22**:N1–5. https://doi.org/10.1656/045.022.0104

Smith CF, Schuett GW, Earley RL *et al*. The spatial and reproductive ecology of the copperhead (*Agkistrodon contortrix*) at the northeastern extreme of its range. *Herpetological Monographs* 2009;**23**:45–73. https://doi.org/10.1655/08-026.1

Smith RM. Spatial ecology of the timber rattlesnake (*Crotalus horridus*) and northern pine snake (*Pituophis melanoleucus*) in the Pine Barrens of New Jersey. Ph.D. Thesis, Drexel University, 2013.

Stelkens RB, Schmid C, Selz O *et al*. Phenotypic novelty in experimental hybrids is predicted by the genetic distance between species of cichlid fish. *BMC Evolutionary Biology* 2009;**9**:283. https://doi.org/10.1186/1471-2148-9-283

Tetzlaff SJ, Vizentin-Bugoni J, Sperry JH *et al*. Fission–fusion dynamics in the social networks of a North American pitviper. *Ecology and Evolution* 2023;**13**:e10339. https://doi.org/10.1002/ece3.10339

Tozetti AM, Vettorazzo V, Martins M. Short-term movements of the South American rattlesnake (*Crotalus durissus*) in Southeastern Brazil. *Herpetological Journal* 2009;**19**:201–6.

Van Devender TR, Betancourt JL, Wimberly M. Biogeographic implications of a packrat midden sequence from the Sacramento Mountains, south-central New Mexico. *Quaternary Research* 1984;**22**:344–60. https://doi.org/10.1016/0033-5894(84)90028-0

Volpe EP. Evolutionary consequences of hybrid sterility and vigor in toads. *Evolution* 1960;**14**:181–93. https://doi.org/10.2307/2405825

Waldron JL, Lanham JD, Bennett SH. Using behaviorally-based seasons to investigate canebrake rattlesnake (*Crotalus horridus*) movement patterns and habitat selection. *Herpetologica* 2006;**62**:389–98. https://doi.org/10.1655/0018-0831(2006)62[389:ubstic]2.0.co;2

Wastell AR, MacKessy SP. Spatial ecology and factors influencing movement patterns of desert massasauga rattlesnakes (*Sistrurus catenatus edwardsii*) in southeastern Colorado. *Copeia* 2011;**2011**:29–37. https://doi.org/10.1643/ce-09-122

Wickham H. *ggplot2: Elegant Graphics for Data Analysis*. New York: Springer-Verlag New York, 2016. https://ggplot2.tidyverse.org

Wickham H, Averick M, Bryan J *et al*. Welcome to the tidyverse. *Journal of Open Source Software* 2019;**4**:1686. https://doi.org/10.21105/joss.01686

Zancolli G, Baker TG, Barlow A *et al*. Is hybridization a source of adaptive venom variation in rattlesnakes? A test, using a *Crotalus scutulatus* × *viridis* hybrid zone in southwestern New Mexico. *Toxins* 2016;**8**:188. https://doi.org/10.3390/toxins8060188