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How Does Behavior Impact Hybridization Dynamics in Systems Lacking Significant
Prezygotic Isolating Mechanisms? A Case Study of Free-Ranging Rattlesnakes Across a
Hybrid Zone

A Dissertation submitted in partial satisfaction
of the requirements for the degree of

Doctor of Philosophy

in

Evolutionary Biology

by

Dylan Wallace Maag

June 2023

Dissertation Committee:
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Committee Co-Chairperson

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University of California, Riverside
San Diego State University

ACKNOWLEDGEMENTS

DEDICATION

I would like to dedicate this dissertation to my wife, Dr. Chelsea Maag. Chelsea has been

a constant wellspring of support and love for me and my research during my PhD.

Without her I would not be where I am today. She has been with me every step of the

way during this phase in my life and has provided emotional, practical, and financial

support that were all invaluable to me. Thank you for being the best wife I could ever

have and I love you.

ABSTRACT OF THE DISSERTATION

How Does Behavior Impact Hybridization Dynamics in Systems Lacking Significant
Prezygotic Isolating Mechanisms? A Case Study of Free-Ranging Rattlesnakes Across a
Hybrid Zone

by

Dylan Wallace Maag

Doctor of Philosophy, Graduate Program in Evolutionary Biology
University of California, Riverside and San Diego State University, June 2023
Dr. Rulon W. Clark and Dr. Polly Campbell, Co- Chairpersons

Defining a species is notoriously difficult, as often individuals in previously well-defined

species groups are found to hybridize in some portion of their range. Because they

represent a test of the barriers that lead to reproductive isolation between lineages,

hybridization events are useful "natural laboratories" for developing a deeper

understanding of how reproductive isolation is (or is not) maintained, and what traits

either strengthen or weaken it. Rattlesnakes provide a unique opportunity to study

interspecific hybridization due to the common occurrence of hybridization between

lineages in nature, the seeming general weakness of prezygotic reproductive barriers, and

the establishment of effective techniques for monitoring the behavior and ecology of free-

ranging individuals. I used a naturally occurring hybrid zone between Mojave (*Crotalus*

scutulatus) and Prairie (*C. viridis*) Rattlesnakes as a case study for examining potential

extrinsic barriers to hybridization that could develop from interactions between

behavioral expression and ecological conditions. I used a combination of radiotelemetry, fixed-field videography, and laboratory behavioral assays to compare the spatial ecology, hunting behavior, and differential expression of behavioral types and syndromes in individual snakes sampled from across this hybrid zone, and used genomic analyses to quantify the ancestry of individuals in my analyses. I found that parental and hybrid individuals were generally similar to one another in many behaviors and in the variability of behavioral traits, suggesting relatively conserved behavioral phenotypes within these lineages. However, I found some potentially important differences in certain aspects of spatial (movement frequency and core space use patchiness) and hunting (chemosensory behaviors and abandonment time of the hunting site) behaviors. I also found that Prairie Rattlesnakes were significantly more likely to rattle defensively than hybrid or Mojave Rattlesnakes, and, furthermore, hybrid rattlesnakes that had a greater portion of their genome derived from Prairie Rattlesnakes were more likely to rattle defensively. My research highlights the potential utility of using behavioral expression in free-ranging animals to identify differences between lineages that could impact further reproductive isolation and other evolutionary dynamics of hybrid zones.

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INTRODUCTION

General Background

Species, although central to our system of biological organization, have historically been

notoriously difficult to define (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. This hybridization between

lineages has been a topic of interest in the field of evolution since the process of natural

selection was first recognized (Darwin 1859). Modern evolutionary studies have shown

that these zones of hybridization can give us critical insights into the processes of

introgression, speciation, and genetic variation (Harrison and Larson 2014).

Consequently, research on hybrid zones is now recognized as a valuable approach for

further developing a fundamental understanding of microevolutionary patterns and

processes mediated by individual variation in traits.

Individual organisms are the most fundamental level at which selection operates,

and the behavior of individuals mediates their interaction with the environment (ecology).

Understanding the behavioral trait variation between individuals of mixed ancestry

(hybrids) is necessary to determine why particular hybrid zones exist and predict how

they may shift in response to future changes in climate or habitat availability. However,

most behavioral studies of hybridization (especially in herpetofauna) focus solely on

species with significant prezygotic isolating barriers—mechanisms that limit

reproduction across species boundaries. These species often have sexually selected traits

that are expressed differentially across the zone and that strongly affect mate choice and

reproduction (e.g., Abt & Reyer, 1993; Brown, 1971; Doherty & Gerhardt, 1984; Feller et al., 2016; Kozak & Boughman, 2015; Malmos et al., 2001; Maroja et al., 2009; Melo et al., 2009; Pfennig, 2007; Smadja et al., 2004). However, hybridization also frequently occurs between species that readily breed and produce viable hybrids (see previous examples but also, Green 1996; Ribi and Oertli 2000; Perry et al. 2001; Mallet et al. 2007; Jančúchová-Lásková et al. 2015; Cordonnier et al. 2019). Even though these species lack significant prezygotic isolating mechanisms, hybridization is still often limited to a narrow geographic area with limited introgression of alleles across the zone, indicating that postzygotic isolating mechanisms are limiting the survival or reproduction of individuals with hybrid genotypes. Less behavioral work has been done in systems where postzygotic isolating mechanisms limit hybridization, although there is a rich literature using genomic tools and computational models to estimate fitness for hybrid and parental populations (Barton and Hewitt 1985; Gompert et al. 2017). Typically, these studies use field sampling across a zone of hybridization to characterize morphology, rates of gene flow, and relative reproductive success at the population level (e.g., Mckenzie et al., 2015; Zbawicka et al., 2014), but do not directly examine behavior or ecological relationships of free-ranging individuals within the zone. The focus of these studies tends to be on effects of intrinsic barriers (traits that inherently decrease the fitness of hybrid individuals; reviewed in Coyne & Orr, 2004), hybrid inviability (e.g., Fitzpatrick, 2004; Moore, 1951; Nurnberger et al., 1995) or physiological sterility (e.g., Jančúchová-Lásková et al., 2015; Peterson et al., 2005; Volpe, 1960). Thus, we still have a limited understanding of how extrinsic barriers—which stem from how hybrids interact

with their environment, conspecifics, and heterospecific individuals—lead to postzygotic isolation.

Further progress in this area requires a deeper understanding of how an individual's ancestry impacts trait variation, and how that variation mediates survival and reproduction in nature. It is understandable that few relevant studies have been conducted, as tracking individuals within hybrid zones to gather information on their behavior and ecology in nature is logistically difficult for most species. Gene flow and reproduction at the population level is mediated by individual dispersal and spatial ecology, habitat selection, foraging, and mating behaviors—all of which must be measured in parental and hybrid individuals in a comparative fashion. An ideal model system would focus on relatively sedentary species for which researchers have developed detailed field tracking and monitoring approaches at the individual level, and which was also amenable to observation and manipulation under laboratory conditions in large numbers.

A hybrid zone that is uniquely suited for such a study can be found in southwestern New Mexico, U.S.A., where two species of pitvipers, Prairie (*Crotalus viridis*) and Mojave Rattlesnakes (*C. scutulatus*), hybridize across a restricted geographic area (Zancolli et al. 2016). Rattlesnakes have become model organisms for the study of spatial and foraging ecology in nature (Reinert 1984, 1992; Clark 2016). Compared to most vertebrates, these thick-bodied, low-energy specialist predators are relatively immobile, large enough to carry a long-term radio transmitter, and occur at very high population densities (Nowak et al. 2008). The highly seasonal reproductive ecology of

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these species is also well known (Duvall et al. 1992; Aldridge and Duvall 2002; Schuett et al. 2002) and signatures of interspecific hybridization have been found throughout the *C. scutulatus* (Schield et al. 2018) and *C. viridis* + *C. oreganus* (Schield et al. 2019; Nikolakis et al. 2022) complexes, suggesting the prezygotic isolating mechanisms are relatively weak and intrinsic and extrinsic postzygotic isolating mechanisms are shaping the hybrid zones (Nikolakis et al. 2022). These features make the hybrid zone in New Mexico an ideal system to study the effects of postzygotic isolation mechanisms on hybridization. Characterizing this hybrid zone will be a crucial first step in developing a detailed understanding of what factors result in the apparently stable existence of this zone, but also limit it to a narrow geographic area.

My dissertation research is a central piece of a burgeoning research collaboration that is broadly focused on assessing the fundamental question of how species maintain their distinct identity in the face of ongoing introgression. Once we understand the extent and relationship of variation in ecologically relevant behavioral traits, my data will be integrated with further genetic, morphological, venomic, energetic, and thermal ecology data in order to test hypotheses related to fitness tradeoffs that both favor and limit hybrid zones. My research, in combination with these collaborative efforts, has the potential to make this rattlesnake hybrid system a transformative example of hybridization in nature that will address several fundamental questions concerning reproductive isolation and other evolutionary dynamics.

General Methods

Study Sites

The hybrid zone is located within the Cochise Filter Barrier (CFB, modified from Castoe et al. 2007; Figure I.1). The CFB is a transitional region between the Chihuahuan and Sonoran deserts and is frequently implicated in lineage divergence due to climatic shifts induced by glacial cycling. Since the CFB is considered a region of "soft" allopatric divergence, gene flow across the barrier is still possible through the dispersal of some individuals across the region (Pyron and Burbrink 2010). Because there is not a major physical barrier separating the two deserts, the CFB has frequent secondary contact and hybridization between lineages.

Within the CFB the hybrid zone occupies a valley between two mountain ranges, the Peloncillo and Animas, in the extreme southwest of New Mexico, U.S.A. (Figure I.2). Within this valley, and the valleys on either side, there are sporadic homesteads with various amounts of active pasture/agricultural land. Hybrid snakes are found in a narrow band (~12 km) of transitional/mosaic habitat in the center of the valley, with parental populations located on either side of the bordering mountain ranges (Zancolli et al. 2016). On the southwestern side of the hybrid zone the Mojave Rattlesnake (*Crotalus scutulatus*) site (31.891703° N, 109.034757° W) is characterized as a lowland scrub desert macrohabitat. This habitat has large continuous stands of Mesquite, *Prosopis glandulosa,* and patches of tuft grasses, *Aristida* spp.*, Bouteloua* spp.*, Eragrostis intermedia, Erioneuron pulchellum, Hilaria mutica, Koeleria pyramidata, Leptochloa dubia, Lycurus phleoides, Muhlenbergia* spp.*,* and *Sporobolus* spp., interspersed with

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various cacti species, *Opuntia* spp. The northeastern side of the hybrid zone is the site of

the other parental population of Prairie Rattlesnakes (*C. viridis*; 32.259056° N,

108.844943° W). This habitat is generally a short-grass prairie habitat type with similar

plant species to the Mojave Rattlesnake habitat except that the Mesquite is less common

and restricted to a riparian corridor that runs through the site. This corridor was created

by an ephemeral stream that fills with water for a few days after large precipitation events

in the summer during the monsoons and with snowmelt from the mountains in the early

spring. Fourwing Saltbush, *Atriplex canescens*, is the dominant shrub species in the

Prairie Rattlesnake habitat. Within the hybrid zone (32.152532° N, 108.914127° W), in

the middle of the valley, the natural macrohabitat in this site transitions from a Creosote,

Larrea tridentata, dominated lowland desert to an arid short-grass prairie, similar to the

Prairie Rattlesnake habitat, across a southwest/northeast gradient, with large Mesquite

stands in the middle between the two habitat types. Scattered throughout the hybrid zone

are large patches of barren soil that are devoid of almost all vegetation, mammal burrows,

and middens.

Across all three active seasons of data collection, 2019–2021, the average

temperature was 28.0 °C and ranged from 4.67–48.5 °C. For the first data collection

season, late May to late August 2019, the average temperature was 28.7 °C and ranged

from 5.6–45 °C. Total accumulated rainfall for those months was 1507 cm. The second

season, late May to late August 2020, the average temperature was 28.8 °C and ranged

from 5.6–48.5 °C, with a slightly higher total accumulated rainfall of 1780 cm. The last

collection season was from mid-May to early September and the average temperature was

26.8 °C and ranged from 4.7–46.8 °C. Total accumulated rainfall for those months was 1640 cm (https://www.wunderground.com/, station PF01). Across all three years the vast majority of the rainfall was during the monsoon season which started in early to mid-July. All three sites would sporadically flood in lowland spots, leading to a seasonal abundance of annual forbs as ground cover. These plant community shifts were the most dramatic at the Prairie Rattlesnake site and the least at the hybrid site.

Snake Sampling and Surgical Procedures

I collected and sampled all rattlesnakes encountered via road and visual encounter surveys within and adjacent to the hybrid zone. Upon capture I recorded GPS coordinates (precision: ± 5 m) and assigned a putative status (SCVI = hybrid, CRVI = *Crotalus viridis*, and CRSC = *C. scutulatus*) to each individual based on physical features (tail banding pattern, head scalation, and facial coloration). At the end of each night, snakes were transported back to a field station in Rodeo, NM. After processing, I released the snakes at the point of capture. Every snake was processed at the field station by assaying behavioral types (Chapter 3), marking with a Passive Integrated Transponder (PIT) tag, sampling tissue and venom, and measuring morphometrics, coloration, and scalation. In addition to tissue samples, some whole snakes were donated to collaborators in the laboratory of Dr. Castoe of the University of Texas at Arlington (UTA) for functional genomic work and full genome sequencing.

A subset of captured snakes was implanted with very-high frequency (VHF) radio transmitters so they could be tracked for collection and estimation of spatial and hunting

behaviors (Chapters 1 & 2). I anesthetized 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 snake's

body mass. I released snakes at their point of capture after a 24–72 h recovery period.

During recovery, snakes were housed in their own individual containers at a temperature

range of 22–26 °C and provided 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).

Determination of Ancestry

In collaboration with Dr. Castoe's research group, I estimated ancestry coefficients for

each snake following a modified version of the STRUCTURE-based (Pritchard et al.

2000; Evanno et al. 2005; Earl and VonHoldt 2012) approach used by Schield et al.

(2019). We extracted DNA from tissue samples for genomic analyses. We sequenced the

full genomes of a subsample of individuals, and used a double-digest RAD sequencing

approach to quantify hybrid genotypes for the remainder. Both types of genomic data

were included in the STRUCTURE-based pipeline to produce a hybrid index score (HI)

that reflected the percentage of the genome that assigned to each parental lineage. For

analyses using categorical identification of parentage, we classified any snake with an HI

between 5 and 95% as a hybrid (Figure I.3). Seven individuals had HIs estimated using

both whole genome and RAD sequencing in order to cross-validate the RAD sequencing

approach. Estimates between the two were similar (Table I.1), and only one of these seven individuals was classified differently when using the two approaches (this snake had an HI of 0.933 (Hybrid) with full genome sequencing and a 0.999 (Prairie Rattlesnake) with RAD sequencing).

Due to extraction or sequencing failures, a small number of snakes used in various analyses did not have HI estimates, including two of the 40 snakes analyzed for their diet (one putative Prairie and one putative hybrid rattlesnake; Chapter 2). I elected to keep those snakes in the analysis in order to maximize sample size, as my dietary results are descriptive (I did not have sufficient samples for statistical analyses), and I could be confident in my ancestry estimate based on the geographic locale and morphology of these two samples. I also was not able to obtain HI estimates for six of the 185 snakes analyzed for their behavioral types and syndromes (Chapter 3; based on morphology and geography, one putative *C. scutulatus*, three putative *C. viridis*, and two putative *C. scutulatus* x *viridis*). I re-ran all analyses in Chapter 3 with and without these individuals and found no difference in the overall patterns (see Tables A.1–A.3 for analyses without these individuals), and thus chose to report results in Chapter 3 with all samples included.

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Figures & Tables

Figure I.1: Modified rom Castoe et al. (2007), showing the general distribution of the Sonoran and Chihuahuan Deserts of the southwestern United States and Central Mexico. The Cochise Filter Barrier is located at their meeting in southeastern Arizona and southwestern New Mexico. My site is directly at the Cochise Filter Barrier in the southwestern most county of New Mexico, USA (Hidalgo Co.).
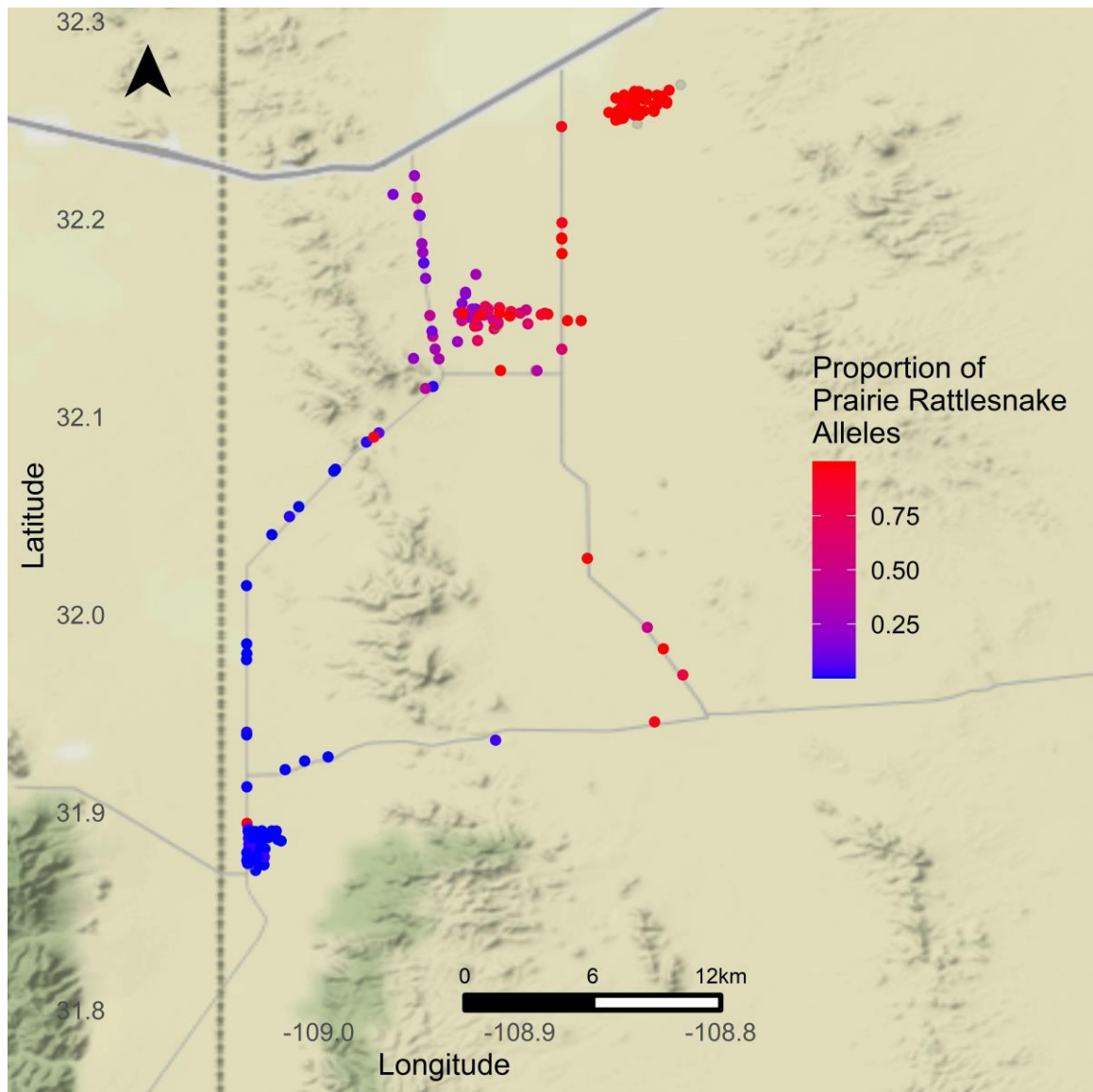
Figure I.2: Hybrid indices (proportion of genome estimated to be derived from *C. viridis*) for all snakes in dataset (n = 195). Colored points indicate snakes that have hybrid indices for them (n = 189; *Crotalus scutulatus* = 67; *C. viridis* = 56; *C. scutulatus* x *viridis* = 66). Greyed out points indicate individuals without a hybrid index estimate that were still included in some portion of my analyses (n = 6; Putative *C. scutulatus* = 1; Putative *C. viridis* = 3; Putative *C. scutulatus* x *viridis* = 2).
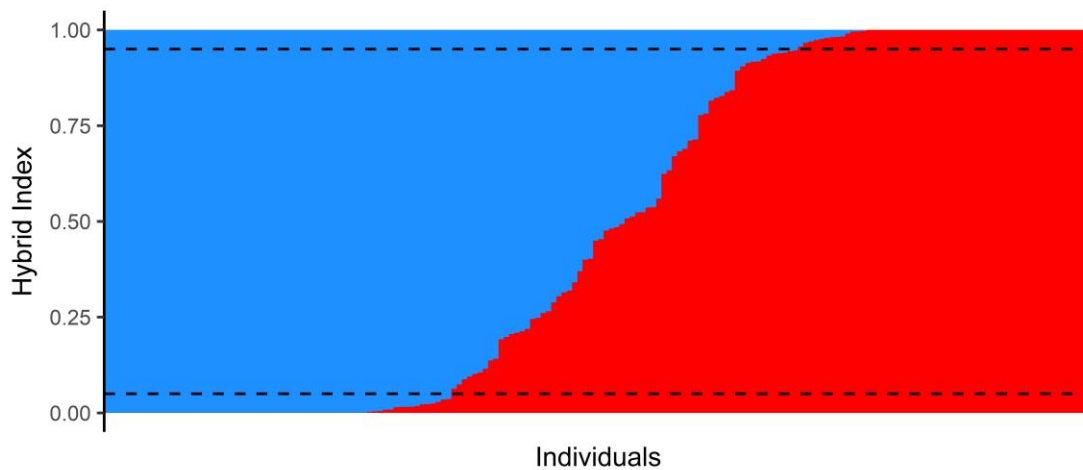
Figure I.3: Hybrid Indices (HI) for all 189 snakes with estimated HIs by either whole genome or ddRAD sequencing techniques. Blue signifies the proportion of Mojave Rattlesnake (*Crotalus scutulatus*) ancestry and red for Prairie Rattlesnake (*C. viridis*). The dashed lines indicate the cut off used for classifying hybrid individuals (0.05 and 0.95).

Table I.1: The seven rattlesnakes that had their hybrid indices (HI) estimated using both Whole Genome (WGS) and ddRAD sequencing techniques.

Snake ID	WGS HI	ddRAD HI
CRSCGG21	0.0153	0.0000
CRSCII21	0.0000	0.0000
CRSCN21	0.0345	0.0000
SCVIEE19	0.9329	0.9999
SCVIF21	0.9663	0.9654
SCVIG21	0.3696	0.4010
SCVIP20	0.9999	0.9999

Chapter 1: Hybrids in space: examining spatial ecology of rattlesnakes across an

interspecific hybrid zone

Abstract

Hybridization between species provides unique opportunities to understand the evolutionary processes that lead to reproductive isolation and speciation. Hybrid individuals often survive and reproduce in only limited geographic areas, and the extrinsic factors that limit zones of hybridization are poorly understood for most systems. For example, although the movement of individual organisms through natural habitats is a fundamental factor shaping survival and reproductive success, studies that compare the spatial ecology of hybrids to parental lineages in nature are relatively rare. Crotaline snakes (pitvipers) exhibit relatively high levels of interspecific hybridization, with apparently few pre-zygotic isolation barriers. Although there is a diverse literature focused on Crotaline spatial ecology, no previous studies have examined how spatial ecology may vary across hybrid zones. My research compares the spatial ecology of individuals across two lineages of hybridizing rattlesnakes, Mojave and Prairie Rattlesnakes, across a naturally occurring hybrid zone in southwestern New Mexico, USA. Using radiotelemetry, I measured the movement parameters and Utilization Distributions (UD) of free-ranging Mojave, Prairie, and hybrid snakes. Generally, individuals in these three lineages were similar to each other. However, Prairie Rattlesnakes had significantly longer durations between movements (1.87 days between moves) compared to the Mojave Rattlesnakes (1.55 days). Hybrid rattlesnakes were similar to parentals in most aspects of movement parameters. Although hybrids did show increased patchiness of core UDs, this is likely related to the increased patchiness of the habitat in areas where they occurred. Overall, I did not find evidence for overt extrinsic

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barriers to hybridization associated with spatial ecology and conclude that the similarities

between the parental species likely increases the probability of hybridization.

Introduction

Historically, research on traits contributing to postzygotic isolation of lineages has been heavily focused on intrinsic factors relating to interactions between genes and the genome, such as hybrid inviability and physiological sterility (General Background). Less work has been done analyzing traits contributing to the extrinsic factors (i.e., interactions between genes and the environment) that lead to postzygotic isolation (General Background). One reason behind this bias is the logistical problems involved with measuring traits in hybrids that contribute to extrinsic barriers, as such traits must be examined *in situ* to understand how they relate to hybridization dynamics. Quantifying details of spatial ecology, foraging ecology, reproductive behaviors, and predation all generally require individuals in nature to be intensely monitored for long durations. For many species, this usually requires some combination of radio/GPS tracking, long term mark-recapture sampling, and detailed behavioral observations of free-ranging individuals, all of which involve significant investments of time and resources. Nevertheless, overcoming these logistical hurdles is necessary in order to evaluate potential extrinsic barriers to hybridization.

The isolation of lineages can be a direct product of the distinct spatial ecology of a species, including the nature and timing of movements related to their resource use and reproductive behaviors. Findings to date focusing on the spatial ecology of hybrids vary depending on the system in question. For example, a study of wolves (*Canis lupus* and *C. lycaon*), coyotes (*C. latrans*), and their hybrids found that social groups of different lineages had spatially segregated home ranges (Benson and Patterson 2013). Although a

similar pattern of spatial segregation was found in two species of woodrats (*Neotoma bryanti* and *N. lepida*) and their hybrids, the spatial segregation was sex-specific, with females exhibiting more separation by lineage than males (Shurtliff et al. 2013). Hybrid lineages can also become more isolated due to an increase in the variability of movements. For example, hybrid Swanson's thrushes (*Catharus ustulatus ustulatus* and *C. u. swainsoni*) were found to have more variability in migratory pathways and locations of overwintering sites than individuals of either parental population. These intermediate routes and overwintering sites were presumably less optimal than those used by parental individuals, resulting in decreased fitness of hybrids (an extrinsic barrier, Delmore and Irwin 2014). Conversely, if the lineages involved in a hybrid zone exhibit overlap in their spatial and temporal use of resources, then gene flow and hybridization between the two parental species can be enhanced. Austin et al. (2019) found that hybrids of Balearic (*Puffinus mauretanicus*) and Yelkouan Shearwaters (*P. yelkouan*) shared similar foraging areas to one parental lineage during the breeding seasons, which presumably led to backcrossing between the spatially overlapping parental and hybrid individuals. These few case studies indicate that a detailed understanding of the spatial behaviors of individuals involved in a hybrid zone can give critical insights into the role of postzygotic isolation and the traits influencing extrinsic barriers, but relevant data is lacking in many systems. An ideal experimental system would focus on species for which researchers have developed detailed methodological approaches for quantifying spatial ecology at the individual level, such as Crotaline snakes.

Crotaline snakes (pitvipers) are somewhat unique among vertebrates in that they exhibit high levels of hybridization and interspecific gene flow, with few apparent pre-mating barriers to hybridization (Zancolli et al. 2016; Schield et al. 2018, 2019; Myers 2021; Nikolakis et al. 2022; Roldán-Padrón et al. 2022). They have also become model systems for the study of spatial ecology (e.g., Reinert and Zappalorti 1988; Dreslik 2005; Waldron et al. 2006; Cardwell 2008; Greenberg and Mcclintock 2008; Hayes et al. 2008; Smith et al. 2009; Roth 2009; Hoss et al. 2010; Degregorio et al. 2011; Davis et al. 2015; Maag et al. 2022). Generally, Crotaline snakes are sit-and-wait ambush hunters of small mammals and lizards that move infrequently over relatively short distances. These movements are typically between sites that offer refuge (Cardwell 2013; Gardiner et al. 2015; Maag et al. 2022) and hunting opportunities (reviewed in Maag and Clark 2022). Movements during their active season (i.e., when not overwintering) are often broken up by multiple days of inactivity (DeSantis et al. 2020). Thus, the home range sizes of these snakes tend to be relatively small, but also variable. Differences between the sexes are common, with males typically exhibiting larger home ranges due to their increased body size and long distance movements during the breeding season (e.g., Cardwell 2008; Degregorio et al. 2011; Bailey et al. 2012). Non-pregnant females show similar patterns of space use, except they do not generally exhibit increased movement during the breeding season. Pregnant females typically move very short distances (Fitch and Shirer 1971; Hamilton 2009; Degregorio et al. 2011; Wastell and MacKessy 2011; Shipley et al. 2013; Maag et al. 2022) when they do move, and prioritize sites for optimal thermoregulation to gestate young (Johnson 1995; Crane and Greene 2008). The

increased movement of reproductively active males is thought to be a reproductive

strategy (Aldridge 1993; Holycross 1995; Schuett et al. 2002), because males moving

longer distances are more likely to locate scent trails of receptive female mates (Duvall et

al. 1992). Both males and females are only active on the surface during the active season

(late spring to late fall depending on the species and population) and typically return to a

hibernaculum (either communally or individually) for overwintering.

Both Prairie (*Crotalus viridis*) and Mojave (*C. scutulatus*) Rattlesnakes generally

follow the typical pattern of Crotaline spatial ecology with a few exceptions. Although

Prairie Rattlesnakes have been studied more intensively than Mojave Rattlesnakes, most

of this work has been focused on northern and high altitude populations of Prairie

Rattlesnakes, which may exhibit very different patterns of seasonal migration (reviewed

in Jorgenson et al. 2008). Northern Prairie Rattlesnakes exhibit variable home range sizes

(0.19–31.4 ha; Macartney et al. 1988; Shipley et al. 2013) and are known for their long

(up to 20 km; Jorgenson et al. 2008) migrations to and from hibernacula (Chiszar et al.

2014). The more limited data on Mojave Rattlesnakes indicates that they have similar

home range sizes (2–52.6 ha; Cardwell 2008), but do not exhibit long migrations to and

from hibernacula.

Although signatures of interspecific hybridization have been found throughout *C.

scutulatus* (Schield et al. 2018) and *C. viridis + C. oreganus* (Schield et al. 2019;

Nikolakis et al. 2022) complexes and extrinsic isolation mechanisms are likely in the *C.

viridis + C. 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 behavior, and ultimately, reproductive success. Infrequent or short distance movements could be insufficient for locating suitable refugia or prey, and more limited movements during the breeding season would negatively impact male reproductive success. Additionally, unsuitable patterns of movement could leave snakes vulnerable to 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 behavior (Maag and Clark 2022).

In order to examine the potential role of movement ecology and space use in hybridization dynamics, I used established telemetery methods (Reinert 1992) to quantify spatial ecology of individuals across the Mojave/Prairie hybrid zone (General Background). More specifically, I quantified daily movement distances, movement frequencies, and the pattern of space use during the active season and analyzed these spatial behavioral patterns in relation to hybrid ancestry. Due to potentially decreased efficiency in the physiological and metabolic traits underlying locomotion or navigation, I hypothesized that hybrid individuals would display transgressive patterns of movements and spatial distribution in comparison to individuals of parental species. Specifically, I predicted hybrids would display shorter and less frequent movements, with smaller Utilization Distributions (UD) than parental snakes, thus contributing to an extrinsic barrier to further hybridization (Zancolli et al. 2016). Hybrid males with these traits

would be expected to be less successful at finding females, which could relate to an extrinsic barrier limiting the geographic extent of the hybrid zone.

Methods

I captured and implanted Very High Frequency (VHF) radio transmitters (Wildlife Materials SOPI-2380) into individual Prairie (*Crotalus viridis*), Mojave (*C. scutulatus*), and hybrid (*C. scutulatus* x *viridis*) rattlesnakes using previously described methods (General Methods). I 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 a week, from mid-May through late-August or early-September (depending on the year). Upon locating a snake, I recorded UTM coordinates with a handheld GPS (MotionX-GPS app v24.4; precision: ± 5 m). I also noted the body position and behavior of the snake when it was visible (moving, within a retreat site, hunting/eating, and resting [found on the surface not moving but not in a stereotyped ambush posture, Reinert et al. 2011, Fig. 4]). I used GPS coordinates and straight-line distances between successive points to quantify movement patterns and estimate spatial Utilization Distributions (UD—a home range is a form of a UD and the terms are sometimes used synonymously, but I use UD because I was not able to track snakes for their entire active season). To account for any lingering effects of the capture and surgery process, only GPS locations after the first location where the snake was actively seen performing surface activity (hunting, moving, or reproductive behaviors) were retained for analyses. In arid ecosystems, heavy rains can drive changes in

ecological communities (Ostfeld and Keesing 2000). Because these sites exhibit strong

seasonal patterns of precipitation, I also examined changes in spatial behavior across the

wet and dry seasons. I determined the onset of the wet season each year using

precipitation data logged by the weather stations in closest proximity to my three field

sites (KNMANIMA5, KNMRODEO1, and KNMLORDS12;

https://www.wunderground.com/; Table A.4).

Statistical Analyses

I 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, I used Paired Wilcoxon Signed Rank tests to

determine if 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 (greater than five unique GPS positions and were tracked for at least a 30 day

period) and to facilitate comparisons to other studies, I estimated their spatial UDs using

100% minimum convex polygons (MCP) as well as 95, 75, and 50% kernel density

estimators (KDE). For KDEs, I used h-ref and *ad-hoc* (Kie 2013) to select the smoothing

parameter (h), and 95, 75, and 50% Brownian-bridge KDEs (bbKDE). I used bbKDE as

the primary estimator to compare the lineages 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 centers.

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 my sampling design because the time between tracking events can vary for extraneous reasons (temporary signal loss, inclement weather, etc.). I also calculated the patchiness of the UDs by counting the amount of discontinuous 50% bbKDEs areas for each snake.

I used either a Linear Model (LM) or Generalized Linear Model (GLM) framework to assess the relationship between an individual's ancestry and its spatial ecology. Genetic lineage (Prairie, Mojave, or hybrid, as determined by its hybrid index; General Methods) was used as an independent variable for all models. For each dependent variable (individual mean daily movement distance, movement frequency, UD size, UD patchiness) I created three models with the following fixed factors: genetic lineage, genetic lineage + Snout-Vent Length (SVL), and genetic lineage*SVL. I included SVL since the length of the snakes could influence their movement due to their serpentine movement. I used Akaike's information criterion (AIC_C) to select which of the three models best fit the data. Due to the limited number of non-pregnant females in the hybrid (n = 4), Prairie (n = 0) and Mojave (n = 5) populations, I used only males for these analyses, but I have reported the data from females for comparative purposes in Table A.5. 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 determine between-lineage differences in variance of the data existed, I used the Bartlett's test of homogeneity of variances when the data fit a normal distribution or could be transformed to fit one, and a 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 lineages, to determine which pairs of lineages were driving the pattern, I performed three additional tests to address all of the pairwise comparisons and adjusted the p-values with the Holm adjustment.

All statistical analyses were done in R (v. 3.6.3, 2020) using the following packages: tidyverse (Wickham et al. 2019), Hmisc (Harrell Jr et al. 2020), gridExtra (Auguie 2017), MuMIn (Barton 2020), adehabitatHR (Calenge 2006), ggplot2 (Wickham 2016), emmeans (Lenth 2021). Values are reported as mean ± 1 SEM.

Results

Overall, 56 snakes were implanted with radio transmitters (22 Mojave (*Crotalus scutulatus*), 16 Prairie (*C. viridis*), and 18 hybrid (*C. scutulatus* x *viridis*); Tables A.6–A.8). However, I was unable to obtain sufficient spatial data for analysis on four hybrid and one Prairie individual. One additional hybrid rattlesnake became pregnant in 2021, and so only her 2020 data is reported. The remaining 51 snakes were tracked on average for 65.8 ± 5.94 days (Mojaves: 47.6 ± 5.15, Prairies: 104 ± 13.1, hybrids: 53.7 ± 7.62), yielding on average 55.3 ± 4.64 spatial points per snake (Mojaves: 39.9 ± 4.43, Prairies: 80.1 ± 9.55, hybrids: 52.9 ± 8.17).

Movements

I was able to measure movement parameters for 42 male snakes (17 Mojave, 15 Prairie, and 10 hybrid rattlesnakes). DMD and FM both conformed to normality after FM underwent a natural logarithmic transformation. For daily distance moved (DMD) the most informative models included the independent variables of genetic lineage and genetic lineage + SVL, so I report the results of the model with genetic lineage as the only independent variable. For the number of days between movements (FM) the model containing only the snakes' lineage as the independent variable had the lowest AIC_C. Males of the three lineages did not significantly differ in DMD (F = 0.304; df = 2, 39; p = 0.739). Averaged across the lineages, males moved 67.2 ± 4.88 m per day (Mojave = 71.9 ± 7.92 m/day; Prairie = 63.4 ± 6.53 m/day; Hybrid = 65.1 ± 12.6 m/day). Male rattlesnakes did differ in their FM (F = 3.68; df = 2, 39; p = 0.034). Mojave Rattlesnakes moved more frequently (one move every 1.55 ± 0.067 days or every 37.2 ± 1.61 hr), than Prairie Rattlesnakes (one move every 1.87 ± 0.098 days or 44.8 ± 2.36 hr; t-ratio = -2.65, p = 0.031). Hybrid rattlesnakes moved no more or less often than Mojave (t-ratio = -0.044, p = 0.834) or Prairie Rattlesnakes (t-ratio = 0.137, p = 0.205), moving every 1.64 ± 0.148 days (or 39.5 ± 3.56 hr). Variation in DMD and FM was not different between the three lineages (K^2 = 2.28, 1.03; df = 2,39, 2,39; p = 0.320, 0.597; respectively; Figure 1.1).

Spatial Utilization

Thirty-three male rattlesnakes (13 Mojaves, 14 Prairies, and 6 hybrids) had sufficient samples for UD estimation. MCP and KDE UDs resulted in similar patterns between the parental and hybrid lineages, thus only bbKDE were used in further analyses of UD area. MCP and the other KDE averages and SEM for the genetic lineages are reported in Table A.9. However, two individuals had insufficient data for Brownian Bridge Kernel Density Estimator (bbKDE) analysis (one Mojave and one Prairie). Additionally, I found that snakes tracked for two consecutive years had similar UD sizes by a Paired Wilcoxon Signed Rank test regardless of if I used the MCPs or bbKDEs ($V = 19, 16$; $n = 10, 10$; $p = 0.43, 0.28$; respectively). Thus, I averaged the respective UD size estimations between the years for those snakes.

The snakes' UD areas conformed to normality after a square-root transformation. The UD models containing genetic lineage and genetic lineage + SVL as independent factors had the lowest AIC_C; therefore, I report the results from the model containing genetic lineage as the only predictor variable. Male UD size estimated by bbKDE did not differ significantly between the lineages ($F = 2.62$; $df = 2, 28$; $p = 0.091$). Male rattlesnakes had an average UD area of 47.9 ± 6.36 ha (Mojave = 34.6 ± 5.83 ha; Prairie = 65.7 ± 11.4 ha; Hybrid = 35.8 ± 13.8). I found no difference in the variance in UD areas between lineages ($K^2 = 2.62$; $df = 2, 28$; $p = 0.270$; Figure 1.2).

The model that best explained the patchiness of the core UDs contain genetic lineage + SVL. The patchiness of the core UDs was significantly different between the lineages ($X^2 = 19.8$; $df = 2, 29$; $p < 0.001$). Patchiness of the core UDs was greater in

hybrid rattlesnakes than either parental population (Prairie: z-ratio = 2.91, p = 0.010; Mojave: z-ratio = 4.17, p < 0.001), and Prairie and Mojave Rattlesnakes both had similarly continuous core UDs (z-ratio = 1.39, p = 0.344). However, no difference in the variation of the patchiness was found between any of the lineages (F = 0.629; df= 2, 29; p = 0.540; Figure 1.3). SVL was also found to have a significant relationship with the snakes' core UD patchiness (X^2 = 4.37; df = 1, 29; p = 0.037), with longer snakes having a less patchy core UD. Examples of a typical UD for each of the lineages are depicted in Figure 1.4.

Seasonal Shifts

Twenty-three male rattlesnakes (4 Mojaves, 14 Prairies, and 5 hybrids) 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 Wilcoxon signed rank tests: V = 0, 21; n = 6, 6; p = 0.031, 0.031; respectively) but not during the wet season (Paired Wilcoxon signed rank tests: V = 5, 14; n = 6, 6; p = 0.313, 0.563; respectfully). Thus, I randomly selected one of the years of each of these six snakes to keep for the analyses. Since some snakes decreased their DMD and/or FM from the dry to the wet season, resulting in negative values, I scaled all of the data by adding a constant to every value and subtracting the smallest negative number of each dataset from each value.

The model examining seasonal changes in snake movement containing only genetic lineage as the independent factor had the lowest AIC_C. The overall change in

snakes' DMD was not significantly different between the lineages ($X^2 = 6.00$; df = 2, 20; p = 0.050; Figure 1.5). Male snakes increased daily movement distance in the wet season by 60.1 ± 11.5 m (Mojave = 26.6 ± 3.70 m; Prairie = 78.1 ± 16.5 m; Hybrid = 36.4 ± 15.3 m). Additionally, I found no differences between the lineages in FM in dry and wet seasons ($X^2 = 0.900$; df = 2, 20; p = 0.639; Figure 1.5). All male snakes increased movement rate in the wet season (average number of days per move decreased by 0.272 ± 0.191; Mojave = 0.139 ± 0.341; Prairie = 0.185 ± 0.231; Hybrid = 0.624 ± 0.573). Lastly, I found no differences in dry and wet season variance in DMD (F = 2.87; df = 2, 20; p = 0.080; I used Levene's test because data was non-normal after transformation) and FM ($K^2 = 1.47$; df = 2; p = 0.480). There was not sufficient tracking data across seasons to investigate seasonal shifts in UD size between lineages.

Discussion

Overall, my analysis of spatial ecology revealed that snakes of hybrid ancestry are broadly similar to parental snakes, exhibiting typical Crotaline patterns of movement frequency, space use, and seasonal variation in movement patterns. These results did not reveal any clearly transgressive or abnormal movement patterns associated with hybridization except in the patchiness of male UDs (Figure 1.3). However, spatial behavior in Crotaline snakes and many other animals is typically associated with resource acquisition, and a heterogeneous distribution of resources across the landscape could result in more patchy utilization distributions.

Pitvipers, like many animals, exhibit preferences for particular habitat features, and the availability of preferred habitat can often drive patterns of space use (e.g., Reinert 1984; Cardwell 2013; Maag et al. 2022). The Mojave/Prairie Rattlesnake hybrid zone spans an area of transition between lowland scrub desert and short-grass prairie; thus, each lineage occupies areas with a different habitat type. Both parental habitats appear more homogeneous than the transitional and heterogeneous habitat found in the middle of the hybrid zone (General Methods; "Study Sites"). The area within the hybrid zone contains intermittent barren patches of soil with sparse vegetation cover and almost no mammal burrows or middens (*Per. Obs.*, D. Maag)—such areas are likely to be largely devoid of prey and habitat structures that offer protection from predators and extreme weather. Therefore, snakes within the hybrid zone are likely restricted to using habitat corridors around these barren patches, which is likely driving the significant increase in UD patchiness (subsequent analyses will quantitatively examine the microhabitat preferences of individuals across this hybrid zone). It is also possible that the increased patchiness found in hybrid rattlesnakes' UDs is an indirect result of fragmented habitat driving a heterogenous distribution of females. Male rattlesnakes exhibit long distance mate searching behaviors during the mating season to find females, and then engage in an extended period of courtship (Schuett and Gillingham 1988), with a prolonged period of attendance before and after coitus as a form of mate guarding behavior (Duvall et al. 1992). Thus, if the females were less uniform in their distribution in the middle of the hybrid zone, then this would be reflected in the patchiness of the male UDs I documented.

Seasonal precipitation patterns were also a dominant feature of the environment throughout the hybrid and parental habitats. Even though lineage was not quite a significant factor (P = 0.050) for the model examining changes in the distance moved per day in the dry and wet season, Prairie Rattlesnakes increased their daily movement distance around twice (78.1 ± 16.5 m) as much as either Mojave or hybrid rattlesnakes (26.6 ± 3.70 m, 36.4 ± 15.3 m, respectively). Since the majority of the Prairie Rattlesnakes tracked during both the dry and wet seasons resided at the Prairie site (60%), the relatively larger change in movement could be due to more exaggerated habitat change (flooding and annual plant growth) I observed at this site compared to the Mojave site and hybrid zone (*Per. Obs.*, D. Maag). The parental lineages have similar mating seasons overlapping the dry-to-wet season transition (Prairie = mid-summer to fall, [Aldridge 1993; Holycross 1993]; Mojave = June to October, [Jacob et al. 1987; Goldberg and Rosen 2000; Schuett et al. 2002; Cardwell 2008]). Both of these characteristics point to the apparent similarity between these species' reproductive strategies and behaviors, which may be a key factor predisposing them to hybridization.

Outside of the breeding season, regular movements between sites were associated with ambush hunting behavior, with snakes of all three lineages exhibiting the typical Crotaline 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. I did find a statistical difference in the frequency of movements between the parental lineages. However, this difference was minor, with the Mojave Rattlesnakes moving every 1.55 days and Prairie Rattlesnakes every 1.87 days. Individuals within lineages were also

highly variable in movement frequency (Figure 1.1), and it is likely that the minor difference in movement is due more to variation in habitat features or other conditions between the sites, rather than intrinsic differences between lineages.

More broadly, differences in hunting behavior can drastically affect the spatial behavior of animals (e.g., Secor 1995; Montgomery 2005). Pitvipers often use ambush hunting as their primary predation strategy (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 Mojave/Prairie hybrid zone (see Chapter 2), possibly contributing to the similarity in their basic spatial ecology as well.

Unfortunately, the limited sample size of adult females precluded statistical analysis or comparisons between the lineages for female spatial ecology. However, as a whole, non-pregnant females moved far shorter distances per day over the active season when compared to 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 ± 0.243 days/move; males = 1.69 ± 0.059 days/move) and had smaller UDs (95% bbKDEs for females = 8.01 ± 3.50 ha; males = 47.9 ± 6.36 ha). This pattern is consistent across a

number of different species of Crotaline snakes, and is thought to be driven by male-specific mating movements (e.g., Cardwell 2008; Degregorio et al. 2011; Bailey et al. 2012).

Although behavioral traits such as habitat preference can shape space use, patterns of space use and movement frequencies also could directly impact the risk of predation. Like many species, rattlesnakes largely appear to rely on crypsis to avoid their own predators, and past studies have found that rattlesnakes appear to be more vulnerable to their own predators when moving between sites rather than when sitting in ambush coils (Maag and Clark 2022). Even though 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.

I not only found that male hybrid rattlesnakes had similar spatial behaviors as the parental species, but also that the parental lineages themselves are broadly similar in spatial ecology. Estimates of home ranges of Prairie Rattlesnake males range between 0.3–31.4 ha (Shipley et al. 2013), and the more limited data for Mojaves indicates a somewhat larger home range of 14.6–52.6 ha (Cardwell 2008). Although my estimates of MCP size (Table A.9) for Mojave rattlesnakes are in line with previous studies, my average MCP estimate for Prairie Rattlesnakes (36.6 ha) is larger than what is typically reported for northern populations of this species, despite the fact that I was not able to include spatial data for the entire active season, possibly reflecting significant intraspecific variation in spatial behaviors of this lineage. Additional metrics also indicate that intraspecific space use can vary substantially between sites. Male Mojave

Rattlesnakes in my study showed longer daily movement distances than members from a California population (71.9 m this study versus 38 m Cardwell 2008), and male Prairie Rattlesnakes move more often (1.87 days between moves this study v. 2.38–4.76 in Jorgenson et al. 2008). Habitat structure, predator communities, and prey resources vary substantially between different geographic 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.

While I didn't find evidence for transgressive or abnormal movement behaviors, other studies have shown that space use and movement behavior can be a critical factor shaping hybridization dynamics. Delmore and Irwin (2014) found hybrid Swanson's thrushes to be intermediate and more variable in their migratory movements when compared to the parentals, which resulted in decreased fitness of the hybrids. Although we did not monitor movements of our snakes outside the summer active season, we did find that all three lineages are similar in daily movement distances, UD sizes, and in their changes in movements from dry to wet seasons, indicating that general movement ecology in our hybrid system is very consistent. Other studies have highlighted the importance of spatial segregation between hybrid and parental individuals and how it affects current hybridization. Hybrids of Balearic (*Puffinus mauretanicus*) and Yelkouan (*P. yelkouan*) Shearwaters that display overlap in their spatial use with the parental lineages promote continued admixture (Austin et al. 2019), while hybrids between two wolf species (*Canus lupus* and *C. lycaon*) and coyotes (*C. latrans*) that are spatially segregated from parental lineages impede it (Benson and Patterson 2013). My sampling

design did not allow me to evaluate this possibility, because I purposefully selected parental sites outside the hybrid zone to maximize the probability that I was comparing hybrids to individuals without admixed genomes. However, I did find overlap between lineages with some parental individuals (both Prairie and Mojave) found within the hybrid zone (Figure I.1; Tables A.6–A.8), making it possible that future studies could more closely examine factors leading to spatial segregation.

A major and unanticipated shortcoming of my analysis was the limited sample of female snakes found in these areas, despite extensive search effort. Females could be more detrimentally affected by hybridization, and critical extrinsic barriers could be linked to aspects of female spatial ecology that I was not able to quantify in detail. Like in all snakes (except for Boids and Pythonids), rattlesnakes follow ZW sex determination in which the females are the heterogametic sex (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). Future research into female movements, reproductive behaviors, and microhabitat selection should build on my current analyses. Overall, my study represents an important initial step in determining what extrinsic barriers may exist that limit the spread and extent of this hybrid zone and is one of only a few studies that explicitly examine the role of spatial ecology in shaping hybridization dynamics.

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Figures & Tables
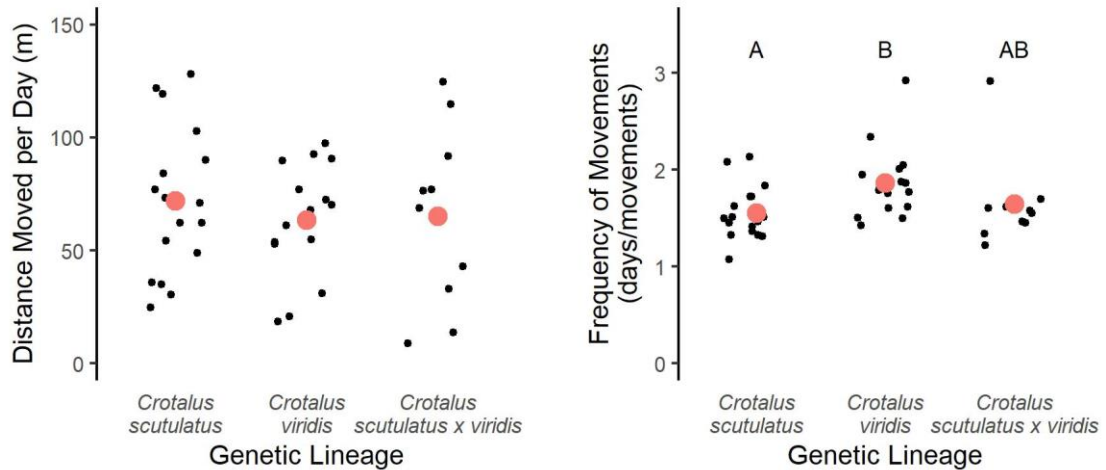
Figure 1.1: Distance moved per day (DMD) and number of days between movements (FM) calculated for each male snake radio tracked. Each black dot represents the overall average for an individual snake and the red dots represent the averages for the lineages. Dots are jittered horizontally to better differentiate individuals from each other. No differences were found in the means of DMD ($F = 0.304$; $df = 2, 39$; $p = 0.739$). Male rattlesnakes did differ in their FM ($F = 3.68$; $df = 2, 39$; $p = 0.034$). Mojave Rattlesnakes moved more frequently, one move every 1.55 ± 0.067 days (or every 37.2 ± 1.61 hr), than Prairie Rattlesnakes, one move every 1.87 ± 0.098 days (or 44.8 ± 2.36 hr; t-ratio = -2.65, $p = 0.031$). Hybrid rattlesnakes moved neither more or less often than Mojave (t-ratio = -0.044, $p = 0.834$) or Prairie Rattlesnakes (t-ratio = 0.137, $p = 0.205$), moving every 1.64 ± 0.148 days (or 39.5 ± 3.56 hr). The variances of these spatial metrics were the same between the three lineages (DMD: ($K^2 = 2.28$; $df = 2, 39$; $p = 0.320$; FM: $K^2 = 1.03$; $df = 2, 39$; $p = 0.597$). DMD and FM were calculated assuming straight-line movements of the snakes.
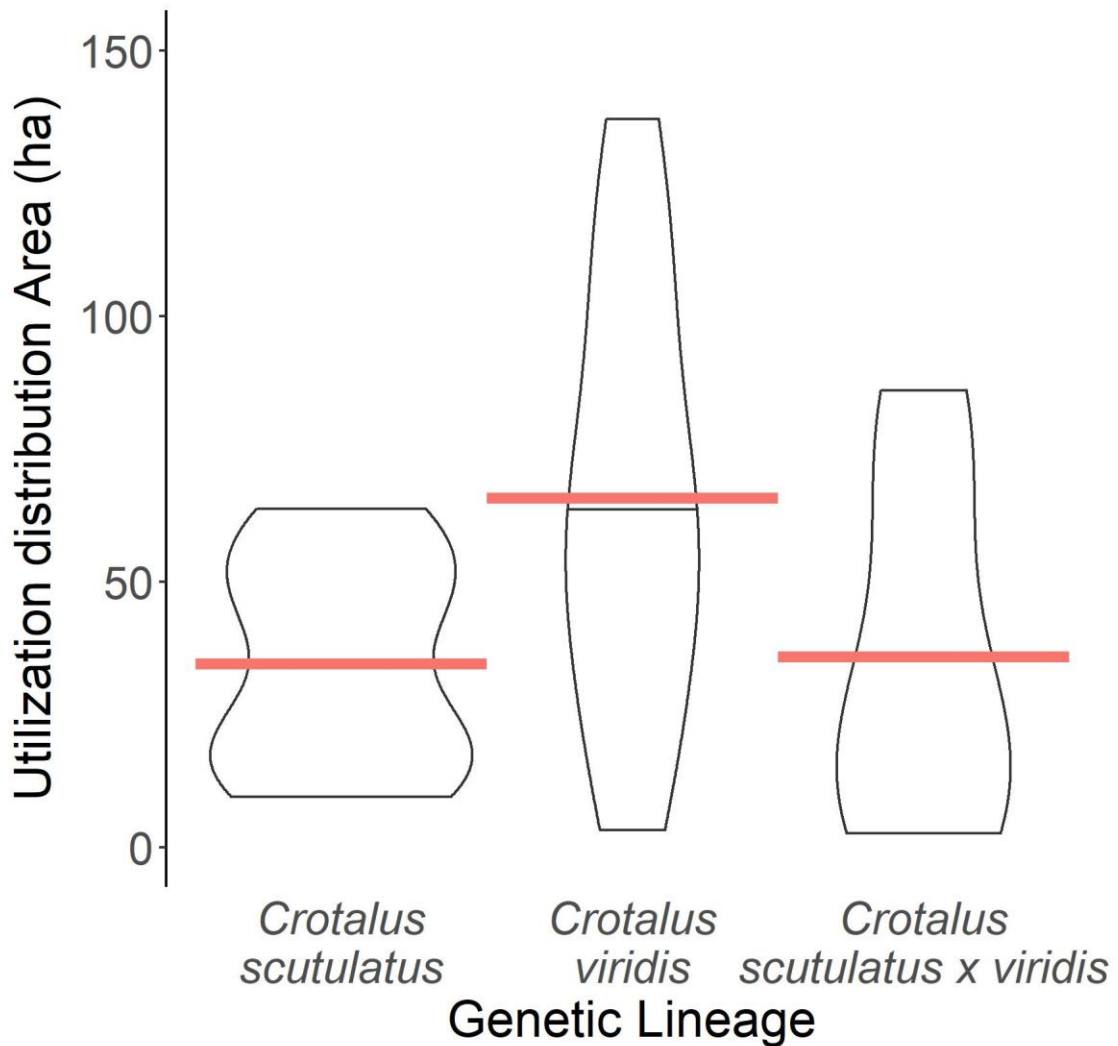
Figure 1.2: Violin plots of 95% Utilization distributions (UD) estimated by Brownian Bridges Kernel Density Estimators (bbKDE) from radio tracked male snakes during their active season, May–September. Size of UDs did not differ significantly across lineages (F = 2.62; df = 2, 28; p = 0.091). Variance was not statistically different between the lineages (K^2 = 2.62; df = 2, 28; p = 0.270). The red line indicates mean values and letters above the box plots indicate statistically significant groupings. Sample sizes: *Crotalus scutulatus* = 12, *C. viridis* = 13, *C. scutulatus* x *viridis* = 6.
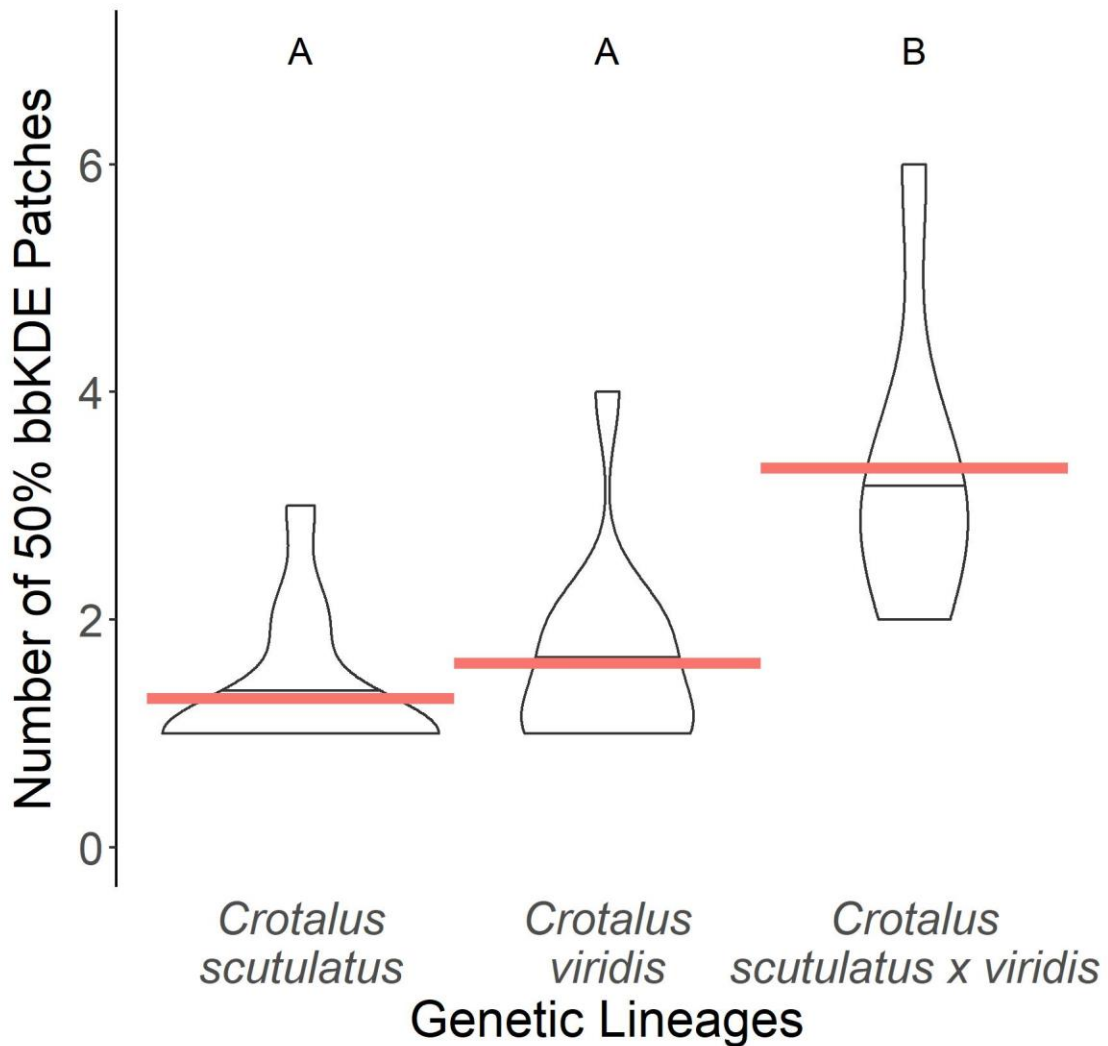
Figure 1.3: Violin plots of the core Utilization distributions (UD) patchiness from radio tracked snakes during their active season, May–September. Patchiness was determined by the number of disconnected 50% bbKDE isopleths. Patchiness of the core UDs were different between the lineages (X^2 = 19.8; df = 2, 29; p < 0.001). *Post-hoc* Tukey tests found that connectivity in the core UDs of the parental linages were similar (z-ratio = 1.39; p = 0.344), while hybrids had more disconnected core UDs (*Crotalus scutulatus* v *C. scutulatus* x *viridis*: z-ratio = 4.17; p < 0.001; *Crotalus viridis* v *C. scutulatus* x *viridis*: z-ratio = 2.91; p = 0.010). No difference in the variation of the patchiness was found between any of the lineages (F = 0.629; df= 2, 29; p = 0.540). Red lines indicate mean values and letters above the box plots indicate statistically significant groupings. Sample sizes: *Crotalus scutulatus* = 13, *C. viridis* = 13, *C. scutulatus* x *viridis* = 6.
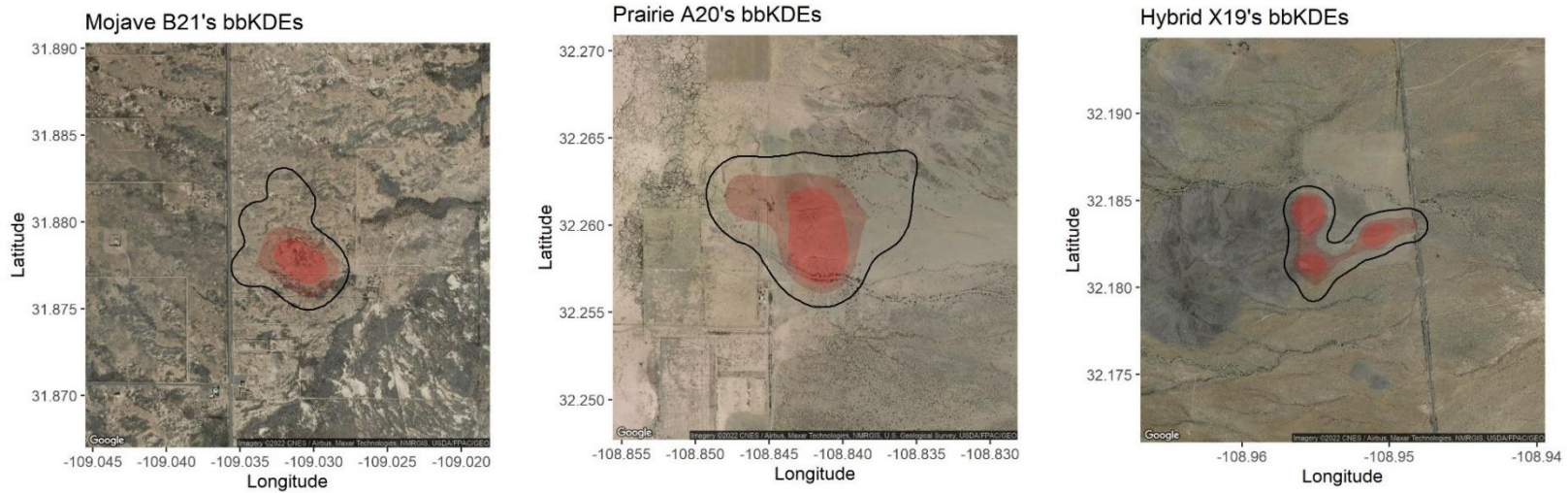
Figure 1.4: Representative Utilization distributions (UD) of a *Crotalus scutulatus* (left), *C. viridis* (middle), *and C. scutulatus* x *viridis* (right) estimated by Brownian bridge Kernel Density Estimators (bbKDE). The black line is the border of the 95% bbKDE, the light red shading is the 75% bbKDE, and the dark red shading indicates the 50% bbKDE.
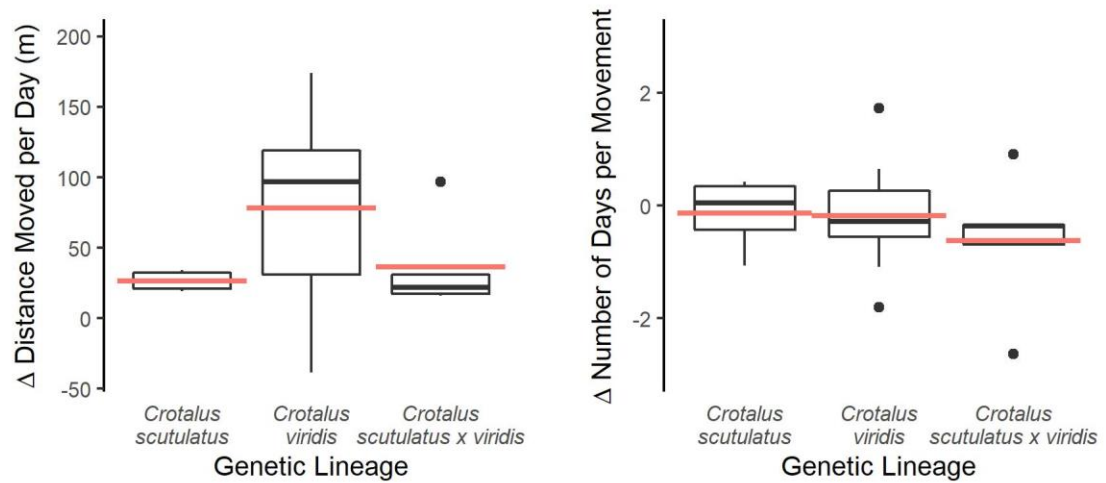
Figure 1.5: Box plots of the change in distance moved per day (DMD) and number of days between movements (FM) between the dry and wet seasons for males within the same year. Red lines indicate mean values. Lineage was not a significant factor affecting the change in DMD ($X^2 = 6.00$; df = 2, 20; p = 0.050) or FM ($X^2 = 0.900$; df = 2, 20; p = 0.639). Variance in DMD and FM was also not significantly different between lineages (DMD: (F = 2.87; df = 2, 20; p = 0.080; FM: $K^2 = 1.47$; df = 2; p = 0.480). DMD and FM were calculated assuming straight-line movements of the snakes. Sample sizes: *Crotalus scutulatus* = 4, *C. viridis* = 14, *C. scutulatus* x *viridis* = 5.

Chapter 2: Comparative analyses of hunting behavior and feeding ecology of Prairie (*Crotalus viridis*) and Mojave (*C. scutulatus*) Rattlesnakes across a hybrid zone

Abstract

Predators must contend with numerous biotic and abiotic challenges to successfully find and capture prey. Thus, traits impacting hunting behavior are often adapted to local conditions and can impact reproductive isolation between lineages. Research on the feeding ecology of animals in hybrid zones has shown that hybrids may exhibit transgressive or novel behaviors that could contribute to post-zygotic isolating mechanisms between lineages, but empirical studies of hunting behaviors across hybrid zones are lacking for many taxa. I undertook the first such study for a hybrid zone between two snake species, (Prairie and Mojave Rattlesnakes, *Crotalus viridis* and *C. scutulatus*), leveraging established methods to quantify hunting behaviors, prey communities, and diet of parental and hybrid free-ranging snakes across the zone in southwestern NM, USA. I found that all three lineages were generally similar in their hunting behavior and diet. However, compared to Prairie and hybrid snakes, Mojave Rattlesnakes (*Crotalus scutulatus*) exhibited significantly more chemosensory activity while hunting at night and abandoned their ambush sites earlier in the morning. Prey availability was also similar across the hybrid zone, with Kangaroo Rats (*Dipodomys spectabilis*, *D. merriami*, and *D. ordii*) as the most common small mammal in both habitat surveys and frequency of encounters with hunting rattlesnakes. Analysis of prey remains in stomachs and feces also showed broad similarity in diets, with all lineages relying primarily on small mammals, and secondarily on lizards as prey. These results suggest that the hunting behaviors of Mojave, Prairie, and their hybrid rattlesnakes do not appear to be contributing to extrinsic barriers to hybridization.

Introduction

All organisms need to assimilate energy from the environment to survive and reproduce.

For predators, their success in acquiring energy depends directly on their ability to find,

subdue, and consume other animals. Anatomical, physiological, and behavioral traits

related to this process are consistent targets of natural selection due to their impacts on

the predator's growth, development, and fecundity (reviewed in Schoener 1971). The

hunting behavior of predators must also account for complex interactions with the biotic

and abiotic environment. Predators not only interact with their prey, but also compete for

resources with conspecific and heterospecifics. Additionally, predators need to contend

with obstacles imposed by the abiotic environment (light levels, geological structure,

terrestrial vs aquatic, etc.) to effectively hunt. Because of their close association with

fitness, the physiological and behavioral traits associated with hunting behaviors could

play a major role in shaping reproductive isolation between closely related lineages. High

levels of interspecific competition between hybridizing lineages could create an extrinsic

barrier to gene flow by reinforcing the partitioning of foraging niches. Conversely, if

interspecific competition is low then extrinsic barriers could weaken due to the relaxed

selection pressure on traits associated with predation.

   Past research on hybrid zones has found important links between feeding ecology

and hybridization dynamics. When parental lineages have similar foraging traits, many

hybrids display a phenotype that is similar to one or both parental phenotypes (Vamosi et

al. 2000; Sas et al. 2005; Peters and Kleindorfer 2015). If competition is high and the

hybrids are less fit, extrinsic barriers to hybridization will increase. Conversely, if there is

a lack of competition and contact between the hybrid and parental individuals as they access the same food source, extrinsic barriers will weaken. When parental lineages differ in their foraging traits, many hybrids display intermediate phenotypes in foraging and diets. However, intermediacy is not synonymous with "maladaptive", as Grant and Grant (1996) postulated that the high survival of Darwin's finches hybrids was due to their ability to better utilize the fluctuating seed banks of the island due to El Nino cycles.

Hybrids may also be intermediate between parentals on average, but much more variable, as increased variation in phenotype is commonly found across hybrid zones (Barton 2001; Mallet 2007; Rieseberg et al. 2007). Thus, hybrid lineages could express more novel or extreme (transgressive) traits compared to parental populations (Rieseberg et al. 1999; Stelkens et al. 2009; Harrison and Larson 2014). If these traits allow hybrids to fill empty transgressive niches, subsequent adaptive evolution could lead to transgressive segregation (Seehausen 2004). For example, transgressive foraging traits can allow the hybrids to exploit food that is unavailable to parental individuals: hybrid cichlids were more efficient than parentals at exploiting novel food types, but less efficient with food types that were routinely encountered by either parental lineages (Selz and Seehausen 2019). Another study found that hybrid gulls (*Larus galucescens* x *L. occidentalis*) had more fish in their diets than parental individuals. The high fish diets of hybrids was thought to have increased growth and survival of their chicks, leading to higher reproductive success of hybrids compared to parental gulls (Good et al. 2000). It is possible that traits associated with feeding represent extrinsic factors shaping hybridization in other systems as well, but data are generally lacking.

The prevalence of hybridization found between several lineages of crotaline snakes (pitvipers) represent a unique opportunity to further explore the relationship between feeding ecology and hybridization dynamics. Pitviper hunting behaviors and diets are relatively well studied due to the development of novel techniques for quantifying hunting behaviors and diet in these systems (Clark 2016). Additionally, hunting efficiency is especially relevant to fitness as crotaline reproductive success is generally food-limited (Taylor and DeNardo 2005; Taylor et al. 2005; Waldron et al. 2013).

Most pitvipers are sit-and-wait ambush hunters that use chemosensory cues to locate an appropriate ambush site. Ambush hunting in pitvipers typically involves a series of behaviors expressed across distinct stages of a predatory encounter. Upon adopting stereotypical coiled body positions (e.g., Reinert et al. 2011, Fig. 4), pitvipers then spend prolonged periods of time waiting in ambush, motionless, with the exception of periodic chemosensory probing or mouth gaping—behaviors related to their continued evaluation of chemical cues from potential prey or predators in the local environment (Barbour and Clark 2012). When potential prey come within range, pitvipers rapidly strike, envenomate, and typically release prey immediately (Hayes 1991; Hayes and Duvall 1991). Pitvipers then use specific chemosensory cues to locate and ingest the carcass of the prey after it has succumbed to envenomation (referred to as Strike Induced Chemosensory Searching, SICS—see Teshera and Clark 2021 for a review). As with many hunting behaviors, this sequence of events involves a series of complex behaviors and decisions that are influenced by the behaviors of prey and constraints imposed by

environmental conditions, hence most predatory encounters are not successful. Typically, rattlesnakes striking at small mammals successfully envenomate their prey in less than half of encounters (Clark 2016; Whitford et al. 2017, 2019). Thus, even relatively minor differences in hunting performance could impact the relative fitness of individual snakes.

Both Mojave (*Crotalus scutulatus*) and Prairie (*C. viridis*) Rattlesnakes hybridizing in southwestern New Mexico (General Methods) specialize on small mammals as prey (Garrigues III 1962; Reynolds and Scott Jr. 1982; Holycross 1993; Rothe-Groleau and Fawcett 2022). However, Prairie Rattlesnakes may also incorporate lizards and, to a lesser extent, amphibians and birds into their diet (Stabler 1948; Ludlow 1981; Hayes 1992; Chiszar et al. 1993; Reed and Douglas 2002). Both species rely on ambush hunting as their primary strategy for prey capture (Hayes and Duvall 1991; Cardwell 2008). Additionally, an experimental study indicated that Prairie Rattlesnakes exhibit an ontogenetic shift in their preference, favoring lizard prey as juveniles and mammalian prey as adults (Saviola et al. 2012).

I integrated a number of approaches to examine the feeding ecology of parental and hybrid snakes across the New Mexico hybrid zone. I hypothesized that hybrid individuals would show transgressive patterns of hunting behavior when compared to the parents. Specifically, I predicted that hybrids would exhibit fewer and less successful prey encounters or lower levels of effort, thus contributing to an extrinsic barrier to further hybridization (Zancolli et al. 2016). I also evaluated the hypothesis that hybrid individuals exhibit a transgressive diet, specializing on prey that are either not present in habitats occupied by parental individuals, or prey that are typically rejected by parentals.

To characterize the hunting behavior of snakes, I tracked free-ranging individuals via radiotelemetery and used fixed-field videography to quantify rates of chemosensory probing and mouth gaping, site abandonment times, outcomes of prey encounters, and prey encounter rates. To compare the availability of principle prey species across different habitats used by snakes, I quantified the relative abundance of small mammals using live trap grids and catch-per-unit effort metrics. Lastly, to examine the diets of individuals, I quantified the relative frequency of mammal and lizard remains in the fecal and stomach contents of rattlesnakes.

Methods

Scaled Mass Index

Using the mass and SVL data from adult (male and non-pregnant female) and juvenile snakes, we calculated the body condition of the snakes using the scaled mass index (SMI), as this has been shown to be a more precise indicator of body condition when comparing individuals across different body sizes (Peig and Green 2009). We treated each "genetic lineage" (i.e., *C. scutulatus*, *C. viridis*, hybrids) as a separate sample when calculating the SMIs and created separate indices for adults and juveniles. To analyze the relationship between body condition and genetic lineage, we used two separate linear models (LM) after verifying normality, one for adult and one for juvenile snakes. For each model, we used SMI as the dependent variable, and genetic lineage (determined by its hybrid index, see below) and sex as independent variables.

Fixed-field Videography

To gather data on the hunting behavior and diet of individuals, I used a modified version of the fixed-field videography approach described in Clark (2016). I used radiotelemetry to locate free-ranging individuals (see Chapter 1 for telemetry details) and then deployed videography units when I found individuals in stereotyped ambush coils (Reinert et al. 2011). Field videography units consisted of a near-infrared (IR) sensitive surveillance camera mounted 1 m from coiled snakes approximately 45° to the left or right side of the head of the snake depending on the local habitat structure. A separate near-IR light was positioned ~3 m from the snake to illuminate a 1 m^2 area with IR light that was visible to the camera but could not be detected by animals (Figure 2.1). Cameras recorded continuously at 1 frame every two seconds and increased to 1 frame per second (fps) when they detected motion in the frame. Videos recorded in this fashion allowed me to calculate rates and outcomes of predatory encounters as well as abandonment times of individuals (snakes in this habitat retreat to thermal refugia during the heat of the day). Cameras were relocated as necessary when snakes changed ambush locations. Video footage was scored independently by two observers blind to the hybrid index of the snakes in order to reduce human error in quantifying relevant metrics. Reviewers extracted data on snake hunting behaviors (chemosensory probing and mouth gaping, abandonment times, and outcomes of prey encounters) and prey encounter rates (Clark et al. 2016). Reviewer scores were averaged to obtain final values; however, when reviewer scores differed in the number or occurrence of snake hunting behaviors, outcomes of prey encounters, or the abandonment times, a third individual independently reviewed the

video footage. If a mistake was found, then the third reviewer's score was used; otherwise, I averaged the behavioral scores of the third reviewer and the reviewer who had the next closest score to create a final score.

To analyze the relationship between hunting behavior and genetic lineage, I used Linear Models (LM) when the data could be transformed to conform to a normal distribution and showed no signs of having differences in variances across the genetic linages. When either of these assumptions could not be met, I used a Generalized Linear Model (GLM) framework. The individual's genetic lineage (determined by its hybrid index; General Methods) was used as the independent variable for all models. For each dependent variable (hunting frequency [proportion of nights that the snake was hunting or hunted while tracked], probing rate, gaping rate, prey encounter rate, strike frequency, successful strike frequency, and abandonment time) I created three models with the following fixed factors: genetic lineage, genetic lineage + Scaled Mass Index (SMI; a measure of body mass correcting for size differences following Peig and Green 2009), and genetic lineage * SMI. I used AIC_C to select which of the three models best fit the data. I included SMI to account for differences in hunting behaviors based on the body condition of the snakes (e.g., a snake with a lower body condition might hunt for longer or more often to try to improve its SMI). When more than one model was within 2 ΔAIC_C of the top model, I chose to only analyze the simplest model (the model with the fewest number of independent variables). Lastly, I used either a Bartlett's test, if the data conformed to normality or could be transformed to normality, or a Levene's test (if the

data had a non-gaussian distribution) to assess if the variation between the three lineages was equal.

Due to the limited sample of individuals, I did not incorporate sex as a factor in the analysis. Past studies of crotaline hunting behavior indicate that the sexes do not differ in the metrics I calculated (reviewed in Clark 2016). I calculated the hunting frequency of the snakes by counting the proportion of nights that a snake was found on the surface in a stereotyped ambush coil and/or eating a food item. Rates of probing, gaping, and prey encounters were calculated from the total amount of video recorded via field videography units for each individual snake. Because past studies on rattlesnakes indicate that probing and gaping rates differ between daytime and nighttime hours (Barbour and Clark 2012), I conducted separate daytime and nighttime analyses for the rates of these behaviors. A prey encounter was counted when a prey item was seen in the field of view of the camera and was in front of the snake (i.e., in the 180° semicircle around the head of the snake with the head positioned at the midpoint of the semicircle). I calculated the individual rate of predatory strikes as the number of strikes towards a prey item divided by the total number of prey encountered by that snake, and successful strike rate as the number of predatory strikes where the snake contacted the prey item divided by the total number of strikes. I calculated abandonment time of day as the time (to the nearest minute) that the snake left the ambush position and moved out of the frame of the camera. Because most behavioral count data were left skewed and zero-inflated, I followed the recommendation of Smithson and Verkuilen (2006) and transformed the data using a beta distribution.

Prey Availability

To determine if all snakes encountered the same types of prey, I only used the prey encounter frequencies from known prey items. I then grouped together all known prey types encountered by snakes while hunting into the following five categories: (1) non-predatory birds, (2) kangaroo rats (*Dipodomys spectabilis*, *D. merriami*, and *D. ordii*), (3) all other rodents, (4) lizards, and (5) toads. Due to the nature of the data, I created four GLMs using the beta distribution with a zero-inflation transformation (Smithson and Verkuilen 2006). Each model had prey encounter frequency as the dependent variable and the following fixed factors: prey + prey:lineage, prey + prey:lineage + SMI, and prey + prey:lineage + SMI+ SMI:lineage. I used AIC_C to select which of the three models best fit the data. When more than one model was within 2 ΔAIC_C of the top model, I chose to only analyze the simplest model (the model with the fewest number of independent variables).

I used trapping surveys to characterize the abundance small mammals, which are the most important class of available prey (both parental species are considered small mammal specialist as adults; Ludlow 1981; Reynolds and Scott Jr. 1982; Holycross 1993; Salazar and Lieb 2003; Zancolli et al. 2019). Trap lines (HB Sherman Live Traps 3310A) were deployed for 4–10 consecutive nights across all three of the sites where snakes were monitored with radiotelemetery (General Methods). Trap lines contained 15–25 trapping stations 15 m apart from each other, each with two traps per station. Traps were opened between 18:30 and 22:00, and were closed between 00:00 and 3:40, depending on the time of sunset. Most traps were baited with sterilized sunflower seeds. However, the traps

at every fifth station the traps were baited with dry cat food to attempt to sample

carnivorous small mammals (e.g., *Onychomys* spp.). Each small mammal captured was

identified to at least the genus level, marked with unique ear tags, and measured for mass,

body length, hindfoot length, and tail length.

I calculated an index of mammal abundance for each trap night and line (number

of unique captures/hours of trapping) for each collection site. The data could not be

transformed to conform to normality, so I created three GLMs with the index of

abundance as the dependent variable and the following combinations of independent

variables: site (Mojave site, Prairie site, or hybrid zone), site + prey (kangaroo rat or not),

and site * prey. I used AIC_C to select which of the three models best fit the data. When

more than one model was within 2 ΔAIC_C of the top model, I chose to only analyze the

simplest model (the model with the fewest number of independent variables).

Lastly, I conducted visual encounter surveys for herpetofauna (presence and

absence of toad and lizard prey species) at all three sites. These surveys were *ad-hoc* and

the effort was broadly similar across the sites. Thus, even though the sampling effort was

equivalent between the three collection sites, I consider these comparisons to be tentative.

Diet analysis

While fixed-field videography for quantifying feeding ecology works well to eliminate

bias due to differential digestion of prey (Glaudas et al. 2017), it can suffer from small

sample sizes. Thus, I combined video diet data with data from other sources. Fecal

samples were collected from animals being held for behavioral analysis (Chapter 3) and

frozen. I then soaked, thawed, and dried samples in 70% alcohol and examined them under a dissecting microscope to identify hairs, teeth, scales, and other prey remains (Salazar and Lieb 2003; Weatherhead et al. 2009; Hamilton et al. 2012). I also palpated and identified any stomach contents from individuals during post-capture processing and recorded any incidental feeding observations seen during field monitoring.

I used BORIS v. 7.4.11 to review video and quantify behaviors (Friard and Gamba 2016). I used R (v. 3.6.3, 2020) for statistical analysis, using the following packages: tidyverse (Wickham et al. 2019), Hmisc (Harrell Jr et al. 2020), nortest (Gross and Ligges 2015), MuMIn (Barton 2020), emmeans (Lenth 2021), betareg (Francisco, Cribari-Neto, Zeileis 2010), car (Fox and Weisberg 2019), ggplot2 (Wickham 2016). When necessary, I performed *post-hoc* multiple comparison tests using a Tukey adjustment. Values are reported as mean ± 1 SEM.

Results

Scaled Mass Index

Body condition (SMI) of adult snakes in the three genetic lineages (*C. scutulatus*, *C. viridis*, and hybrids) differed significantly ($F = 46.1$; df = 2,131, $p < 0.001$; Figure 2.2A), but SMI was not different between the sexes ($F = 2.04$; df = 1,131; $p = 0.155$). Overall, individuals of *C. scutulatus* were in better condition ($> $ SMI) than *C. viridis* or hybrids (*post-hoc* Tukey: t-ratios = 5.44, 9.47; $p < 0.001$, < 0.001, respectively), and *C. viridis* were in better condition ($> $ SMI) than hybrids (*post-hoc* Tukey: t-ratio = 3.70; $p = 0.001$).

Body condition (SMI) of juvenile snakes also differed between genetic lineages

(F = 37.7; df = 2,59, p < 0.001; Figure 2.2B) but not between sexes (F = 0.041; df = 1,59;

p = 0.841). Like the adults, juveniles of hybrids had the lowest body condition compared

to *C. scutulatus* and *C. viridis* (*post-hoc* Tukey: t-ratios = 8.28, 5.54; p < 0.001, < 0.001,

respectively). Unlike the adults, the parental lineages were similar in body condition

(*post-hoc* Tukey: t-ratio = 1.96; p = 0.131).

Hunting behaviors

Out of the 51 snakes radio tracked, I was able to gather hunting data through

fixed-field videography on 40 of them (17 *Crotalus scutulatus*, 13 *C. viridis*, and 10 *C.*

scutulatus x *viridis*). On average I recorded 4.8 ± 0.574 hunting nights for each snake

(2.88 ± 0.382 nights for *Crotalus scutulatus*, 6.31 ± 1.12 nights for *C. viridis*, and 6.10 ±

1.37 nights for *C. scutulatus* x *viridis*).

Snakes in the three lineages did not differ in hunting frequency. Overall, snakes

were found in ambush hunting coils on 60% ± 2.5% of the nights that they were tracked

using radiotelemetry. The most informative models analyzing variation in hunting

frequency were those that contained genetic lineage and genetic lineage + SMI, so I

report results of the model with genetic lineage as the only predictor variable. This model

showed that individuals in different lineages hunted at an equivalent frequency (F = 2.83;

df = 2,50; p = 0.068) and also did not differ in the variance of hunting frequency (K^2 =

2.93; df = 2,50; p = 0.231; Table 2.1).

Snakes in different lineages did exhibit differences in chemosensory behaviors while nocturnally hunting. The most informative models for nocturnal probing were those that contained genetic lineage and genetic lineage + SMI after a log-transformation of the data. Thus, I report results of the model with genetic lineage as the only predictor variable. Overall, the lineages exhibited significantly different nighttime probing rates ($F = 12.0$; $df = 2,37$; $p < 0.001$). Mojaves exhibited 0.113 ± 0.011 probes per min, or one probe every 8.9 minutes. This was significantly more frequent than probes of Prairie (*post-hoc* Tukey: t-ratio = 4.36, $p < 0.001$) and hybrid rattlesnakes (*post-hoc* Tukey: t-ratio = 3.79, $p = 0.002$). Prairie and hybrid snakes had similar probing rates (0.061 ± 0.007 and 0.063 ± 0.006 per min; respectively), which were no different from each other (*post-hoc* Tukey: t-ratio = -0.229, $p = 0.972$). This equates to Prairie rattlesnakes probing once every 16.3 minutes and hybrids probing once every 16.0 minutes (Figure 2.3; Table 2.1).

Regarding nocturnal mouth gaping rate, the most informative models contained genetic lineage and genetic lineage + SMI after a square-root transformation of the data. Therefore, I report the results of the model containing only genetic lineage as a predictor variable. The rate of mouth gaping differed between lineages ($F = 5.36$; $df = 2,37$; $p = 0.009$; Figure 2.4). Mojaves gaped around 1.75 times more often (0.021 ± 0.002 gapes per min or one gape every 48 minutes) than Prairie Rattlesnakes (*post-hoc* Tukey: t-ratios = 3.168; $p = 0.008$), which gaped 0.012 ± 0.002 times per min (once every 83.8 min). Hybrid snakes gaped 0.014 ± 0.002 times per min or once every 72.9 min and were not statistically different from Mojave or Prairie Rattlesnakes (*post-hoc* Tukey: t-ratios =

2.03, -0.853; p = 0.120, 0.673; respectively). I found no between-lineage difference in the variances of either nocturnal probing or gaping rates (K^2 = 0.274, 1.72; df = 2,37; p = 0.872, 0.423, respectively). Daytime probing and mouth gaping rates for all snakes were extremely low compared to nighttime rates, (0.007 ± 0.002 per minute or one probe per 2.5 hour; 0.002 ± 0.001 per min or one gape per 11 hours; Table 2.1) and this small sample precluded statistical comparisons for daytime rates.

The three rattlesnake lineages did not differ in their prey encounter rates or outcomes. For prey encounter rates, the most informative model set included the simplest model (genetic lineage as sole predictor variable). Strike rates and strike success rates were best explained by models containing genetic lineage or genetic lineage + SMI. For all three metrics I report the results from models with genetic lineage as the sole predictor variable. The three lineages were not statistically different in prey encounter rates (X^2 = 0.254; df = 2,36; p = 0.881), strike rates (X^2 = 3.16; df = 2,30; p = 0.206), or successful strike rates (X^2 = 0.924; df = 2,19; p = 0.630). I also found no differences in the variances of these hunting metrics between lineages (Prey encounter rate: F = 0.400; df = 2,36; p = 0.674; Strike rate: F = 0.067; df = 2,30; p = 0.935; Successful strike rate: F = 0.796; df = 2,19; p = 0.466; Table 2.1). Overall, snakes encountered 0.004 ± 0.001 prey per min, or one prey item every 4.29 hours while hunting. During these prey encounters snakes struck $31.7\% \pm 5.87\%$ of the time. Snake strikes were successful (i.e., the strike contacted the prey item) $27.1\% \pm 8.03\%$ of the time. Hence, for every hour of hunting effort, there is a ~2% probability that the snake will successfully strike an encountered prey item.

Snakes from different lineages abandoned their hunting coils at different times of the morning. Because the most informative models for ambush coil abandonment times contained the genetic lineage and genetic lineage + SMI of the snakes as the predictor variables, I report the results of the model contain genetic lineage as the only predictor variable. The lineages differed in the time of day they abandoned ambush sites to seek thermal refuge (F = 7.32; df = 2,36; p = 0.002; Figure 2.5). Mojave Rattlesnakes left their hunting location earliest at an average time of 05:40 (*post-hoc* Tukey: t-ratio = -3.18, -3.26; p = 0.008, 0.007; relative to Prairie and hybrid Rattlesnakes, respectively) while Prairie and hybrid snakes left their ambush sites several hours later (average time of abandonment at 07:37 and 07:49, respectively; *post-hoc* Tukey: t-ratio = -0.306, p = 0.950). I did not find any between-lineage differences in the variances of abandonment times (K^2 = 5.71; 2, 36; p = 0.057).

Prey availability

The most informative models for prey encounters contained only prey type as the predictor variable, indicating that lineages did not differ overall in the type of prey encountered while hunting. Overall, snakes encountered different types of prey (kangaroo rats, lizard, toad, avian, other rodents) at different rates (X^2 = 20.1, df = 4;178, p < 0.001, Figure 2.6). Kangaroo rats were encountered significantly more often than all other prey types (toads, lizards, and other rodents; *post-hoc* Tukey: z-ratios = -3.55, 3.83, 3.15; p = 0.004, 0.001, 0.014, respectively), except for birds (*post-hoc* Tukey: z-ratio = -2.23, p = 0.168). All other prey items were encountered similarly (*post-hoc* Tukey: z-ratios < 1.61;

p > 0.493). Even though birds were frequently recorded with my camera traps, snakes

were never observed striking at them and they were not present in fecal/gut contents (see

below). Thus, I do not consider birds to be important prey items. All other encountered

known prey types were struck at by snakes at similar rates. Snakes struck at 33.8% of the

kangaroo rats encountered, 44.4% of toads encountered, 26.7% of other small mammals

encountered, and 12.5% of lizards encountered. Snakes were also equally successful at

striking all prey types (~1/3 strikes were successful). Kangaroo rats were successfully

struck in eight out of the 24 attempts, toads were successfully struck 2 out of the 4

attempts, other small mammals 1 out of the 4 attempts, and the one strike against a lizard

was not successful.

The most informative model for small mammal abundance included collection

site, prey category (kangaroo rat species or other rodent species), and their interaction as

predictor variables. Small mammal trapping yielded similar abundances of rodent species

across the three sites ($X^2 = 4.09$; df = 2,219; p = 0.130). Reflecting the predatory

encounter rates, kangaroo rats (unique captures: *Dipodomys merriami* = 89.4%; *D.

spectabilis* = 4.98%; *D. ordii* = 4.36%; *D.* spp. = 1.25%) were captured 1.8 times more

often than all other rodent species ($X^2 = 28.3$; df = 1,219; p < 0.001). I found a significant

interaction between the relative abundance of kangaroo rats and trapping site ($X^2 = 20.0$;

df = 2,219; p < 0.001; Figure 2.7). The Mojave Rattlesnake site had an equal abundance

of kangaroo rats and all other rodent species combined (*post-hoc* Tukey: z-ratio = 0.337,

p = 0.999), while the other two sites exhibited 2–4 times more kangaroo rats than all

other rodent species combined (*post-hoc* Tukey: z-ratios = 2.87, 4.73; p = 0.047, < 0.001; respectively).

Visual encounter surveys for small lizards and toads that represent prey items revealed no major differences between the three sites (Table 2.2). The lizard/toad species richness between the sites are almost even with 10 species present at the Prairie and Mojave sites, and 12 at the hybrid site. Although I was not able to estimate the abundance of each species, qualitatively I did not see major differences in lizard/toad abundance.

Stomach Contents and Fecal Samples

I collected and analyzed fecal samples from a total of 33 adult rattlesnakes (Mojaves = 10, Prairies = 12, hybrids = 11) and 20 juveniles (Mojaves = 11, Prairies = 5, hybrids = 4; Table A.10). I also palpated seven prey items from the stomachs of anesthetized snakes (2 juvenile Mojaves, 1 adult Prairie, 2 adult and 2 juvenile hybrid rattlesnakes; Table A.11). These resulted in a total of 66 individual prey items, all of either mammalian (46) or squamate (lizard; 20) origin (Figure 2.8). Due to sample size limitation within each lineage, I did not perform any statistical tests between the lineages. However, snakes of all lineages had similar diets within age classes (Figure 2.8), and when compared as a whole (i.e., all lineages combined), adults and juveniles differed in diet ($X^2 = 6.47$; df = 1; p = 0.011). Juveniles relied equally on lizards and mammals as prey (51.7% of prey items of juveniles are small mammals), whereas adults shifted to a primarily mammalian diet (83.8% of prey items of adults are small mammals).

Discussion

Overall, I found that the feeding behavior, prey availability, and diet of Mojave, Prairie, and hybrid rattlesnakes was remarkably similar, with only minor differences among the three lineages and geographic locations. Snakes at all sites exhibited comparable rates of hunting behavior, encountered and successfully attacked prey at similar rates, and had broadly overlapping diets. Interestingly, we found that the body condition index of hybrids fell below values of the parental species, indicating that they were less robust insofar as general health. Generally, this pattern indicates that factors other than differences in hunting behavior or diet underlie the relatively poorer body condition of hybrid snakes.

The hunting ecology of rattlesnakes can drastically affect their reproductive success (Taylor and DeNardo 2005; Taylor et al. 2005; Waldron et al. 2013). Thus, differences in the hunting efficiency and diet of individuals can result in differences in their relative fitness. However, conversely to my hypothesis, hybrid rattlesnakes did not display transgressive patterns of hunting behavior. Rather, hybrids were either no different than parental individuals or very similar to Prairie Rattlesnakes when parental lineages differed in some metric. Prairie and hybrid rattlesnakes exhibited similar rates of chemosensory behavior while in ambush (chemosensory probing and gaping rates, Figures 2.3 & 2.4). Chemosensory probing is thought to be a mechanism rattlesnakes use to continually reevaluate hunting locations by sampling local chemical cues with the vomeronasal organ (Barbour and Clark 2012). Mouth gaping appears to be functionally related to chemosensory probing, apparently serving to clear the vomeronasal organ,

located on the roof of the mouth (Graves and Duvall 1985). Barbour and Clark (2012) proposed that increased rates of probing during nighttime resulted both from snakes compensating for reduced visual information and from body movements being less conspicuous to visually-oriented snake predators at night. My study represents the first direct comparison of the rates of these behaviors across populations or species. Surprisingly, I found that Mojave Rattlesnakes had consistently higher frequencies of chemosensory probing and mouth gaping during the night than Prairie Rattlesnakes. It is unclear why this might be the case, but it may be indicative of underlying differences in sensory systems that are not yet understood. Although comparative data on interspecific variation in crotaline sensory systems are generally lacking, pitvipers do vary in the external and internal anatomy of their facial pits (IR sensory organs), which affects their sensory fields and causes small differences between species in the spatial resolution of the system (Bakken et al. 2012). Additionally, outside of pitvipers, a number of other closely related predator species have been shown to differ in sensory ability: different species of water snakes (*Nerodia* spp.) vary in the size of their eyes in relation to the proportion of frogs in their diets. The difference in eye morphology indicates that increased visual sensitivity may coevolve with specialization on a prey item with an explosive saltatory escape response (Camp et al. 2020). Differences in visual systems or acuity have also been found across closely related species of Darters (*Etheostoma* spp.), cichlids (*Pundamilia* spp.), Tamarins (*Saguinus* spp.), and a number of bird and lanternfish species (Carleton et al. 2005; Surridge et al. 2005; Gumm et al. 2012; Martin 2017). Thus, although further research would be needed to confirm, it is possible that

Prairie and hybrid rattlesnakes, differ from Mojave Rattlesnakes in some aspects of chemosensory or visual acuity that influence their relative frequency of chemosensory investigation while in ambush. Although increased movements associated with chemosensory probing might be expected to make individuals less cryptic, I found no evidence of a functional tradeoff associated with higher rates of chemosensory behaviors—the more active Mojave Rattlesnakes exhibited similar encounter rates and outcomes with potential prey (Table 2.1) and encountered the fewest predators compared to other lineages (Maag and Clark 2022).

Prey encounters and outcomes were similar across rattlesnake lineages. Given that all three sites also had an equivalent abundance of small mammals (Figure 2.7), hybrid snakes seem to be just as effective as parentals at locating profitable ambush sites. Furthermore, hybrid rattlesnakes exhibited similar strike rates and strike success rates when encountering prey (Table 2.1). Across all lineages, kangaroo rats were the most frequently encountered prey type (Figure 2.6). The success rate of rattlesnakes in my study when striking toward kangaroo rats (33.3%) was also similar to strike success rates of sidewinder rattlesnakes (*Crotalus cerastes*) attacking kangaroo rats (34.8–46.9%; Whitford et al. 2017, 2019). The similarities between the primary prey (kangaroo rats) and hunting efficiency for Sidewinders, Prairie, Mojave, and hybrid rattlesnakes suggests that rattlesnake hunting behaviors may be broadly conserved across the genus.

I found that lineages differed in their abandonment times, indicating a significant difference in daytime hunting frequencies. Mojave Rattlesnakes tended to abandon their ambush coils earlier in the morning than either Prairie or hybrid rattlesnakes (Figure 2.5).

The small mammals that make up the bulk of the diet of all three lineages are nocturnal, whereas lizards eaten occasionally by these snakes are exclusively diurnal. Thus, the tendency to remain in ambush into the daylight hours exhibited by Prairie and hybrid rattlesnakes could reflect an increased reliance on lizards as prey items. The preliminary data from dietary analyses support this pattern (Figure 2.8), as adult Mojaves had the lowest proportion of lizards in their diet overall. However, dietary data was relatively limited in sample size, and statistical analyses of these patterns would require a larger sample of fecal or stomach contents. Nevertheless, the initial pattern in both behavior and diet indicates a potentially important fundamental difference between the lineages, with Mojaves showing increased reliance on mammalian prey and Prairies and hybrids showing a stronger tendency to use morning hours to hunt diurnal lizards.

I also found that the relative abundance of small mammals (Figure 2.7) and richness of the ectothermic prey types (toads and lizards, Table 2.2) was similar between each of the prey communities at the three sites. Across all sites, kangaroo rats were the most abundant rodents in both trapping surveys and field encounters with snakes. Although birds were commonly seen in the vicinity of snakes via videography (Figure 2.6), I never recorded predatory strikes by snakes at birds, and no bird remains were present in stomach or fecal samples. Although past studies have found that Prairie Rattlesnakes occasionally prey on birds (Ludlow 1981; Hayes 1992), such occurrences could be relatively rare, or derived from scavenging (Ryan Hanscom, *Per. Comm.*) or nest raiding (*Per. Obs.*) rather than ambush hunting.

Both of the parental species are frequently characterized as rodent generalists as adults, with an ontogenetic shift away from lizards as prey of juvenile snakes (Garrigues III 1962; Reynolds and Scott Jr. 1982; Holycross 1993; Rothe-Groleau and Fawcett 2022); my data generally support this pattern. Further exploration of the potential differences between lineages would require more detailed behavioral data on the foraging ecology of juvenile snakes—data that are generally lacking due to constraints on the size of radio transmitters—as well as an increased sample size for each of the lineages.

Some shortcomings of my approach to quantifying hunting behaviors are inherent to fixed-field videography. Once snakes struck and released prey items they generally left the field of view of the camera while using chemosensory trailing behavior to locate and ingest prey carcasses. Thus, the frequency and timing at which prey succumbed to venom are unknown. Prey can survive envenomation through either physiological venom resistance (Robinson et al. 2021) or rapid escape which physical removes the snake, lowering the time the snake has to inject its venom (Whitford et al. 2017, 2019). The process of chemosensory trailing to locate prey carcasses can also be prolonged, and occasionally lead to failure on the part of the snake to locate the carcass (Teshera and Clark 2021). Thus, it is possible that differences exist between the lineages at these stages of the hunting process, and additional field methodologies would be required to evaluate this possibility (such as animal-borne accelerometry; see Hanscom et al. 2023).

Overall, the parental species hybridizing in this area are generally similar in their hunting behaviors and diet, with hybrids largely resembling one or both of the parental lineages in different metrics of foraging ecology. Comparable patterns have been reported

in other hybrid systems. Hybrids and parentals of two species of Darwin's finches (*Camarhynchus parvulus* and *C. pauper*) were found to be broadly similar in their foraging behaviors, with parental lineages differing only in the canopy height at which they foraged. In this system, hybrids foraged at heights matching one of the parental lineages, *C. parvulus* (Peters and Kleindorfer 2015). Similarly, hybrid sticklebacks (*Gasterosteus aculeatus*) were shown to have high degrees of dietary overlap between both the benthic and limnetic parental individuals (Vamosi et al. 2000). Additionally, hybrids between two toads (*Bombina bombina* x *variegate*) were found to more closely resemble one of the parental species (*B. bombina*) in terms of prey diversity in diet (Sas et al. 2005).

The general pattern of hybrids using prey resources or exhibiting foraging behaviors that match one parental lineage, but not the other, could have a number of implications for understanding extrinsic barriers to hybridization. In systems where food resources drive spatial behaviors, hybrids and parentals that exhibit similarities in foraging ecology could encounter each other more frequently, leading to increased back-crossing within one parental lineage. Because hybrid zones are often found in novel or transitional habitats where resources are unique, hybrids that specialize on a particular resource would be expected to have much more limited success in parental habitat types (bound hybrid superiority; Arnold and Hodges 1995; Arnold and Martin 2010), limiting the degree of spatial overlap and introgression into the associated parental lineage. However, when individuals are found in multiple types of habitats with variable food resources then habitat type, rather than lineage *per se*, would be expected to drive

variation in feeding behaviors. For example, hybrid woodrats (*Neotoma bryanti* x *N. lepida*) were found to have diets that were more dependent on habitat than lineage (Nielsen et al. 2023). Neither of these factors appears to be a major extrinsic barrier to hybridization in the Mojave/Prairie Rattlesnake hybrid zone, as I found that the prey communities are similar in abundance and composition across the region. Additionally, individuals from all three lineages have been found within the area of most frequent hybridization, implying that hybrid individuals spatially overlap with both parental types (Chapter 1). Thus, it is unlikely that hunting behavior or feeding ecology shape hybridization dynamics in this snake system. Given that our study revealed that both adult and juvenile hybrids were in significantly poorer body condition when compared to parental snakes of either lineage, perhaps metabolic efficiency or other intrinsic physiological processes are driving differences in fitness and post-zygotic lineage isolation, an intriguing area of research that should be undertaken in future studies.

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Figures & Tables

Figure 2.1: A typical example of the fixed-field videography unit used to study foraging ecology. The set-up consists of a near-infrared (IR) sensitive surveillance camera mounted 1 m from coiled snakes approximately 45° to the left or right side in front of the snake's head followed by a separate IR light positioned around 3 m away from the front of the snake to illuminate a 1 m^2 area around the snake. This unit is recording a Prairie Rattlesnake (*Crotalus viridis*), with the snake in the lower right corner of the photograph.
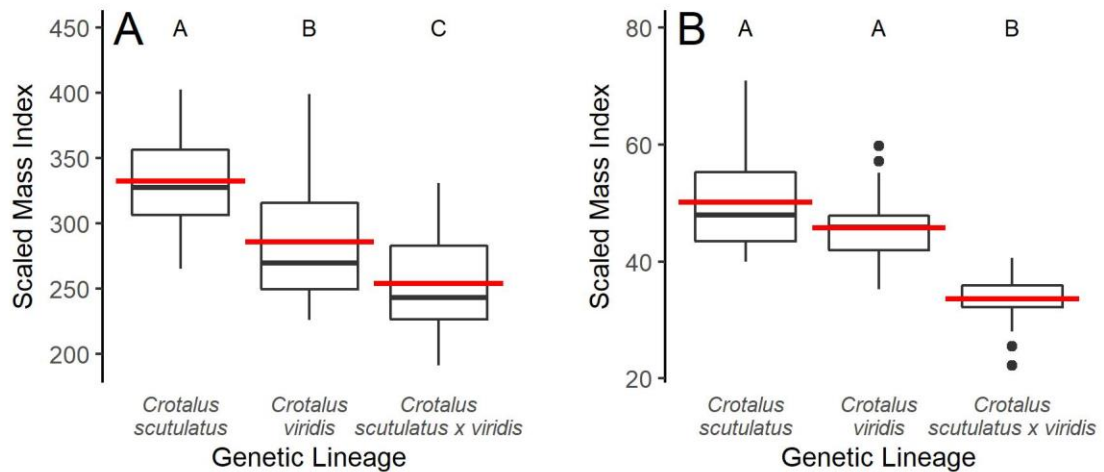
Figure 2.2: Box plots of body condition (SMI) for adults (A) and juveniles (B). Body condition of adult and juvenile snakes in the three genetic lineages differed significantly (F = 46.1, 37.7; df = 2,131, 2,59; p < 0.001, < 0.001; respectively). However, no differences in body condition were detected between sexes in either age group (F = 2.04, 0.041; df = 1,131, 1,59; p = 0.155, 0.841; respectively). Within adults, Mojaves were in better body condition than either Prairie or hybrid rattlesnakes (*post-hoc* Tukey: t-ratios = 5.44, 9.47; p < 0.001, < 0.001, respectively) and Prairie Rattlesnakes were in better condition than hybrids (*post-hoc* Tukey: t-ratio = 3.70; p = 0.001). Within juveniles, Mojave and Prairie Rattlesnakes did not differ in body condition (post-hoc Tukey: t-ratio = 1.96; p = 0.131), while hybrids were in worse condition than either parental lineage (post-hoc Tukey: t-ratios = 8.28, 5.54; p < 0.001, < 0.001, respectively). Red lines indicate lineage means. Letters above boxplots indicate statistically significant groupings. Sample sizes: Adults—*C. scutulatus* = 45, *C. viridis* = 41*, C. scutulatus* x *viridis* = 49; Juveniles—*C. scutulatus* = 23, *C. viridis* = 16*, C. scutulatus* x *viridis* = 24.
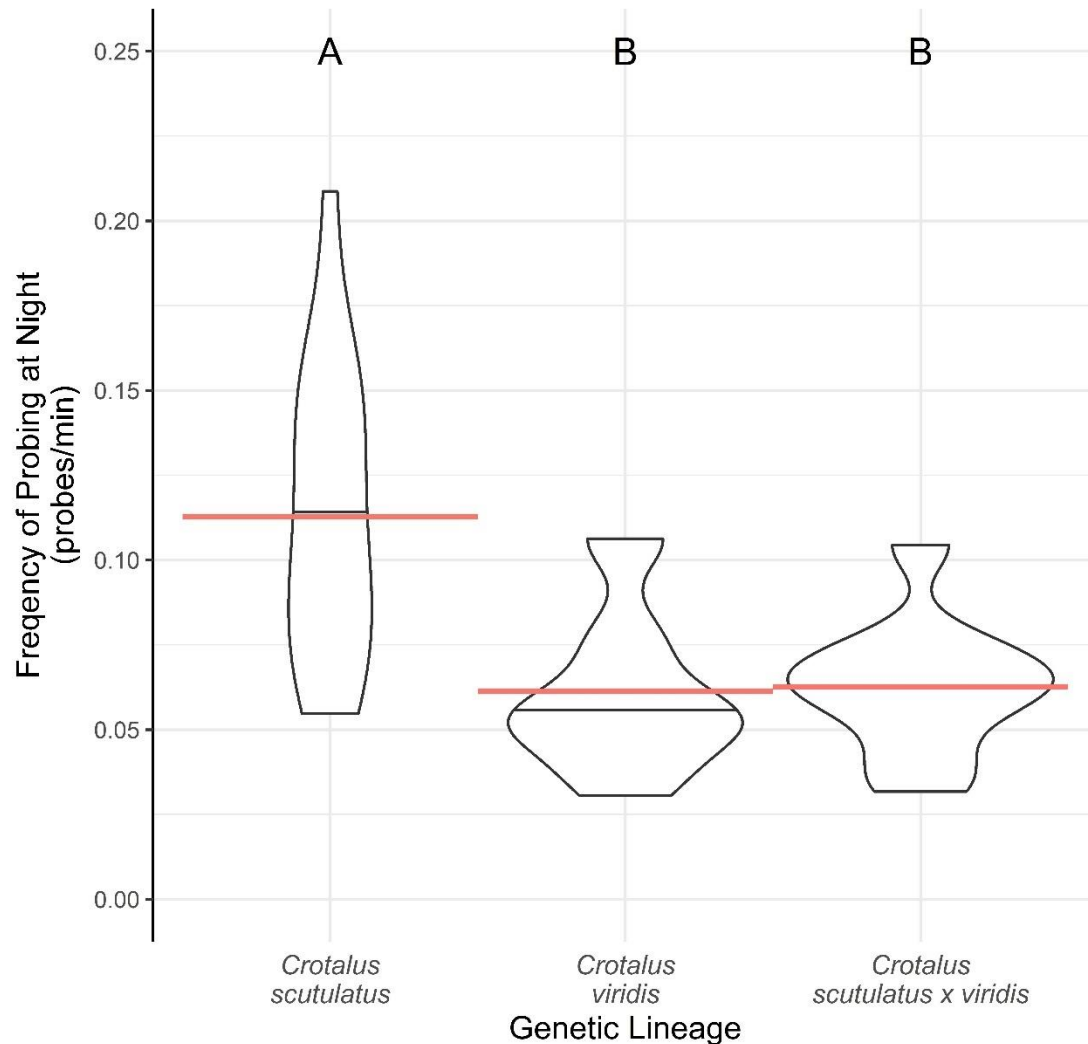
Figure 2.3: Violin-plots of rate of chemosensory probing while hunting at night in ambush coils. The probing rate for each individual was calculated by dividing the total number of times a snake probed during the nocturnal hours by the total amount of nighttime foraging effort. The genetic lineages exhibited different probing rates (F = 12.0; df = 2, 37; p < 0.001). *Crotalus scutulatus* probed more often than *C. viridis* (*post-hoc* Tukey: t-ratio = 4.36, p < 0.001) and *C. scutulatus* x *viridis* (*post-hoc* Tukey: t-ratio = 3.79, p = 0.002), which were no different from each other (*post-hoc* Tukey: t-ratio = -0.229, p = 0.972). Variance was not different between the lineages (K^2 = 0. 274; df = 2, 37; p = 0.872). Red lines indicate the lineage mean. Letters above the violin-plots indicate statistically significant groupings. Sample sizes: *C. scutulatus* = 17, *C. viridis* = 13*, C. scutulatus* x *viridis* = 10.
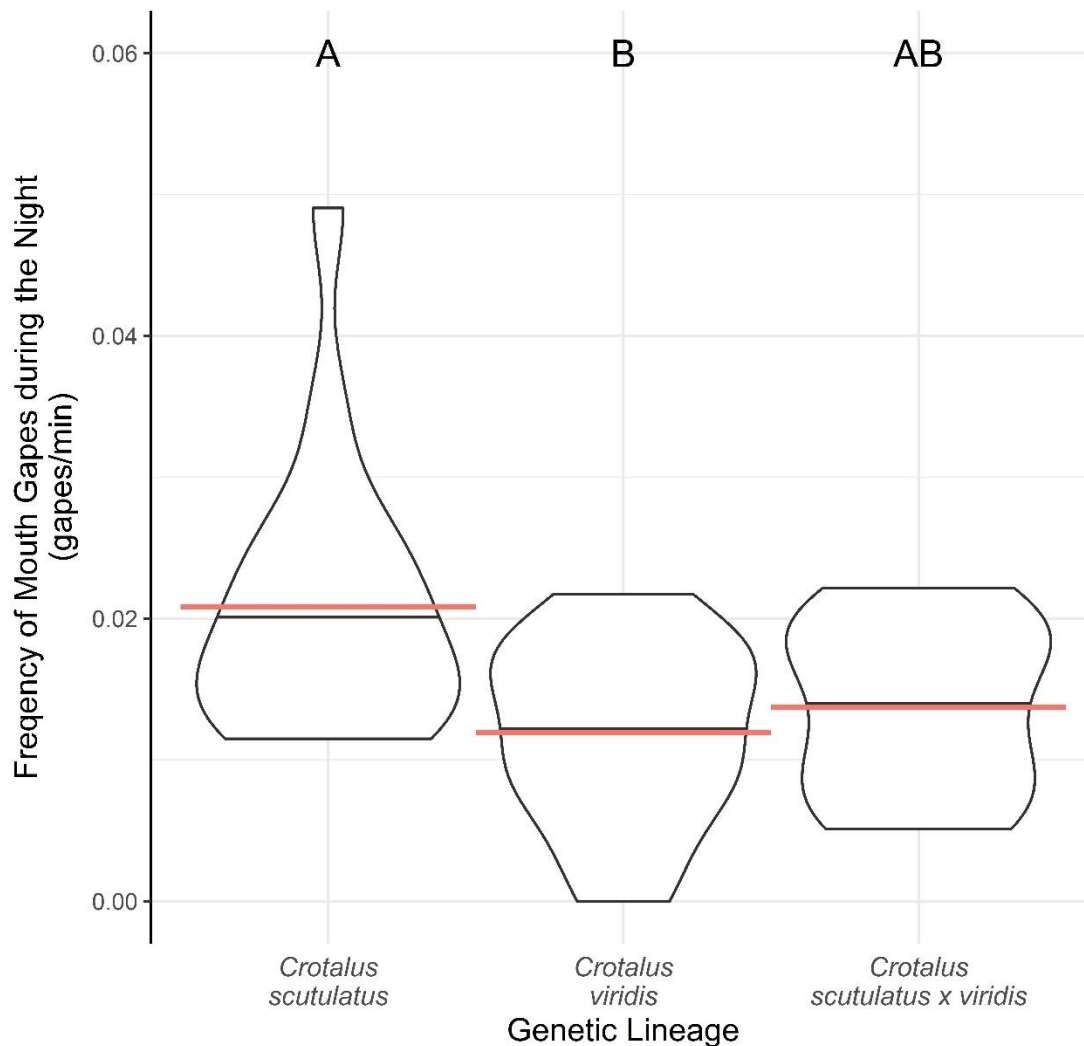
Figure 2.4: Violin-plots of mouth gaping while hunting at night on the surface of the habitat. The gaping rate for each individual was calculated by dividing the total number of times a snake mouth-gaped during the nocturnal hours by the total amount of nighttime foraging effort. The genetic lineages displayed different gaping rates (F = 5.36; df = 2, 37; p = 0.009). *Crotalus scutulatus* gaped more often than *C. viridis* (*post-hoc* Tukey: t-ratio = 3.168; p = 0.008) but not *C. scutulatus* x *viridis* (*post-hoc* Tukey: t-ratio = 2.03, p = 0.120), which were no different from each other (*post-hoc* Tukey: t-ratio = -0. 853, p = 0.673). Variance was not different between the lineages (K^2 = 1.72; df = 2, 37; p = 0.423). Red lines indicate the lineage mean. Letters above the violin-plots indicate statistically significant groupings. Sample sizes: *C. scutulatus* = 17, *C. viridis* = 13, *C. scutulatus* x *viridis* = 10.
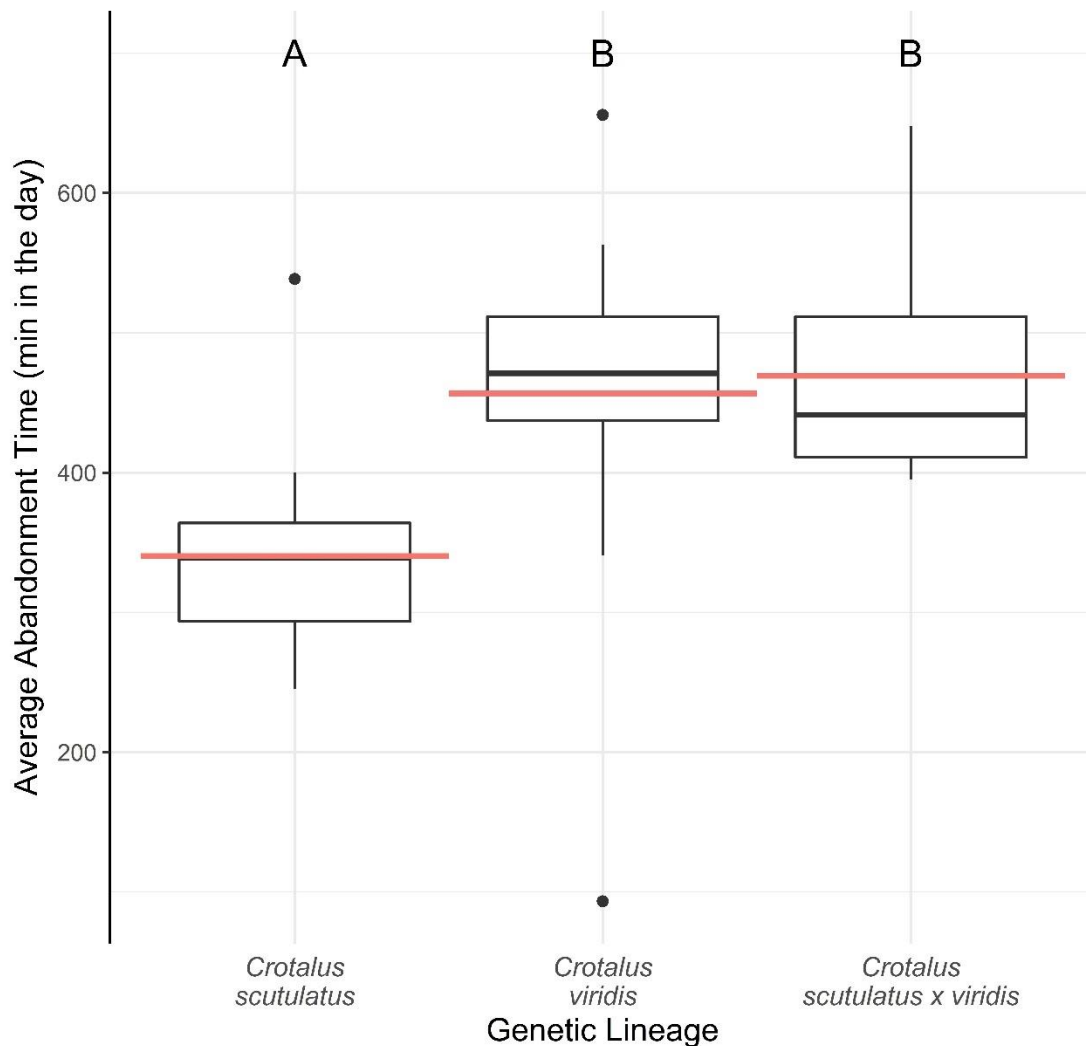
Figure 2.5: Box plot of the average time of day (minutes after midnight) that individual snakes abandon their ambush coils. The genetic lineages differed in abandonment time (F = 7.32; df = 2, 36; p = 0.002). *Crotalus scutulatus* abandoned ambush sites at an average of 5:40, earlier than *C. viridis* (*post-hoc* Tukey: t-ratio = -3.18, p = 0.008) and *C. scutulatus* x *viridis* (*post-hoc* Tukey: -3.26, p = 0.007). *C. viridis* and *C. scutulatus* x *viridis* were no different from each other (*post-hoc* Tukey: -0.306, p = 0.950), abandoning on average at 7:37 or 7:49, respectively. Variance was not different between the lineages (K^2 = 5.71; 2, 36; p = 0.057). Red lines indicate the lineage mean. Letters above the violin-plots indicate statistically significant groupings. Sample sizes: *C. scutulatus* = 16, *C. viridis* = 13, *C. scutulatus* x *viridis* = 10.
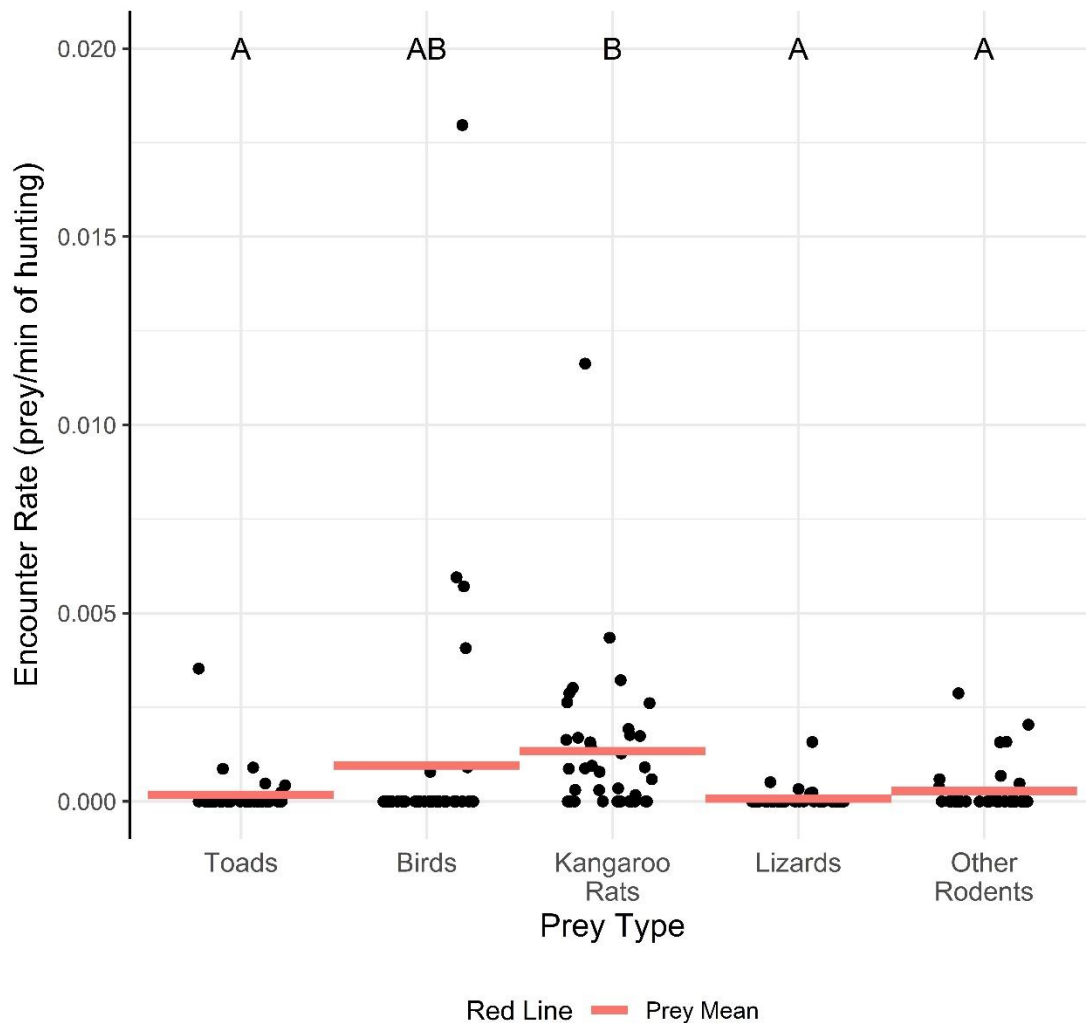
Figure 2.6: Strip chart of prey encounter rates. Overall, rattlesnakes in the three lineages encountered prey types at different rates ($X^2 = 20.1$; df = 4, 178; p < 0.001). Kangaroo Rats (*Dipodomys* spp.) were encountered significantly more than all other prey types (toads, lizards, and other rodents; *post-hoc* Tukey: z-ratios = -3.55, 3.83, 3.15; p = 0.004, 0.001, 0.014; respectfully) except for birds (*post-hoc* Tukey: z-ratio = -2.23, p = 0.168), while all other prey items were encountered by rattlesnake similarly (*post-hoc* Tukey: z-ratios = -1.32, 0.286, -0.397, 1.6062, 0.924, -0.683; p = 0.678, 0.999, 0.995, 0.493, 0.888, 0.960). Red lines indicate the mean encounter rate of the given prey type for 37 rattlesnakes. Letters above the box plots indicate statistically significant groupings.
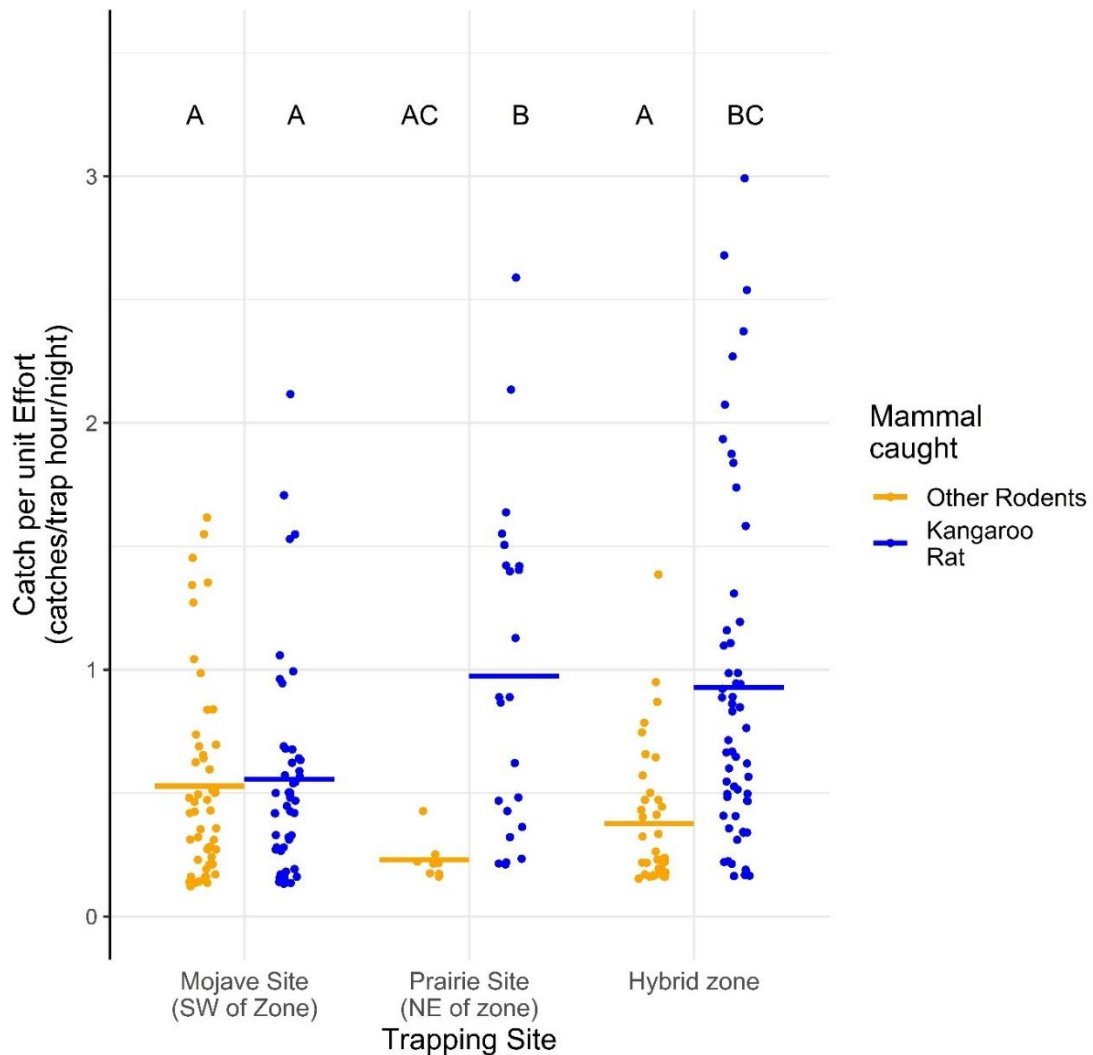
Figure 2.7: Strip chart of the nightly catch per unit effort for small mammal prey types surveyed at each of the three sites. No difference was found between the sites in overall small mammal abundance ($X^2 = 4.09$; df = 2, 219; p = 0.130). Kangaroo rats (*Dipodomys merriami*, *D. ordii*, and *D. spectabilis*) were captured 1.8 times more often than all other rodent species ($X^2 = 28.3$; df = 1, 219; p < 0.001), but this pattern differed by site (site:prey type, $X^2 = 20.0$; df = 2, 219; p < 0.001). The site SW of the hybrid zone used by Mojave rattlesnakes had equal abundance of kangaroo rats and other rodents (*post-hoc* Tukey: z-ratio = 0.337, p = 0.999), whereas kangaroo rats were 2–4 times more common than all other rodents at other sites (*post-hoc* Tukey: z-ratios = 2.87, 4.73; p = 0.047, < 0.001; respectfully). The line segments indicate the mean catch per unit effort for each site across all trapping nights.
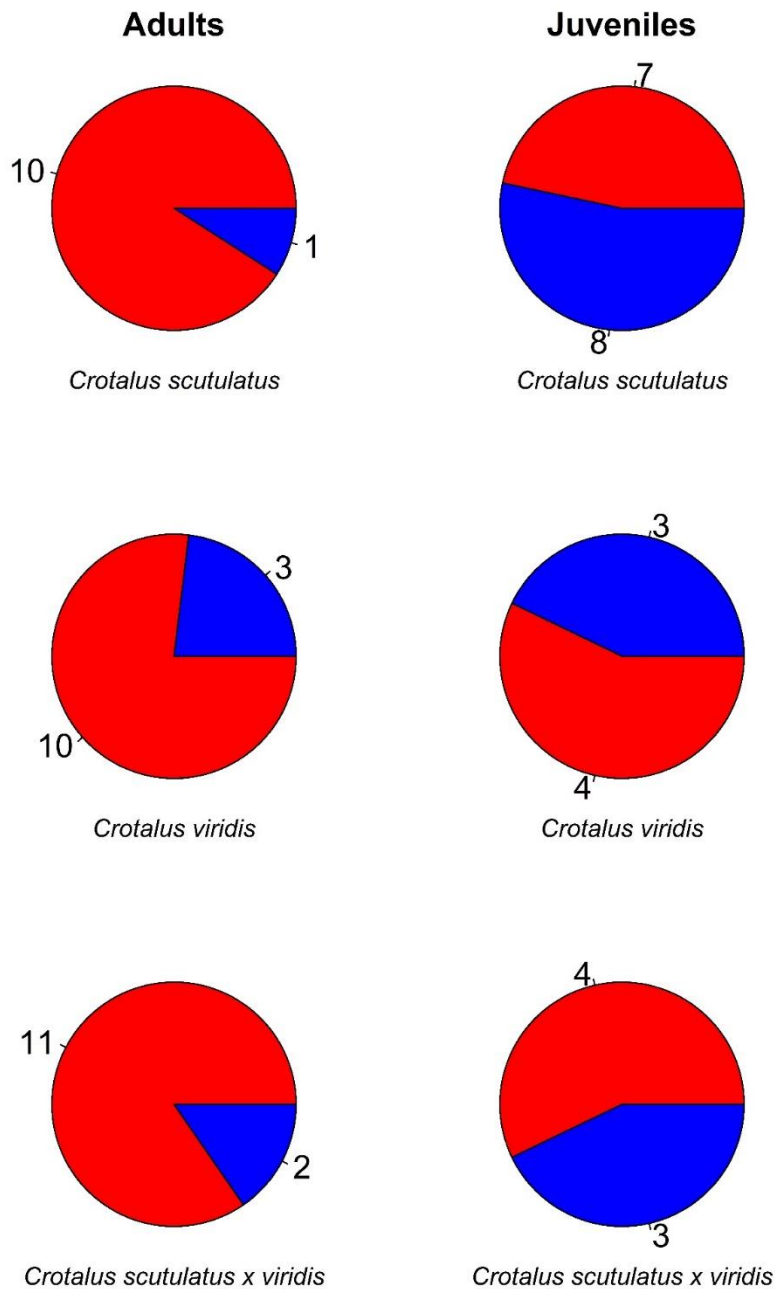
Figure 2.8: Pie charts of the proportion of mammalian and lizard remains found in the fecal samples and stomachs of 60 parental and hybrid rattlesnakes. Red indicates mammal remains; blue indicates lizard remains. The numbers around pie charts indicate sample sizes for the prey types (lizard or mammal) within lineages. Snake sample sizes: Adult *Crotalus scutulatus* = 10, juvenile *C. scutulatus* = 13, adult *C. viridis* = 13, juvenile *C. viridis* = 5, adult *C. scutulatus* x *viridis* = 13, juvenile *C. scutulatus* x *viridis* = 6.

Table 2.1: Snake hunting behaviors for each lineage. Hunting Frequency = number of nights a snake was found hunting divided by total nights tracked; probing & gaping rates (probes or gapes/min) = number of probes or gapes divided by total minutes of nighttime or daytime activity; prey encounter rates (prey/min) = number of prey encounters divided by total minutes of hunting activity; strike rate = number strikes elicited towards a prey item divided by the number of prey encounters; successful strike rate = number successful strikes (i.e., the recordings show the strike contacting the prey) divided by the number of strikes elicited towards a prey item; abandonment time = the time (to the nearest minute) that the snake left the ambush position and moved out of the frame of the camera. Boldened rows denote significant differences between the lineages. Superscripts indicate statistically significant groups by way of a *post-hoc* multiple comparison tests using a Tukey adjustment.

Hunting Behavior	*Crotalus scutulatus*	*Crotalus viridis*	*Crotalus scutulatus* x *viridis*	Test Statistic	P-value
Hunting Frequency	0.619 ± 0.043; *n = 21*	0.661 ± 0.033; *n = 15*	0.517 ± 0.046; *n = 17*	F = 2.283	0.068
Morning Probing Rate	0.007 ± 0.005; *n = 10*	0.005 ± 0.001; *n = 12*	0.008 ± 0.005; *n = 10*	NA	NA
Nighttime Probing Rate	**0.113 ± 0.010^A; *n = 17***	**0.061 ± 0.007^B; *n = 13***	**0.063 ± 0.006^B; *n = 10***	**F = 12.0**	**<0.001**
Morning Gaping Rate	0.003 ± 0.003; *n = 10*	0.001 ± 0.001; *n = 12*	0.001 ± 0.001; *n = 10*	NA	NA
Nighttime Gaping Rate	**0.021 ± 0.002^A; *n = 17***	**0.012 ± 0.002^B; *n = 13***	**0.014 ± 0.002^{AB}; *n = 10***	**F = 5.36**	**0.009**
Prey Encounter Rate	0.004 ± 0.001; *n = 17*	0.005 ± 0.003; *n = 13*	0.003 ± 0.001; *n = 9*	$X^2 = 0.254$	0.881
Striking Frequency	0.250 ± 0.087; *n = 14*	0.459 ± 0.101; *n = 11*	0.240 ± 0.118; *n = 8*	$X^2 = 3.16$	0.206
Successful Strike Frequency	0.286 ± 0.184; *n = 7*	0.347 ± 0.113; *n = 10*	0.100 ± 0.100; *n = 5*	$X^2 = 0.924$	0.630
Abandonment Time	**$05{:}40 \pm 18$ min^A; *n = 16***	**$07{:}37 \pm 37$ min^B; *n = 13***	**$07{:}49 \pm 26$ min^B; *n = 10***	**F = 7.32**	**0.002**

Table 2.2: Presence/absence data of toad and lizard species at field sites southwest of the hybrid zone (Mojave site), northeast of the hybrid zone (Prairie site), and within the hybrid zone. P = species was detected, A = species was not detected.

Site	Year	Toads							Lizards						
		Great Plains Toad (*Anaxyrus cognatus*)	Green Toad (*A. debilis*)	Red-spotted Toad (*A. punctatus*)	Woodhouses Toad (*A. woodhouseii*)	Couchs Space foot (*Scaphiopus couchii*)	Desert Spadefoot (*Spea multiplicate*)	Whiptails (*Aspidoscelis* spp.)	Western Banded Gecko (*Coleonyx variegatus*)	Long-Nosed Leopard Lizard (*Gambelia wislizenii*)	Elegant Earless Lizard (*Holbrookia elegans*)	Texas Horned Lizard (*Phrynosoma cornutum*)	Roundtail Horned Lizard (*P. modestum*)	Fence Lizards (*Sceloporus* spp.)	Common Side-Blotched Lizard (*Uta stansburiana*)
Mojave Site (Southwest of Zone)	2021	P	P	P	P	P	P	P	P	A	A	P	A	P	A
Prairie Site (Northeast of Zone)	2020	P	P	A	A	A	P	A	P	P	A	P	P	A	A
	2021	A	P	A	P	P	A	P	P	P	A	P	P	A	A
Hybrid Zone	2019	P	P	A	A	P	P	P	P	A	P	P	P	P	P
	2020	A	A	A	A	A	A	A	P	P	A	P	P	A	A
	2021	P	P	A	A	P	A	P	P	A	A	P	P	A	P

Chapter 3: Variation in behavioral types and syndromes across a naturally occurring zone of hybridization between Prairie (*Crotalus viridis*) and Mojave Rattlesnakes (*C. scutulatus*)

Abstract

Research into the causes and consequences of individual variability and consistency in behavioral types (i.e., animal personality and behavioral syndromes) has surged in recent decades, and behavioral ecologists have an expanding appreciation for how behavioral types may mediate higher-level population processes. However, research focusing on the role of behavioral types in shaping reproductive isolation or hybridization between lineages is still limited. The behavioral types and syndromes that individuals express in the context of hybridization could either reinforce or weaken barriers to further hybridization between lineages. Case studies have shown that hybridization can have multiple different effects on behavioral syndromes (the correlation of behaviors across context), including both eliminating syndromes present in parental lineages, or generating novel syndromes within hybrid lineages. Here, I took advantage of a naturally occurring Prairie/Mojave Rattlesnake hybrid zone in southwestern NM, USA to assess the consistency of behavioral types and syndromes within parental and hybrid lineages. I used three different behavioral assays to quantify defensiveness and explorativeness, and then determined if behavioral types measured in laboratory assays were correlated with spatial and hunting behaviors collected from free-ranging individuals. I found that all three lineages (Prairie Rattlesnake: *Crotalus viridis*; Mojave Rattlesnake: *C. scutulatus*; hybrids: *C. scutulatus* x *viridis*) were broadly similar, except in a few key differences. *C. viridis* was the most defensive lineage, and hybrid snakes that had a higher proportion of their genome derived from *C. viridis* were also more defensive. Prairie and hybrid rattlesnakes (but not Mojave rattlesnakes) exhibited behavioral syndromes in which

defensiveness and explorative behaviors were negatively related; this relationship was weaker in hybrid snakes making them intermediate between Mojave and Prairie Rattlesnakes. Further research is necessary to determine if these patterns impact hybrid fitness by creating mismatches between behavioral type and predation pressure in natural habitats.

Introduction

Although the behavior of organisms has long been recognized as both a highly variable and evolutionarily labile aspect of phenotype, recent decades have seen an increasing number of studies that document consistent patterns of general behaviors (i.e., behavioral types) expressed within individuals across different contexts (reviewed in Bell et al. 2009; MacKinlay and Shaw 2022). Behavioral ecologists typically make a distinction between consistent behavioral types that vary between individuals (often termed personality, or temperament) and the correlation between two or more behavioral types expressed by an individual across time or context (i.e., behavioral syndromes, Sih et al. 2004; Carter et al. 2013). Animals display behavioral types (also called personality traits) in various contexts as behavioral repertoires that are consistent within the individual but vary between individuals. Behavioral types are often expressed as a spectrum (e.g., bold/shy, explorative/non-explorative, aggression/submission, etc.). For example, bold individuals tend to prioritize high risk/high reward behaviors while shy individuals prioritize the opposite. Individuals that express varying levels of boldness are often found to do so consistently across different contexts (time, age, reproductive status, foraging, social interactions, etc.). The personality traits of an animal, like most behaviors, are moderately heritable, and shaped by both environmental and genetic variation (van Oers et al. 2005).

Although behavioral variation associated with personality and behavioral syndromes has now been studied widely across taxa and in a number of evolutionary contexts (reviewed in van Oers et al. 2005; Biro and Stamps 2008; Dingemanse et al.

2010; Schuett et al. 2010; Sih et al. 2015; Cabrera et al. 2021; Laskowski et al. 2022;

MacKinlay and Shaw 2022), surprisingly little work has been done in the context of

interspecific hybridization. In hybrid populations, genetic and phenotypic variability is

often higher within and between individuals due to interspecific gene flow from different

parental lineages (reviewed in Rieseberg et al. 2007). This admixture is also thought to

lead to the breakdown of suites of correlated traits, which may lead to hybrid inferiority

when co-adapted traits optimize fitness. Because behavioral types and syndromes are

defined by the correlation of traits expressed across contexts, they could break down

across hybrid zones, and in turn represent an extrinsic barrier to further hybridization,

assuming their collapse leads to non-optimal expression of behavioral types in critical

environmental contexts. However, mixed support for this expectation has been shown in

the few studies addressing this question to date.. Hybrids between benthic and

planktivorous morphs of Arctic Charr (*Salvelinus alpinus*) expressed consistent levels of

boldness and sociability, but to a lesser degree than the parental individuals (Horta-

Lacueva et al. 2021). However, this same study did not find evidence for the relaxation of

behavioral syndromes in hybrids when compared to parental individuals. Other studies

have found that behavioral syndromes in hybrids tend to be weaker than in the parental

lineages. Parental salmon (*Salmo salar*) expressed distinct behavioral syndromes

associated with boldness, aggression, and exploration, whereas hybrids only displayed

the boldness syndrome (Islam et al. 2020). Similarly, hybrids of two pufferfish species

(*Takifugu rubripes* and *T. niphobles*) had similar, but statistically weaker, syndromes than

the parentals in a variety of behavioral types, including boldness and feeding (Hosoya et

al. 2015). In contrast, other studies have shown that hybrids express unique or stronger behavioral syndromes. Hybrid swordtails (*Xiphophorus* spp.) displayed a significant behavioral syndrome between boldness and defensiveness that was absent in the parental populations (Johnson et al. 2015). This situation is akin to the expression of transgressive or novel traits seen in many hybrid zones (Rieseberg et al. 1999; Stelkens and Seehausen 2009; Harrison and Larson 2014), which can lead to adaptive evolution through transgressive segregation in the hybrids (Seehausen 2004).

The limited research to date on animal personality in hybrid zones is particularly notable, as a recent review has highlighted the potential for personality to drive reproductive isolation (Ingley and Johnson 2014). Sexual selection can favor either assortative or disassortative mating based on the personality types of individuals, which would in turn shape pre-zygotic isolating mechanisms between species. Personality can also act as a post-zygotic barrier if certain personality types that are over- or under-expressed in hybrids are favored by natural selection in the habitat where hybridization occurs. For example, animal personality can shape spatial behaviors and habitat selection in ways that increase or decrease isolation between lineages, depending on context (Ingley and Johnson 2014). Additionally, a large number of studies have demonstrated how variation related to personality and behavioral syndromes have shaped traits related to reproductive isolation, including variation in general activity levels (e.g., Sinn and Moltschaniwskyj 2005; Harcourt et al. 2009; Wilson and Godin 2009; Michelangeli et al. 2016), exploration patterns (e.g., Reaney and Backwell 2007; Wilson and Godin 2009; Michelangeli et al. 2016; Breck et al. 2019), dispersal (e.g., Fraser et al. 2001; del Mar

Delgado and Penteriani 2008), foraging activity (Sinn and Moltschaniwskyj 2005;

reviewed in Biro and Stamps 2008; Toscano et al. 2016), spatial behaviors (Stuber et al.

2022), anti-predator behaviors (e.g., Réale and Festa-Bianchet 2003; Quinn and

Cresswell 2005; Wilson and Stevens 2005; Sinn et al. 2006; Wilson and Godin 2009),

and reproductive success (Stapley and Keogh 2005; Reaney and Backwell 2007;

reviewed in Biro and Stamps 2008; Sinn et al. 2008; reviewed in Schuett et al. 2010).

However, the literature on animal personality is taxonomically uneven, with

relatively few studies of reptiles (reviewed in Collins et al. 2022; MacKinlay and Shaw

2022). Only one previous study has examined personality in Crotaline snakes (pitvipers);

Gibert et al. (2022) found that individual Western Rattlesnakes exhibited readily

quantifiable defensive and behavioral traits that were consistent within individuals.

Because rattlesnakes also show a strong tendency toward interspecific hybridization

(General Background), they may make an ideal taxon in which to study the role of

personality and behavioral syndromes in shaping hybridization dynamics.

Here, I took advantage of a naturally occurring hybrid zone between Prairie and

Mojave Rattlesnakes to better understand the expression of personality and behavioral

syndromes in relation to interspecific hybridization dynamics. I assayed a large number

of individual rattlesnakes from across this hybrid zone to evaluate their defensiveness and

explorativeness, and potential syndromes between defensiveness and explorativeness. I

also assessed potential behavioral syndromes between personality traits expressed in

laboratory assays and both spatial behaviors (Chapter 1) and hunting behaviors (Chapter

2) measured in free-ranging snakes. Finally, I measured how personality traits and

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syndromes varied between parental and hybrid lineages. I hypothesized that hybrid individuals would be transgressive or intermediate in behavioral types, depending on the relative expression of personality traits in parental species. I also hypothesized that parental populations would show similar behavioral syndromes across defensive and exploratory, and exploratory and spatial/hunting contexts, but that these syndromes would be weaker or absent in the hybrid lineage due to the increased genetic variation of hybrids.

Methods

I captured individual Prairie (*Crotalus viridis*), Mojave (*C. scutulatus*), and hybrid (*C. scutulatus* x *viridis*) rattlesnakes following previously described methods (General Methods). I assayed every animal for defensive and exploratory behaviors within 48 hours of capture before any other procedures were performed on snakes (General Methods; Chapter 1; Chapter 2). All snakes were tested at 22–26 °C to minimize variation in behavior due to body temperature, and I gave individuals at least 4 hours to acclimate to room temperature prior to conducting the assays.

I followed the procedures in Gibert et al. (2022) for conducting and scoring handling, exploratory, and threat assays. Snakes were assayed in a 1.2 x 1.2 x 1.2 m four-walled arena constructed from polyvinyl sheeting and dimly lit with indirect light. I divided the floor of the arena into four equal quadrants using black tape and placed a 10 x 5 x 3 cm black hidebox within each quadrant so that they were equidistant from each other and the center of the arena (Gibert et al. 2022, Fig. 1). I cleaned the entire arena

between trials using a commercial cleaner (Lysol®) and the hideboxes with soap and

water prior to testing each snake. I recorded each of the three assays (handling,

exploratory, and threat) with a Sony Handycam® (model DCR-SR80) mounted above the

arena. Although a few individuals were recaptured and retested, I only used the

behavioral scores from the initial set of assays in all analyses.

Handling Assay

To simulate the snake being grabbed by a perceived predator, I removed snakes with

metal tongs (Midwest Tongs®) from their temporary holding container after the 4-hour

acclimation period and held them 1 m above the center of the arena at midbody for 30 s.

At the 15 s mark I shook the snake back and forth twice. Once 30 s had elapsed, I

released the snake in the center of the arena and left the area, starting the exploratory

assay. Whether or not the snake rattled or struck defensively during the handling assay

was recorded from the video footage.

Exploratory Assay

Snakes were left to explore the arena undisturbed for 60 min. I then reviewed the video

footage after each assay to quantify the duration of time the snake spent within a hidebox,

the number of transitions between quadrants, and the duration that it spent motionless

outside of a hidebox. I considered the snake to have entered a hidebox or a new quadrant

when the entire front half was either obscured from view within the hidebox or had

broken the plane created by the black tape. I considered the snake to be motionless when no detectable movement of its head, body, or tail could be seen or heard.

Threat Assay

After the conclusion of the exploratory assay, I turned on an overhead light to fully illuminate the arena and, using metal tongs, removed all hideboxes and moved the snake into one corner of the arena. I then "threatened" the snake with an inflated balloon on the end of another pair of metal tongs to create a visual, looming stimulus. For each assay the balloon tong was tapped in the center of the floor of the arena five times, around 80 cm from the center of the snake. I then slid the balloon along the floor of the arena and towards the head of the snake until it was around 40 cm from the snake. I then tapped the balloon tong on the floor of the arena another five times. After the initial tapping, I slid the balloon along the floor of the arena again until it touched the snout of the snake. If the snake did not strike at this point of the assay, I introduced a physical threat by tapping the balloon on the head of the snake five times. If at any point the snake struck at the balloon (even if the snake missed) the assay would end, and a positive strike response would be reported for that snake. Otherwise, the assay would end on the fifth tap of the balloon on the snake's head and a negative strike response would be recorded. The threat assay was only performed on snakes captured during the 2020 and 2021 active seasons.

Statistical Analyses

Behavioral assays were scored independently by two different observers to minimize observer bias. If scores were similar (values within 15%), I averaged the scores of the two observers, but if there was a greater than 15% difference between observer scores, the video was scored by a third observer to achieve a consensus score (average of the two scores with < 15% difference).

To assess behavioral types and potential syndromes, I limited analyses to the behaviors that were found to be repeatable in the only previous research assessing repeatability of personality traits in a pitviper (Gibert et al. 2022). Although I could not assess individual-level repeatability of behavioral traits in this study, the traits identified by Gibert et al. (2022) are also broadly similar to other personality traits that have been found to be individually repeatable across a broad sample of non-pitviper snakes, lizards, and other vertebrates (reviewed in Waters et al. 2017; Gibert et al. 2022). Thus, I scored the rattling behavior (whether snake rattled or not during the handling assay) and defensive strike behavior (whether snake struck or not during the threat assay), both of which are indicative of defensive/passive behavioral types (e.g., Scudder and Burghardt 1983; Arnold and Bennett 1984; Goode and Duvall 1988; Herzog et al. 1989; Maillet et al. 2015; Simkova et al. 2017). During the exploratory test, I scored the proportion of time a snake spent in a hidebox, the proportion of time spent motionless (not moving, but not in hidebox), and the number of times the snake transitioned to a new quadrant. The behaviors displayed in these types of open-field tests are typically considered indicative of explorative/non-explorative behavioral types (Carter et al. 2013; Perals et al. 2017).

I created a suite of models using a Generalized Linear Model (GLM) framework to assess variation in defensive and exploratory behavioral types across genetic lineages. All models included either the rattle behavior, proportion of time spent in a hidebox, number of quadrant transitions, proportion of time spent motionless, or strike behavior as the response variables, and genetic lineage, lineage + age (adult or juvenile), lineage * age, and lineage * age + sex as predictor variables. I then used Akaike information criterion (AIC_C) to select which of the four models fit the data the best. If more than one model was within two ΔAIC_C of the best model, then I used the simplest model for analysis (the model with fewest predictor variables). I used a binomial distribution for modelling rattling, striking, and proportion of time motionless, a beta distribution for proportion of time in hidebox, and a quasi-Poisson distribution for the number of quadrant transitions (overdispersed count data). Time spent in hidebox was zero-inflated, so I performed the transformation recommended by Smithson and Verkuilen (2006). I used Tukey tests for *post-hoc* comparisons and Levene's test to compare variation between lineages in exploratory behaviors. To assess if there was a relationship between the value of the hybrid index and behavioral type, I repeated the analyses using only hybrid snakes and incorporating hybrid index (estimate of proportion of genome derived from *Crotalus viridis*) as a fixed factor.

Due to non-normality of predictor variables and the binary nature of response variables, I was not able to use a correlation framework for analyzing behavioral syndromes between defensiveness and explorativeness, and instead used GLMs to test for significant relationships between behavioral traits within each lineage (e.g., Johnson et al.

2015; Dhellemmes et al. 2020; De Meester et al. 2022; Gibert et al. 2022). For each lineage, I constructed binomial GLMs with the three exploratory behaviors as the fixed factors and either rattling or defensive striking as the response variable. Lastly, if any previous models indicated age was a significant factor, I constructed independent models for adults and juveniles.

I also assessed the potential behavioral syndromes between behaviors measured in laboratory assays and movement and hunting behaviors measured while individuals were free-ranging. These analyses were constrained in sample size, as I could only include the subset of radio-tagged individuals with enough spatial and hunting data for statistical analyses. For spatial behaviors, I chose the number of 50% isopleths identified by Brownian Bridge Kernel Density Estimators (bbKDE), distance moved per day (DMD), and the number of days between movements (FM) as behaviors to correlate with explorative activity (Chapter 1). For hunting behaviors, I chose nightly probing rates (NPR), prey encounter frequency (PE), time of day of hunting site abandonment (AT), and the frequency of nights that the snakes were found hunting (HF) as behaviors to correlate with explorative activity (Chapter 2). I performed a Spearman Correlation test on the spatial and hunting behaviors and each of the three exploratory behaviors for each lineage and (in order to maximize sample size) for all lineages combined. Since each comparison consisted of multiple correlation tests, I adjusted the p-values accordingly with a Holm's adjustment. I considered a behavioral syndrome to exist between two behaviors if the correlation between them was significant and if $|r| > 0.3$ (Nyqvist et al. 2012; Lukas et al. 2021).

All behavioral scoring was done from the video footage *post-hoc* using BORIS v.

7.4.11 (Friard and Gamba 2016). All statistical analyses were done in R (v. 3.6.3, 2020)

using the packages tidyverse (Wickham et al. 2019), Hmisc (Harrell Jr et al. 2020),

nortest (Gross and Ligges 2015), psych (Revelle 2021), betareg (Francisco, Cribari-Neto,

Zeileis 2010), ggplot2 (Wickham 2016), emmeans (Lenth 2021), and MuMIn (Barton

2020).

Results

Behavioral types

I conducted behavioral assays on 185 rattlesnakes: 65 Mojave Rattlesnakes (*Crotalus*

scutulatus; adults = 46; juveniles = 19; male = 42; non-pregnant females = 23), 55 Prairie

Rattlesnakes (*C. viridis*; adults = 40; juveniles = 15; male = 41; non-pregnant females =

14), and 65 hybrid rattlesnakes (*C. scutulatus* x *viridis*; adults = 49; juveniles = 16; male

= 43; non-pregnant females = 22). The most informative models for explaining variation

in rattle behavior were those containing genetic lineage + age and genetic lineage * age.

Therefore, I will report the results from the model containing genetic lineage and age as

the two predictor variables. I found that both genetic lineage and age had significant

differences between the groups (X^2 = 20.2, 9.53; df = 2, 1; p < 0.001, = 0.002;

respectively). Mojave Rattlesnakes tended to rattle less often (27.7% of individuals

rattled defensively) than Prairies (60.0%; *post-hoc* Tukey: z-ratio = -3.53, p = 0.001), but

were not different than hybrid rattlesnakes (24.6%; *post-hoc* Tukey: z-ratio = 0.525, p =

0.859). Hybrid rattlesnakes rattled significantly less often than Prairies (*post-hoc* Tukey:

z-ratio = 3.97, p < 0.001; Figure 3.1). Overall, more adult snakes tended to rattle during the assays (42.2%) than juveniles (20%; Figure 3.2).

During the exploratory assays snakes spent an average of 8.17 min in hideboxes (13.6% of the total time), but this was highly variable (Table 3.1). For time spent in hidebox, models with lineage and lineage + age as fixed factors were equally informative, so I chose the simpler model for analysis. However, the relationship between lineage and time in hidebox was not significant (X^2 = 1.79; df = 2, 181; p = 0.408). Lineages also did not differ in the variability of this behavior (F = 1.98; df = 2, 181; p = 0.141).

For the number of quadrant transitions snakes performed, the most informative models were the ones containing genetic lineage * age, and genetic lineage * age + sex, and so I report results from the simpler model. I found no significant relationship between the number of quadrant transitions a snake performed and its genetic lineage, age, or their interaction (X^2 = 4.82, 0.159, 1.94; df = 2,181, 1,181, 2,181; p = 0.090, 0.690, 0.378; respectively). Snakes performed an average of six quadrant transitions during the 60 min exploratory assay, but were again, highly, and equally variable within and between lineages (F = 2.46; df = 2, 181; p = 0.089; Table 3.1).

I found that for the time the snake spent motionless, the most informative models contained genetic lineage and genetic lineage + age as the fixed factors, and so I report the results of the model with only genetic lineage. Again, I found no difference between the lineages in the amount of time that they spent motionless (X^2 = 4.03; df = 2, 181; p = 0.133). Snakes spent an average of 34.8 min (57.9%) motionless outside of a hidebox.

The variability of this trait was also high, but not significantly different between the lineages (F = 1.91; df = 2, 181; p = 0.150; Table 3.1).

For individuals tested using the threat assay (Mojaves = 56, Prairies = 45, Hybrids = 33), the models containing genetic lineage and genetic lineage + age were the most informative, so I report only the results from the genetic lineage model. Overall, only 24.6% of the snakes struck defensively and I found no relationship between the number of snakes that struck during the assay and their genetic lineage (X^2 = 1.09, df = 2, p = 0.579; Figure 3.3).

Behavioral types within hybrids

For my analysis of hybrid index (HI—proportion of genome derived from *C. viridis*) and rattling behavior, the most informative models contained HI, HI + age, and HI * age. Hence, I report the results of the model containing HI as the only fixed factor. Individuals with higher HIs (i.e., more Prairie-like) were more likely to rattle during the handling assay (X^2 = 14.6, df = 1, p < 0.001; Figure 3.4).

The most informative model for time in a hidebox contained HI, HI + age, and HI * age and time spent motionless contained HI and HI * age as the predictor variables, while the best fitting model for the number of quadrant transitions contained HI * age + sex. Therefore, I report the results of the model containing HI as the only predictor variable for the time a hybrid snake spent in a hidebox and motionless. None of the exploratory behaviors (time spent in a hidebox, number of quadrant transitions, and time spent motionless) had a significant relationship with the hybrid index (X^2 = 0.178, 1.44,

0.932; df = 1, 1, 1; p = 0.673, 0.231, 0.334; respectively). I also found that the number of quadrant transitions was not significantly related to age or sex of hybrid snakes (X^2 = 3.21, 1.06; df = 1, 1; p = 0.073; 0.303; respectively), or the interaction between age and HI of the snakes (X^2 = 0.142, df = 1, p = 0.707).

Lastly, the most informative model for striking during the threat assay contained HI, HI + age, and HI * age. Hence, I report the results of the model containing HI as the only fixed factor. I did not find a significant relationship between hybrid index and striking (X^2 = 0.418, df = 1, p = 0.518).

Behavioral syndromes: defensiveness vs. exploration

I found that the number of behavioral syndromes expressed within individuals differed between lineages. Mojave rattlesnakes displayed no behavioral syndromes between any defensive and exploratory behaviors (Table 3.2). Prairie Rattlesnakes exhibited significant relationships between time in a hidebox and defensive striking (i.e., striking during the threat assay), time spent motionless and defensive striking, and quadrant transitions and defensive striking (X^2 = 5.96, 6.70, 5.94; df = 1, 1, 1; p = 0.015, 0.010, 0.015; respectively; Table 3.2). Prairie Rattlesnakes who spent more time within a hidebox or motionless were less likely to strike during the threat assay. However, Prairie Rattlesnakes that performed more quadrant transitions were also less likely to strike during the threat assay. I found only one behavioral syndrome in hybrid rattlesnakes: adult hybrids that had performed more quadrant transitions were less likely to have

rattled during the handling assay ($X^2 = 5.19$; df = 1; p = 0.023; Table 3.2). I did not find

any evidence of behavioral syndromes in juvenile snakes.

Behavioral syndromes: exploration vs. field behaviors

I found no evidence for behavioral syndromes within any of the lineages between their

spatial or hunting behaviors and their exploratory behaviors assayed in the laboratory

(Tables 3.3 & 3.4). After combining data from all lineages, I found a moderately strong

syndrome (r = -0.386, p = 0.032) between spatial behavior in the field and exploratory

behavior in the novel arena, wherein snakes that move more often also tended to

transition between quadrants more often during the exploratory assay (Figure 3.5).

Discussion

The rattlesnake lineages were broadly similar to each other, except in a few key traits.

Prairie Rattlesnakes (*Crotalus viridis*) were more likely to rattle defensively compared to

Mojave (*C. scutulatus*) and hybrid (*C. scutulatus* x *viridis*) rattlesnakes and exhibited the

strongest syndrome between defensiveness (striking) and exploration/activity.

Furthermore, within hybrids, individuals with a larger proportion of their genome derived

from Prairie rattlesnakes were also more likely to rattle, underscoring the likely genetic

basis for this eponymous defensive trait. Across all three lineages I found that snakes

were generally hesitant to strike at threats and exhibited similar levels of exploration

within a novel environment. Although, both Prairie and hybrid rattlesnakes showed

evidence of behavioral syndromes between defensiveness and exploration/activity, hybrids displayed fewer correlated trait pairs.

Because behavioral type or personality, such as I measured here, can have a number of direct and indirect effects on fitness (Smith and Blumstein 2008; Ballew et al. 2017; von Merten et al. 2020), it is important to consider details of how these traits are expressed and measured in different contexts. The only behavioral trait I measured that differed across lineages was the rattling behavior during the handling assay, an expression of defensiveness. Although individuals within species differ in the expression of these traits (Arnold and Bennett 1984; Mayer et al. 2016; Simkova et al. 2017; Gibert et al. 2022; Skinner et al. 2022), Prairie Rattlesnakes as a group were more defensive than Mojave and hybrid rattlesnakes. Like most behaviors, defensive rattling is undoubtedly shaped by both individual experience, ecological contexts, and genetic variability. Prairie Rattlesnakes in this study may have had more experience with predators, as previous work found a higher number of predator encounters in the habitat occupied by Prairie Rattlesnakes compared to the hybrid and Mojave areas (Maag and Clark 2022). Thus, an increased propensity to rattle may be in part a product of phenotypic plasticity driven by selection against individuals that do not react defensively toward potential predators (e.g., Réale and Festa-Bianchet 2003; Bell and Sih 2007; Carter et al. 2010; Kashon and Carlson 2018). However, an increase in the propensity to rattle was also significantly related to proportion of the genome derived from Prairie Rattlesnake (HI) within hybrid individuals. As this group of individuals were found throughout the hybrid zone (General Methods, Figure I.2) this pattern suggests that the defensive rattling behavior also has a

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strong genetic basis. Antipredator behaviors in a number of taxa have been shown to be heritable, including examples in insects (Guzmán-Novoa et al. 2002; Nakayama et al. 2010), mammals (Gammie et al. 2006; Tay et al. 2023), fish (Kim and Velando 2015; Satterfield and Johnson 2020), cephalopods (Sinn et al. 2006), birds (Bize et al. 2012; Jiang and Møller 2017), and lizards (Baxter-Gilbert et al. 2018), but not in other non-avian reptiles. The correlation between HI and rattling is the first evidence we know of pointing toward the genetic basis of an antipredator display in rattlesnakes. Future analyses incorporating genome-wide association studies and transcriptomic approaches could further identify genes or regions of the genome underpinning this behavior.

A large body of literature has shown that behavioral types are often correlated across contexts (Sih et al. 2004), and these behavioral syndromes can impact fitness either synergistically or antagonistically, depending on context. Common behavioral syndromes seen across taxa include a positive correlation between boldness and general activity (Biro and Stamps 2008; Wilson and Godin 2009; Michelangeli et al. 2016, 2019; Lukas et al. 2021), boldness and explorativeness (Wilson and Godin 2009; Michelangeli et al. 2016, 2019; Kudo et al. 2021; Schabacker et al. 2021; Xu et al. 2021; Majelantle et al. 2022), and boldness and foraging (Wilson and Stevens 2005; Carter et al. 2010; Nyqvist et al. 2012). Perhaps because it is associated with locating resources in the environment, the propensity to explore novel environments is often correlated with a number of other traits besides boldness, including general activity (Michelangeli et al. 2019; Muraco et al. 2021; Ferderer et al. 2022), aggressiveness (Schabacker et al. 2021),

sociability, (Dhellemmes et al. 2020), and docility (Agnani et al. 2020; Underhill et al. 2021).

Since hybrid individuals are a product of two distinct parental genotypes, I hypothesized that behavioral syndromes would be generally weaker in hybrids, and my results provide some support for this pattern. The evidence for behavioral syndromes between defensiveness and exploration/activity was absent in Mojave Rattlesnakes, strongest in Prairie Rattlesnakes, and intermediate in hybrid rattlesnakes (Table 3.2). These relationships generally indicate that more defensive individuals exhibit higher levels of activity in novel environments (i.e., are less likely to hide under shelter or sit motionless). However, the propensity to strike during the threat assay and to rattle during the handling assay were inversely correlated with the exploratory movements (i.e., the number of quadrant transitions snakes performed during the open field test) for Prairie and hybrid rattlesnakes, respectively. These response variables, although commonly used across a number of taxa, are somewhat open to interpretation. The time an individual spends in a hidebox or the time it spends sitting motionless could be either indicative of general activity level or associated with bold/shy behavioral types. The time spent motionless could represent a freeze response during a threatening situation (e.g., a novel laboratory environment), so perhaps could serve as a measure of anxiety. However, because these individuals are less likely to strike when threatened directly, perhaps the time a snake spends in a hidebox or motionless are more indicative of general activity. The interpretation is complicated by the fact that it was necessary to handle snakes immediately prior to performing exploration assays, perhaps leaving them in a prolonged

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state of anxiety. Future studies could use alternative methods to assess the relationship more closely between time spent motionless and bold, explorative, or active behavioral types.

Although my findings are in agreement with some other studies showing reduced or weaker behavioral syndromes in hybrids (Hosoya et al. 2015; Islam et al. 2020), this pattern is not universal (Johnson et al. 2015), and hybrids in my system seem to display an intermediate phenotype compared to the parental lineages. Hybridization can generate novel or extreme traits (Rieseberg et al. 1999; Stelkens et al. 2009; Harrison and Larson 2014), and it may be difficult to predict *a priori* how recombination and introgression may affect the strengthening or generation of trait combinations like behavioral syndromes. My findings are similar to patterns found for pufferfish and salmon (Hosoya et al. 2015; Islam et al. 2020) in that hybrid rattlesnakes have similar but weaker syndromes when compared to one parental lineage (Prairie Rattlesnakes), but also mirror results from swordtail fish (Johnson et al. 2015) in that the hybrid lineage had a behavioral syndrome not present in the other parental lineage (Mojave Rattlesnakes). Clearly, more research is needed in order to determine if general patterns apply across taxonomic groups.

In addition to differences between lineages, I also found that behavioral types and syndromes varied across age groups. Across all lineages juveniles did not rattle as readily as adult snakes. These patterns could be driven by higher predation pressure on juveniles, as smaller individuals probably have less effective antipredator behaviors (e.g., shorter effective strike range, smaller venom glands) and are likely to be consumed by a wider

variety of predators (Klauber 1956). Following this logic, adult snakes would be more likely to rattle and draw attention to themselves because of their enhanced ability to effectively strike and envenomate larger mesopredator species. I also found that juvenile snakes did not exhibit the same behavioral syndrome between defensiveness and explorativeness that was found in adults. This was not entirely unexpected, as in general the literature on animal personality has shown that, even when personality traits of juveniles are present, considerable shifts in behavior occur with growth and sexual maturation (reviewed in Cabrera et al. 2021). Although these shifts in personalities across life stages have been found in other snake species, they have not previously been confirmed in rattlesnakes (Simkova et al. 2017). Although additional longitudinal data on individuals would be necessary to confirm, the patterns I documented indicate that snakes might express behaviors more consistently across contexts as they age.

Although I found several behavioral measures were correlated across contexts in the laboratory assay, there was much more limited evidence for behavioral syndromes that spanned the laboratory assays and field-based behaviors. For all adult snakes combined, I found a significant relationship between increased exploration in the laboratory assay (number of quadrant transitions during the exploratory assay) and the field (more frequent daily movements). This syndrome indicates that the behavioral type of an individual could affect metrics associated with spatial ecology, as has been found in other systems (reviewed in Spiegel et al. 2017). Although this relationship makes intuitive sense, supporting evidence in my study is still limited. Most of the spatial and exploratory behaviors showed no syndromes between them across field and laboratory

contexts, and the one that did was moderate (r = -0.386). Additionally, the

explorativeness of the snakes is not linked to any other spatial or hunting behavior, such

as core UD size or frequency of chemosensory probing while in ambush. However, the

laboratory assays and field measures of behavior represent very different contexts.

Compared to an animal's home range in the field, the standardized arena in which

animals are tested in an exploratory assay is small and devoid of sensory cues that

animals use in nature. Additionally, the increased variability in spatial behaviors between

individuals is likely associated with the greater degree of biotic variability across natural

systems and might make it difficult to detect consistent relationships across contexts. It is

also possible that behavioral temperaments are species or lineage specific. The suite of

behaviors I measured in the laboratory assays was based off of a study of Western

Rattlesnake (*C. oreganus*), which displayed individual repeatability for these behaviors

(Gibert et al. 2022). Even though Western, Prairie, and Mojave Rattlesnakes are closely

related and diverged recently (Prairie/Western: 5.24 MYA; Prairie/Mojave: 5.47 MYA;

Schield et al. 2019), it is still possible that interspecific variation exists in behavioral

temperaments and syndromes.

Overall, the parental and hybrid lineages are broadly similar to each other, with

some exceptions. However, the intermediate strength of the defensive/exploratory

behavioral syndrome found in hybrid snakes supports my hypothesis that hybrids would

have weakened behavioral syndromes compared to the parentals due to increased genetic

variability caused by interspecific hybridization (reviewed in Rieseberg et al. 2007), but

only compared to the Prairie Rattlesnakes. The one behavioral syndrome recovered for

hybrids indicates that individuals that are more defensive are also less explorative, a pattern that could indicate some individuals are more sensitive to risk from predation. This same pattern could explain the overall relationship between high levels of exploration correlated with increased movement frequency, as individuals that are more sensitive to risk would be more cautious about exploring new areas. Because predation risk for snakes appears to be highest when they are moving between sites (Maag and Clark 2022), individuals that do not exhibit strong syndromes between defensiveness and explorativeness might be less likely to exhibit appropriate antipredator responses. The fates of the radio-tracked snakes indirectly support this hypothesis (Chapter 1). Out of the 18 hybrid rattlesnakes that I implanted with transmitters, seven (38.9%) of them died before I ended the tracking season. Two of these snakes died of suspected depredation events from large mammals (Table A.8). This was a higher level of mortality than I observed for Mojave (two out of 22 or 9.1% mortality; Table A.6) and Prairie Rattlesnakes (one out of 16 or 6.3% mortality; Table A.7), and none of the parental mortalities were associated with explicit signs of depredation. However, more directed behavioral experiments paired with a long-term mark-recapture program would be necessary to indirectly test the hypothesis that hybrid rattlesnakes are more susceptible to predation.

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Figures & Tables
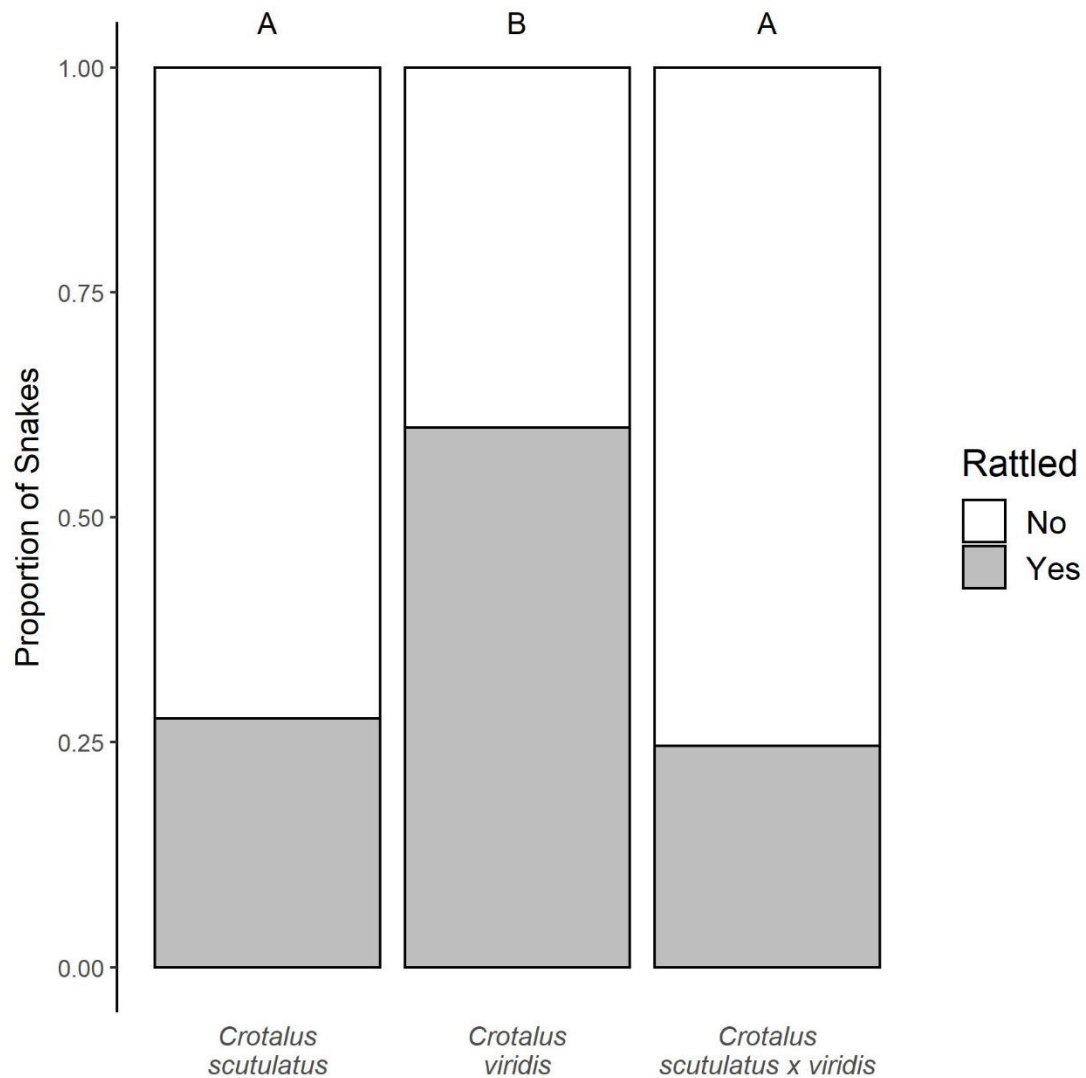
Figure 3.1: Bar graph of the proportions of snakes that either rattled or did not rattle during the handling assay. Genetic lineage did differ in the number of snakes that rattled ($X^2 = 20.2$; df = 2; $p < 0.001$). More *Crotalus viridis* rattled than both *C. scutulatus* and *C. scutulatus* x *viridis* (*post-hoc* Tukey: z-ratio = -3.53, 3.97; p = 0.001, < 0.001; respectively), whereas there was no difference between *C. scutulatus* and *C. scutulatus* x *viridis* (*post-hoc* Tukey: z-ratio = 0.525, p = 0.859). Letters above the bars indicate statistically significant groupings of the genetic lineages. Sample sizes: *C. scutulatus* = 65, *C. viridis* = 55, *C. scutulatus* x *viridis* = 65.
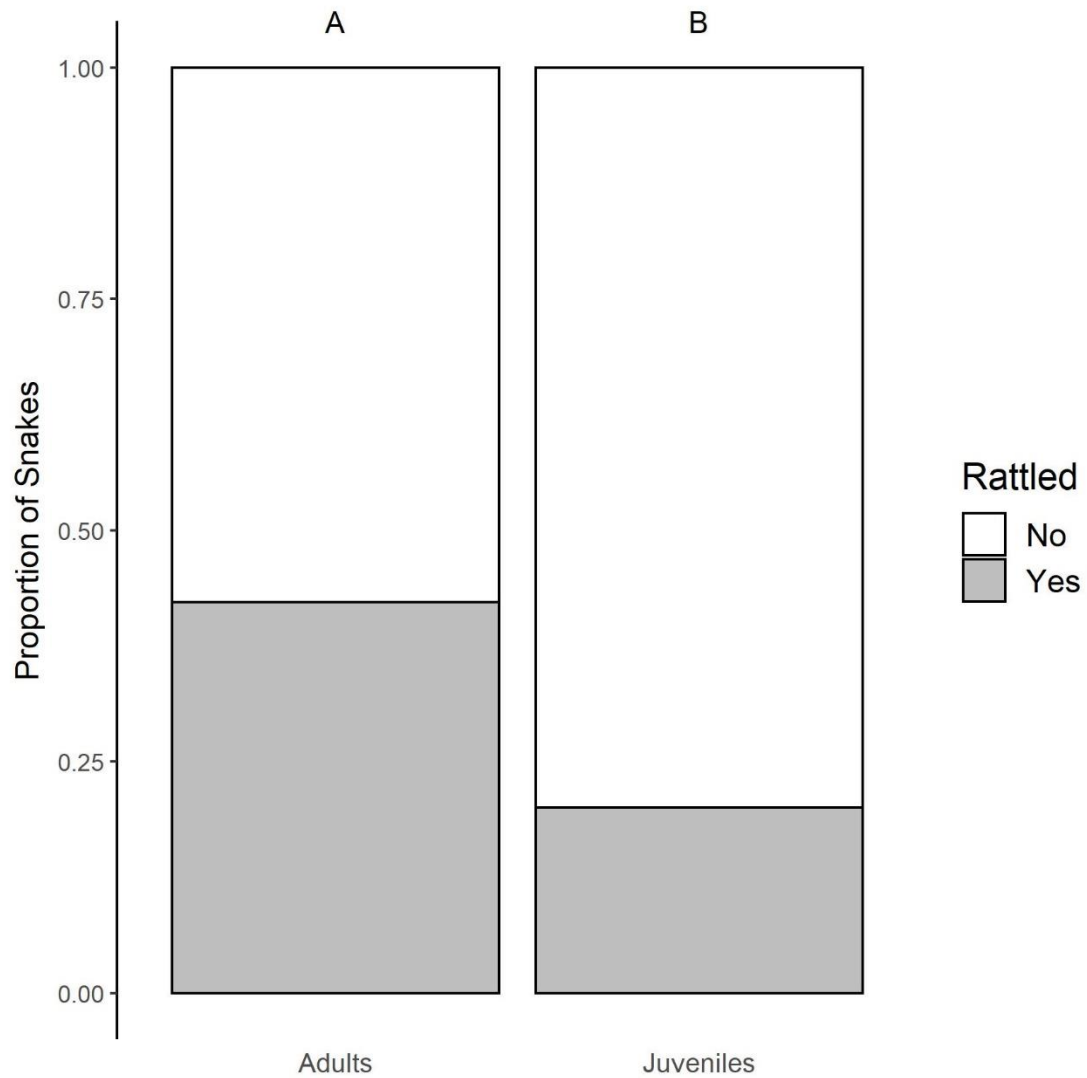
Figure 3.2: Bar graph of the proportions of snakes that either rattled or did not rattle during the handling assay. Age did differ in the number of snakes that rattled ($X^2 = 9.53$; df = 1; p = 0.002). Letters above the bars indicate statistically significant groupings of the genetic lineages. Sample sizes: adults = 135, juveniles = 50.
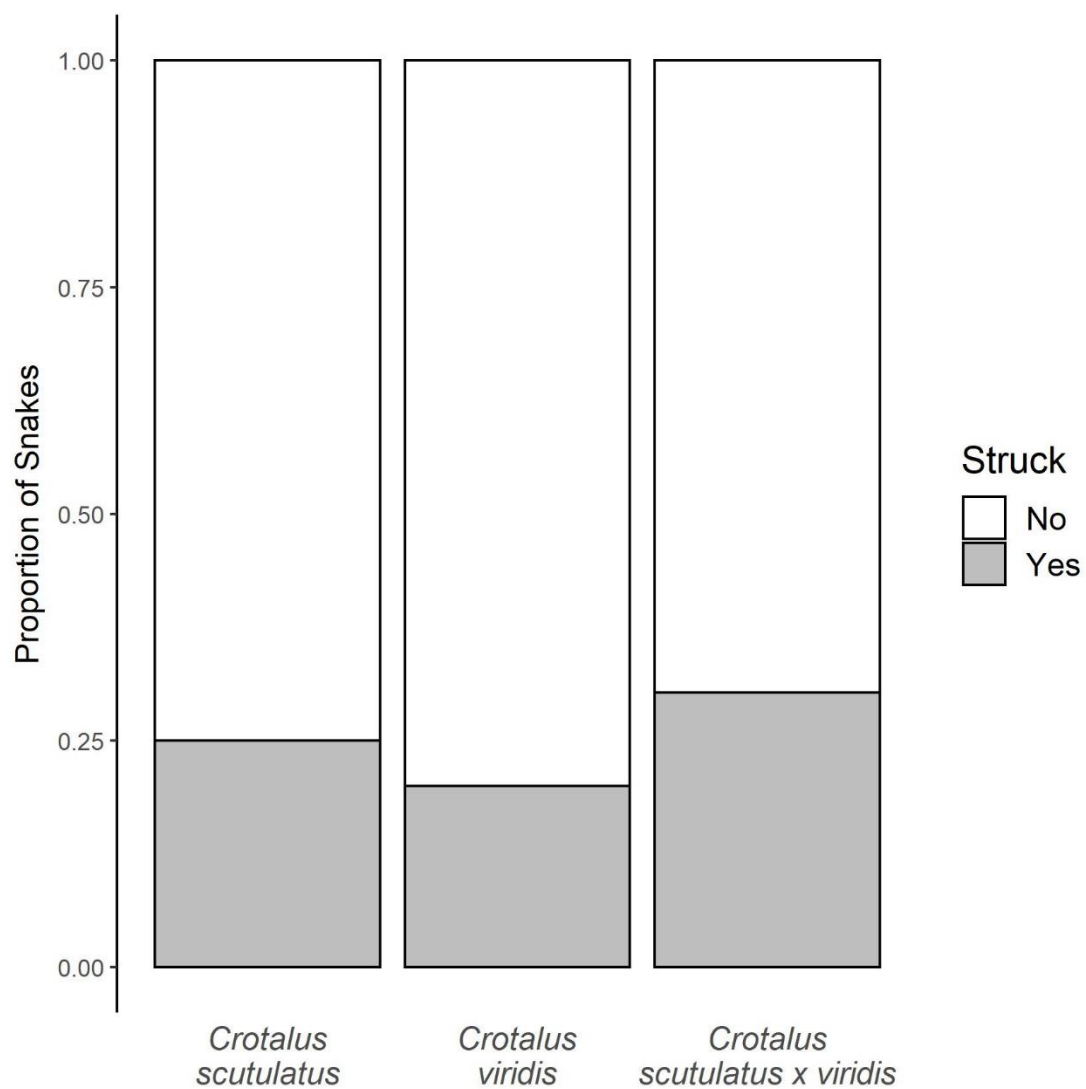
Figure 3.3: Bar graph of the proportions of snakes that either struck or did not strike during the threat assay. No differences were seen between the genetic lineages ($X^2 = 1.09$, df = 2, p = 0.579). Sample sizes: *Crotalus scutulatus* = 56, *C. viridis* = 45, *C. scutulatus* x *viridis* = 33.
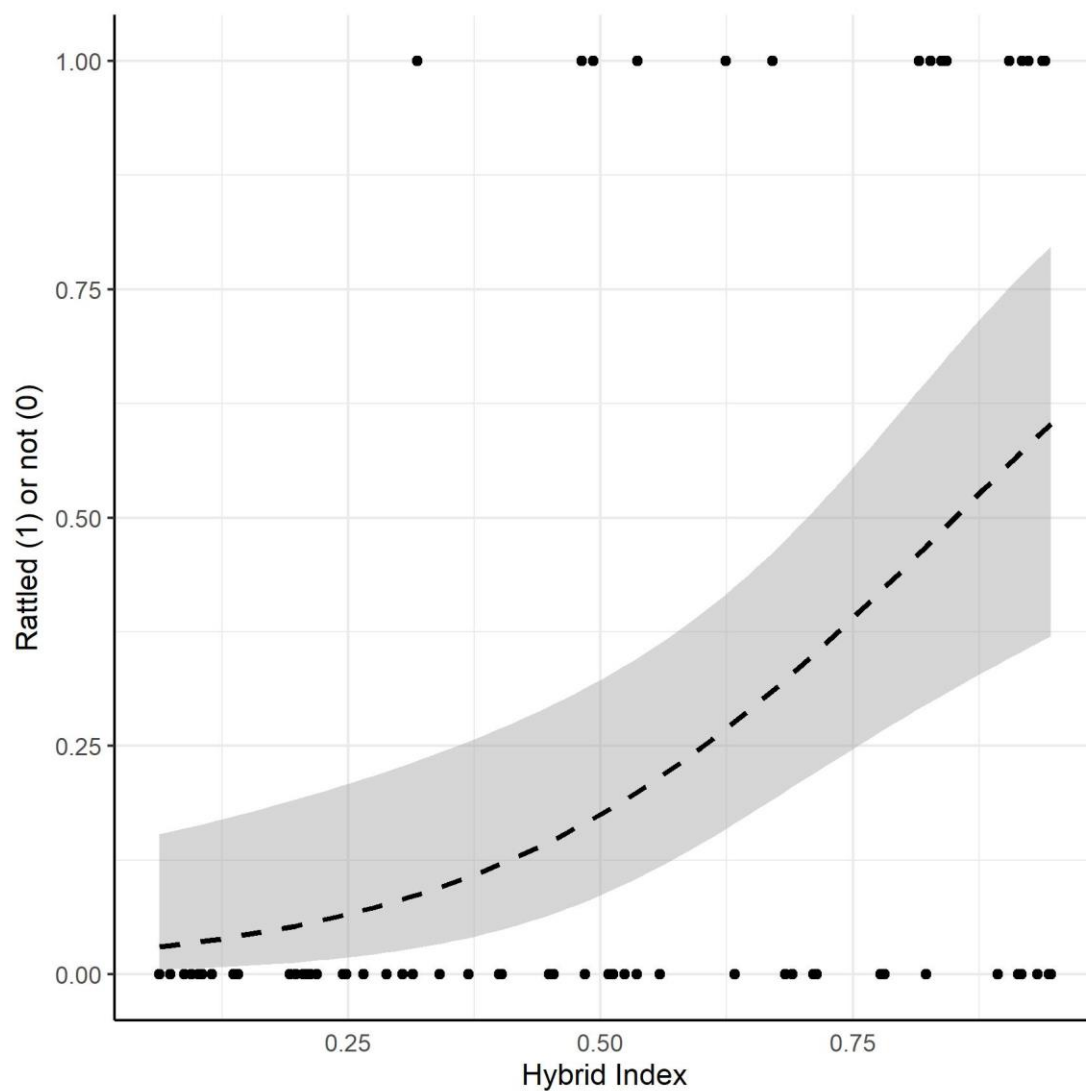
Figure 3.4: Scatter plot of whether a hybrid rattlesnake rattled during the handling assay based on its hybrid index (the proportion of the genome derived from *C. viridis*). The dotted line is the binomial regression line ($X^2 = 14.6$, df = 1, $p < 0.001$) and the shaded region flanking either side of the line is ±1 SE. Sample size = 65.
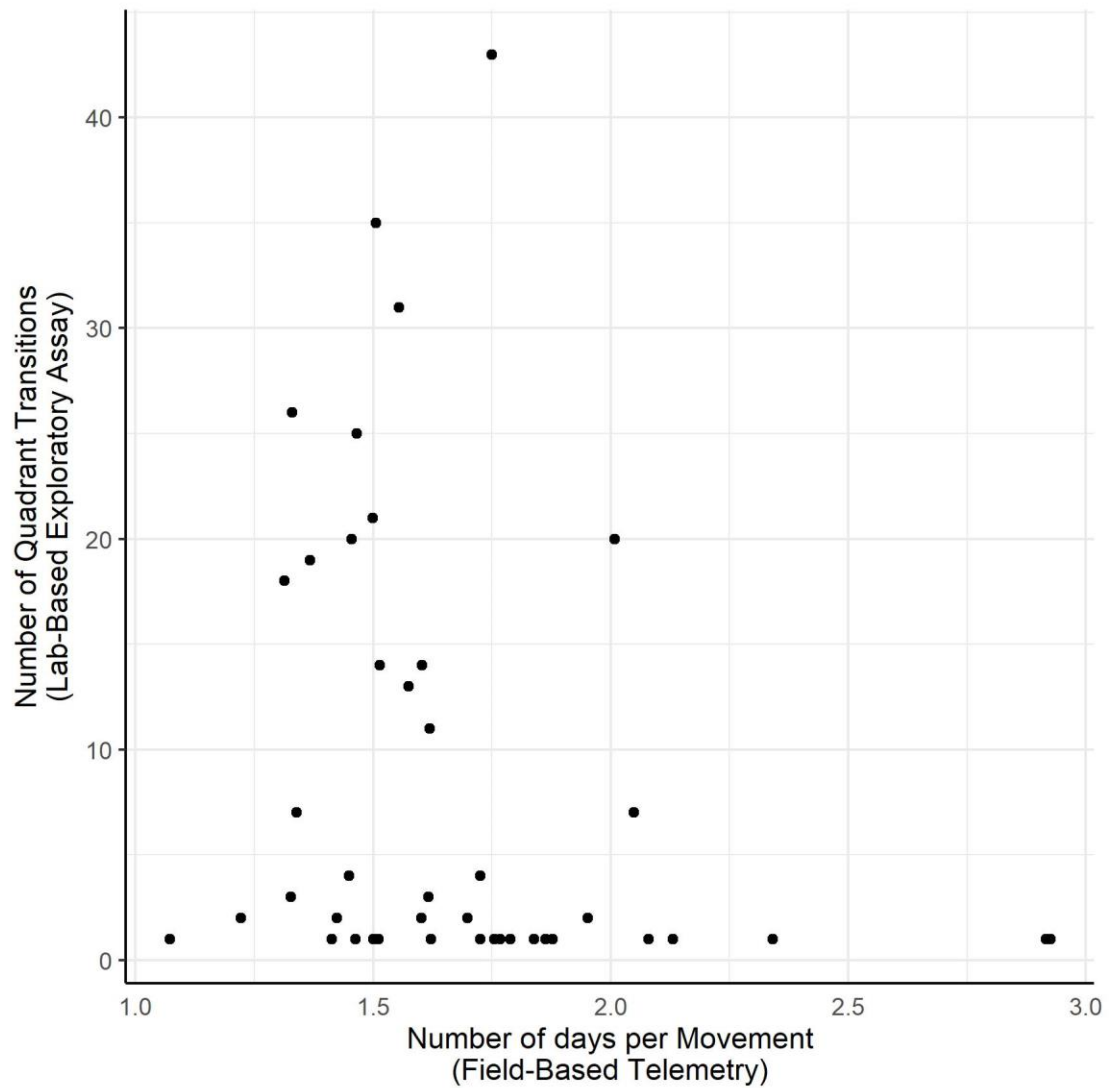
Figure 3.5: Scatter plot showing the negative correlation (behavioral syndrome) between the number of days between movements for individuals when they were free-ranging and the number of quadrant transitions those individuals performed during the exploratory assay. (Spearman correlation, Holm's adjustment: r = 0.-386, n = 43, p = 0.032).

Table 3.1: Exploratory behaviors assayed during the exploratory assay. All had non-significant relationships between the genetic lineages. Time spent in a hidebox and motionless are reported as the proportion of time during the 60-minute assay snakes displayed these behaviors.

Exploratory Behavior	*Crotalus scutulatus* *(n = 64)*	*Crotalus viridis* *(n = 55)*	*Crotalus scutulatus* x *viridis* *(n = 65)*	Chi-squared statistic	P-value
Time spent in a hidebox	0.193 ± 0.043	0.113 ± 0.034	0.100 ± 0.031	1.79	0.408
Time spent motionless	0.481 ± 0.046	0.615 ± 0.046	0.646 ± 0.039	4.03	0.133
Number of quadrant transitions	8.31 ± 1.46	5.82 ± 1.27	4.77 ± 0.894	4.82	0.090

Table 3.2: Behavioral syndromes between defensiveness (handling and threat assays) and exploration. Boldened rows signify the existence of a significant behavioral syndrome identified with binomial Generalized Linear Model approach. Arrows indicate directionality of relationship between defensiveness and explorativeness. TH = Time in a hidebox. TM = Time spend motionless. QT = Quadrant transitions.

Defensive Behavior	Exploratory Behavior	X^2	P	Syndrome Relationship	
				Defensiveness	Explorativeness
Crotalus scutulatus					
Rattling	TH	2.52	0.113	—	—
(Adults)	TM	2.83	0.092	—	—
	QT	0.439	0.508	—	—
Rattling	TH	1.46	0.226	—	—
(Juveniles)	TM	3.40	0.065	—	—
	QT	2.38	0.123	—	—
Striking	TH	0.058	0.810	—	—
	TM	0.412	0.521	—	—
	QT	1.42	0.233	—	—
Crotalus viridis					
Rattling	TH	0.074	0.785	—	—
(Adults)	TM	0.108	0.743	—	—
	QT	1.30	0.234	—	—
Rattling	TH	2.20	0.138	—	—
(Juveniles)	TM	2.54	0.111	—	—
	QT	2.75	0.097	—	—
Striking	**TH**	**5.96**	**0.015**	↓	↓
	TM	**6.70**	**0.010**	↓	↓
	QT	**5.94**	**0.015**	↓	↑
Crotalus scutulatus x *viridis*					
Rattling	TH	1.26	0.263	—	—
(Adults)	TM	3.69	0.055	—	—
	QT	**5.19**	**0.023**	↓	↑
Rattling	TH	2.54	0.111	—	—
(Juveniles)	TM	1.51	0.219	—	—
	QT	2.36	0.124	—	—
Striking	TH	0.224	0.636	—	—
	TM	1.17	0.279	—	—
	QT	0.724	0.395	—	—

Table 3.3: Behavioral syndromes between exploration in laboratory assay and spatial behaviors of free-ranging snakes (Chapter 1). Boldened rows signify the existence of a behavioral syndrome by way of Spearman correlation with Holm's adjusted p-values to account for multiple tests. 50% bbKDE = the number of 50% isopleths estimated from their GPS locations by way of a Brownian Bridge Kernel Density Estimator. DMD = Distance the snakes moved on average per day (m/day). FM = The number of days between movements. NA = not applicable.

Lab-Based Exploratory Behavior	Field Spatial Behaviors	R	Sample Size	P
	Crotalus scutulatus			
Time spent in a hidebox	50% bbKDE	-0.005	14	1
	DMD	-0.095	18	1
	FM	0.265	18	0.863
Number of quadrant transitions	50% bbKDE	0.0029	14	1
	DMD	0.091	18	1
	FM	-0.293	18	0.715
Time spent motionless	50% bbKDE	-0.144	14	1
	DMD	0.036	18	1
	FM	0.029	18	1
	Crotalus viridis			
Time spent in a hidebox	50% bbKDE	-0.289	13	1
	DMD	0.186	15	1
	FM	-0.247	15	1
Number of quadrant transitions	50% bbKDE	-0.293	13	0.664
	DMD	0.361	15	0.560
	FM	-0.227	15	0.664
Time spent motionless	50% bbKDE	0.253	13	0.850
	DMD	-0.261	15	0.850
	FM	0.296	15	0.850
	Crotalus scutulatus x *viridis*			
Time spent in a hidebox	50% bbKDE	NA	6	NA
	DMD	0.406	10	0.488
	FM	-0.174	10	0.631
Number of quadrant transitions	50% bbKDE	-0.338	6	1
	DMD	0.271	10	1
	FM	-0.326	10	1
Time spent motionless	50% bbKDE	0.169	6	0.749
	DMD	-0.612	10	0.180
	FM	0.442	10	0.401
	All Lineages			
Time spent in a hidebox	50% bbKDE	-0.254	33	0.461
	DMD	0.112	43	0.953
	FM	-0.012	43	0.953
Number of planes crossed	50% bbKDE	-0.128	33	0.478
	DMD	0.227	43	0.288
	FM	**-0.386**	**43**	**0.032**
Time spent frozen	50% bbKDE	0.228	33	0.332
	DMD	-0.215	43	0.332
	FM	0.306	43	0.138

Table 3.4: Behavioral syndromes between exploration in laboratory assay and hunting behaviors of free-ranging snakes (Chapter 2). Boldened p-values signify the existence of a behavioral syndrome by way of Spearman correlation with Holm's adjusted p-values to account for multiple tests. NPR = Nightly probing rate (probes/min). PE = Prey encounter frequency (prey/min). AT = Abandonment time. HF = Hunting frequency (number of nights tracked hunting/number of nights tracked). NA = not applicable.

Lab-Based Exploratory Behaviors	Field Hunting Behaviors	R	Sample Size	P
Crotalus scutulatus				
Time spent in a hidebox	NPR	-0.138	17	1
	PE	-0.178	17	1
	AT	0.172	16	1
	HF	-0.038	21	1
Number of quadrant transitions	NPR	0.405	17	0.426
	PE	0.004	17	1
	AT	0.120	16	1
	HF	0.128	21	1
Time spent motionless	NPR	-0.436	17	0.261
	PE	0.457	17	0.261
	AT	-0.235	16	0.761
	HF	0.101	21	0.761
Crotalus viridis				
Time spent in a hidebox	NPR	-0.463	13	0.445
	PE	-0.232	13	0.915
	AT	0.309	13	0.915
	HF	0.247	15	0.915
Number of quadrant transitions	NPR	-0.107	13	1
	PE	-0.612	13	0.105
	AT	0.194	13	1
	HF	0.145	15	1
Time spent motionless	NPR	0.236	13	1
	PE	0.580	13	0.150
	AT	0.005	13	1
	HF	-0.125	15	1
Crotalus scutulatus x *viridis*				
Time spent in a hidebox	NPR	-0.522	10	0.365
	PE	NA	9	NA
	AT	0.522	10	0.365
	HF	-0.325	17	0.365
Number of quadrant transitions	NPR	0.406	10	0.732
	PE	0.288	9	0.904
	AT	-0.135	10	0.904
	HF	0.377	17	0.545
Time spent motionless	NPR	0.079	10	1
	PE	-0.267	9	1
	AT	-0.297	10	1
	HF	0.080	17	1

Table 3.4 continued: Behavioral syndromes between exploration in laboratory assay and hunting behaviors of free-ranging snakes (Chapter 2). Boldened p-values signify the existence of a behavioral syndrome by way of Spearman correlation with Holm's adjusted p-values to account for multiple tests. NPR = Nightly probing rate (probes/min). PE = Prey encounter frequency (prey/min). AT = Abandonment time. HF = Hunting frequency (number of nights tracked hunting/number of nights tracked). NA = not applicable.

Lab-Based Exploratory Behaviors	Field Hunting Behaviors	R	Sample Size	P
	All Lineages			
Time spent in a hidebox	NPR	0.010	40	1
	PE	-0.135	39	1
	AT	-0.062	39	1
	HF	-0.098	53	1
Number of planes crossed	NPR	0.272	40	0.360
	PE	-0.100	39	1
	AT	0.066	39	1
	HF	0.161	53	0.749
Time spent frozen	NPR	-0.298	40	0.227
	PE	0.308	39	0.227
	AT	0.041	39	1
	HF	0.038	53	1

CONCLUSIONS

Interspecific hybridization, once thought to be relatively rare, is proving to be a common occurrence in the evolutionary history of many taxa. Research into the behavior and ecology of hybrid individuals may be key in developing a general understanding of the processes that lead to reproductive isolation and speciation. My research on individual snakes sampled across the Mojave/Prairie Rattlesnake hybrid zone in southwestern New Mexico, USA allowed me to analyze a suite of behavioral and ecological traits that could potentially influence postzygotic isolation of these species. By intensely sampling a relatively large number of animals across multiple seasons in both the field and laboratory settings I was able to quantify the similarity and variability of key traits, and identify potential traits that could be associated with evolutionary dynamics in the hybrid zone.

In terms of spatial ecology, I found that individuals were broadly similar across the hybrid zone, with the exception that hybrids had more patchy core utilization distributions than snakes from either parental lineage. However, this could have been a response by the hybrids to the more heterogeneous habitat structure of the hybrid zone itself. The hunting behaviors of the snakes were also similar across lineages, possibly due to the similarities mirrored in prey communities used by snakes. However, I did find that Mojave Rattlesnakes performed more chemosensory probes while hunting and abandoned their hunting locations earlier in the morning than hybrid and Prairie Rattlesnakes, underscoring a possible difference between lineages in sensory ecology or prey preference. I also found that Prairie Rattlesnakes were more likely to rattle

defensively when handled than either hybrid or Mojave rattlesnakes. Furthermore, hybrid rattlesnakes that had a higher percentage of their genome derived from Prairie Rattlesnakes were more likely to rattle defensively—a finding that provides the first evidence for a strong genetic influence on the propensity to rattle as a defensive display. I also found the strongest evidence for behavioral syndromes between defensiveness and exploration within the Prairie Rattlesnake lineage, and hybrids were intermediate between Prairie and Mojave rattlesnakes in this trait.

Overall, the rattlesnake lineages I studied were broadly similar in ecologically relevant behavioral traits, with a few key differences that could potentially play a role in hybridization dynamics. The patchier utilization distributions exhibited by hybrid snakes could reflect a more heterogenous distribution of resources, with patches of usable habitat isolated within a matrix of unfavorable habitat. It is possible that hybrid snakes are subject to higher risk of predation or higher metabolic costs when moving through this matrix, leading to decreased reproductive success. I also found that hybrid snakes had a weaker and possibly mismatched behavioral syndrome between defensive and explorative behaviors when compared to Prairie Rattlesnakes. Prairie Rattlesnakes that were more defensive were also more explorative, which may help them appropriately respond to predators encountered during exploration activity. Hybrid snakes that do not exhibit this syndrome could be more susceptible to predation. These patterns are all indirectly supported by the higher observed mortality of radio-tracked hybrids during the study.

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Unlike many previous studies, I also did not find significantly more variability in trait expression within the hybrid lineage when compared to parentals. Future research could build on these findings by developing focused approaches to understand the role of these traits in shaping survival and fitness, or reproductive isolation between lineages, as well as expanding analyses to compare other phenotypic traits that might differ between the lineages (microhabitat selection, mate-choice, male-male combat, etc.). Such efforts could yield important findings on the subtleties of how closely related species differ from each other, and how these differences affect reproductive isolation and speciation.

APPENDIX

Table A.1: Alternative results of the relationship between the genetic lineage of individuals to various defensive and exploratory behaviors. These results are from the alternate dataset omitting the six snakes that were unable to have their hybrid indexes estimated in Chapter 3. The significance between these results and the results from the whole dataset found in Chapter 3 did not change.

Factor of Interest	Best Fitting Models' Predictor Variables	Test Statistic of Best and Simplest Model	Degree of Freedom	P Value
Rattle Behavior				
Means	Lineage + Age	Lineage: $X^2 = 21.8$	2	< 0.001
		Age: $X^2 = 7.72$	1	0.005
Time Spent in a Hidebox				
Means	Lineage; Lineage + Age	Lineage: $X^2 = 1.63$	2	0.443
Variance of the Genetic Lineages	NA	$F = 1.65$	2, 175	0.195
Quadrant Transitions				
Means	Lineage * Age; Lineage * Age + Sex	Lineage: $X^2 = 5.23$	2	0.073
		Age: $X^2 = 0.120$	1	0.658
		Interaction: $X^2 = 2.50$	2	0.287
Variance of the Genetic Lineages	NA	$F = 2.56$	2, 175	0.080
Time Spent Motionless				
Means	Lineage; Lineage + Age	Lineage: $X^2 = 3.81$	2	0.149
Variance of the Genetic Lineages	NA	$F = 2.13$	2, 175	0.121
Strike Behavior				
Means	Lineage: Lineage + Age; Lineage * Age + Sex	Lineage: $X^2 = 0.658$	2	0.720

Table A.2: Alternative results of the relationship between the hybrid index (HI) of hybrid snakes to various defensive and exploratory behaviors. These results are from the alternate dataset omitting the six snakes that were unable to have their hybrid indexes estimated in Chapter 3. The significance between these results and the results from the whole dataset found in Chapter 3 did not change.

Best Fitting Models' Predictor Variables	Test Statistic of Best and Simplest Model	Degree of Freedom	P Value
Rattle Behavior			
HI; HI + Age; HI * Age	HI: $X^2 = 14.6$	1	< 0.001
Time Spent in a Hidebox			
HI; HI + Age; HI * Age	HI: $X^2 = 0.178$	1	0.673
Quadrant Transitions			
HI * Age; HI * Age + Sex	HI: $X^2 = 1.44$	1	0.231
	Age: $X^2 = 3.21$	1	0.073
	Interaction: $X^2 = 0.142$	1	0.707
	Sex: $X^2 = 1.06$	1	0.303
Time Spent Motionless			
HI; HI * Age	HI: $X^2 = 0.932$	1	0.334
Strike Behavior			
HI; HI + Age; HI * Age	HI: $X^2 = 0.418$	1	0.518

Table A.3: Alternative results of the behavioral syndromes within each lineage between rattlesnakes' defensive and exploratory behaviors. These results are from the alternate dataset omitting the six snakes that were unable to have their hybrid indexes estimated in Chapter 3. The significance (and directionality of significant syndromes) between these results and the results from the whole dataset found in Chapter 3 did not change. Boldened rows signify the existence of a significant behavioral syndrome identified with binomial Generalized Linear Models.

Defensive Behavior	Exploratory Behavior	X²	P	Syndrome Relationship Defensiveness	Explorativeness
Crotalus scutulatus					
Rattling	Time in a hidebox	2.81	0.094	—	—
(Adults)	Time spent motionless	2.85	0.092	—	—
	Quadrant transitions	0.436	0.509	—	—
Rattling	Time in a hidebox	1.46	0.226	—	—
(Juveniles)	Time spent motionless	3.40	0.065	—	—
	Quadrant transitions	2.38	0.123	—	—
Striking	Time in a hidebox	0.029	0.866	—	—
	Time spent motionless	0.397	0.529	—	—
	Quadrant transitions	1.42	0.233	—	—
Crotalus viridis					
Rattling	Time in a hidebox	0.430	0.512	—	—
(Adults)	Time spent motionless	0.702	0.402	—	—
	Quadrant transitions	0.477	0.490	—	—
Rattling	Time in a hidebox	2.21	0.137	—	—
(Juveniles)	Time spent motionless	2.56	0.133	—	—
	Quadrant transitions	2.78	0.096	—	—
Striking	**Time in a hidebox**	**5.59**	**0.018**	↓	↓
	Time spent motionless	**6.09**	**0.014**	↓	↓
	Quadrant transitions	**5.60**	**0.018**	↓	↑
Crotalus scutulatus x *viridis*					
Rattling	Time in a hidebox	1.26	0.263	—	—
(Adults)	Time spent motionless	3.69	0.055	—	—
	Quadrant transitions	**5.19**	**0.023**	↓	↑
Rattling	Time in a hidebox	2.44	0.118	—	—
(Juveniles)	Time spent motionless	1.47	0.226	—	—
	Quadrant transitions	1.99	0.158	—	—
Striking	Time in a hidebox	0.630	0.427	—	—
	Time spent motionless	1.52	0.217	—	—
	Quadrant transitions	1.20	0.273	—	—

Table A.4: I determined the onset of the wet season each year by using a combination of the accumulated precipitation data logged by the three closest weather stations (KNMANIMA5, KNMRODEO1, and KNMLORDS12; https://www.wunderground.com/) and the explosive growth of annual plants at the sites. Most years the sites had the same start of the wet season however, the Mojave site had a slightly earlier wet season than the other sites due to the isolated nature of the storm systems.

Site	Year	Onset of Wet Season
Mojave Site (Southwest of Zone)	2019	July 5
	2020	August 3
	2021	July 5
Prairie Site (Northeast of Zone)	2020	August 3
	2021	July 16
Hybrid Zone	2019	July 5
	2020	August 3
	2021	July 16

Table A.5: Non-pregnant female spatial behaviors. No female Prairie Rattlesnake were able to be implanted with radio transmitters. Values are the averages for each genetic lineage. DMD = Distance Moved per Day (m). FM = Number of days between moves. MCP = Minimum Convex Polygon. KDE = Kernel Density Estimator (smoothing parameter methods: h-reference *ad-hoc* [Kie 2013]). bbKDE = Brownian Bridge Kernel Density Estimator. MCPs and KDEs are reported in hectares.

Genetic Lineage	Sample Size	DMD	FM	100% MCP	95% KDE	75% KDE	50% KDE	95% KDE	75% KDE	50% KDE	95% bbKDE	75% bbKDE	50% bbKDE
						h-reference			*ad-hoc*				
Crotalus scutulatus	5	24.3 ± 10.1	2.40 ± 0.381	5.17 ± 3.11	15.2 ± 10.6	7.48 ± 5.48	3.49 ± 2.63	7.10 ± 3.46	3.04 ± 1.55	1.27 ± 0.662	8.97 ± 5.79	4.29 ± 2.95	1.99 ± 1.44
Crotalus scutulatus x *viridis*	4 (3 for bbKDEs)	21.4 ± 5.60	2.05 ± 0.298	3.24 ± 1.07	8.17± 2.62	3.92 ± 1.33	1.79 ± 0.647	4.29 ± 1.19	1.77 ± 0.484	0.714 ± 0.196	6.41 ± 0.611	2.93 ± 0.093	1.22 ± 0.089

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Table A.6: Initial capture data for Mojave Rattlesnakes (*Crotalus scutulatus*) implanted with radio transmitters. The first four characters of the Snake ID indicate site (CRSC = Mojave Rattlesnake site SW of the hybrid zone; CRVI = Prairie Rattlesnake site NE of the hybrid zone; SCVI = within hybrid zone). Universal Transverse Mercator (UTM) coordinates are in Zone 12N. Abbreviations: HI = Hybrid Index; Rep. Stat. = Reproductive Status; npF = Non-pregnant Female, M = Male; SVL = Snout-Vent Length (mm); Mov. Calc. = Yes/no for movements statistics; HR Made = Yes/No for 95% Brownian Bridge Kernel Density Estimator; Fate: Unk = Unknown, Trans. = Transmitter, Anthro. = Anthropogenic cause of death (ex., caught in refuse, roadkill).

Snake ID	HI	Sex/ Rep. Stat.	SVL mm	Mass g	Initial Capture Date	Easting	Northing	Date of Implant	Started Tracking	Ended Tracking	Mov. Calc.	HR Made	Fate
CRSCAA21	0.0081	npF	679	219.7	7/14/2021	685980	3529518	7/16/2021	7/22/2021	8/27/2021	Y	Y	Survived
CRSCB21	0.0200	M	662	204.3	6/22/2021	686347	3528533	6/23/2021	6/26/2021	8/31/2021	Y	Y	Survived
CRSCBB21	0.0151	M	900	667.6	7/15/2021	685853	3529587	7/15/2021	7/24/2021	8/30/2021	Y	Y	Survived
CRSCC21	0.0000	M	644	188	6/4/2021	686604	3528098	6/6/2021	6/14/2021	8/27/2021	Y	Y	Survived
CRSCD21	0.0000	M	746	333	6/4/2021	686368	3528366	6/6/2021	6/14/2021	8/27/2021	Y	Y	Survived
CRSCDD21	0.0035	npF	692	268.8	7/16/2021	687154	3530018	7/18/2021	7/30/2021	9/3/2021	Y	Y	Survived
CRSCGG21	0.0153	M	924	605.4	7/19/2021	685873	3529253	7/21/2021	7/27/2021	9/1/2021	Y	Y	Survived
CRSCHH21	0.0000	M	900	570.2	7/19/2021	685828	3528572	7/21/2021	7/27/2021	8/30/2021	Y	N	Survived
CRSCI19	0.0179	npF	733	237.3	7/20/2019	690985	3551901	7/21/2019	7/24/2019 5/29/2020	8/22/2019 8/22/2020	Y	Y	Survived
CRSCI21	0.0241	npF	809	392.8	6/12/2021	685862	3528948	6/12/2021	6/22/2021	8/28/2021	Y	Y	Survived
CRSCII21	0.0000	M	880	487.2	7/19/2021	685796	3528328	7/22/2021	7/27/2021	8/28/2021	Y	Y	Survived
CRSCJ21	0.0000	M	772	338.4	6/12/2021	686399	3529714	6/13/2021	6/28/2021	7/7/2021	Y	N	Unk. (Trans. failure)
CRSCK21	0.0000	M	745	254.3	6/14/2021	685831	3529960	6/16/2021	6/21/2021	7/30/2021	Y	Y	Died (Unk.)
CRSCM21	0.0083	M	771	272.1	6/17/2021	686269	3528936	6/17/2021	6/22/2021	9/1/2021	Y	Y	Survived
CRSCMM21	0.0219	M	706	238.7	7/22/2021	686434	3528275	7/25/2021	8/9/2021	8/27/2021	Y	N	Survived
CRSCN21	0.0345	npF	751	274.2	6/18/2021	686067	3529084	6/20/2021	6/28/2021	9/6/2021	Y	Y	Survived
CRSCP21	0.0000	M	673	230.7	6/25/2021	686502	3528943	6/27/2021	7/5/2021	8/28/2021	Y	Y	Survived
CRSCUU21	0.0000	M	701	231.6	7/30/2021	686513	3528194	8/2/2021	8/6/2021	8/27/2021	Y	N	Survived
CRSCW21	0.0281	M	913	558.5	7/9/2021	686613	3528541	7/11/2021	7/19/2021	8/12/2021	Y	N	Died (Anthro.)
CRSCY21	0.0000	M	861	504	7/12/2021	685869	3530018	7/13/2021	7/16/2021	8/30/2021	Y	Y	Survived
CRSCZ21	0.0000	M	870	430.2	7/13/2021	686299	3528505	7/14/2021	7/16/2021	8/28/2021	Y	Y	Survived
SCVIN19	0.0161	M	686	201.2	6/27/2019	694057	3555076	6/30/2019	7/4/2019	8/20/2019	Y	Y	Survived

Table A.7: Initial capture data for Prairie Rattlesnakes (*Crotalus viridis*) implanted with radio transmitters. The first four characters of the Snake ID indicate site (CRSC = Mojave Rattlesnake site SW of the hybrid zone; CRVI = Prairie Rattlesnake site NE of the hybrid zone; SCVI = within hybrid zone). Universal Transverse Mercator (UTM) coordinates are in Zone 12N. Abbreviations: HI = Hybrid Index; Rep. Stat. = Reproductive Status; npF = Non-pregnant Female, M = Male; SVL = Snout-Vent Length (mm); Mov. Calc. = Yes/no for movements statistics; HR Made = Yes/No for 95% Brownian Bridge Kernel Density Estimator; Fate: Unk = Unknown, Trans. = Transmitter, Anthro. = Anthropogenic cause of death (ex., caught in refuse, roadkill).

Snake ID	HI	Sex/ Rep. Stat.	SVL mm	Mass g	Initial Capture Date	Easting	Northing	Date of Implant	Started Tracking	Ended Tracking	Mov. Calc.	HR Made	Fate
CRVIA20	0.9957	M	875	431.5	5/28/2020	703192	3571398	5/30/2020	6/4/2020 5/27/2021	8/15/2020 8/28/2021	Y	Y	Survived
CRVID20	0.9959	M	760	252.9	6/5/2020	703117	3570967	6/6/2020	6/11/2020 5/27/2021	8/22/2020 8/29/2021	Y	Y	Survived
CRVIF20	0.9973	M	803	382.1	6/7/2020	699961	3562641	6/12/2020	7/2/2020	8/21/2020	Y	Y	Died (Anthro.)
CRVII20	0.9697	M	938	553.2	6/13/2020	702612	3570943	6/15/2020	6/20/2020	8/22/2020	Y	Y	Unk. (Trans. failure)
CRVIII20	0.9999	M	650	185.7	8/25/2020	703293	3571405	8/27/2020	na	na	N	N	Unk. (Trans. failure)
CRVIJ20	0.9999	M	772	325.8	6/13/2020	702924	3571378	6/16/2020	6/20/2020 5/20/2021	8/22/2020 9/1/2021	Y	Y	Survived
CRVIK20	0.9999	M	804	439.2	6/13/2020	702817	3571123	6/17/2020	6/23/2020 6/1/2021	8/24/2020 8/29/2021	Y	Y	Survived
CRVIL20	0.9906	M	796	342.3	6/17/2020	703109	3570708	6/19/2020	6/23/2020 7/18/2021	8/22/2020 9/3/2021	Y	Y	Survived
CRVIM20	0.9999	M	724	273.3	6/20/2020	703385	3570757	6/22/2020	6/26/2020 6/1/2021	8/22/2020 9/2/2021	Y	Y	Survived
CRVIP20	0.9999	M	806	381.1	7/3/2020	703210	3571184	7/5/2020	7/7/2020 6/1/2021	8/22/2020 8/29/2021	Y	Y	Survived
CRVIT20	0.9999	M	748	358.7	7/9/2020	704237	3571063	7/10/2020	7/15/2020 6/13/2021	8/23/2020 9/2/2021	Y	Y	Survived

Table A.7 continued: Initial capture data for Prairie Rattlesnakes (*Crotalus viridis*) implanted with radio transmitters. The first four characters of the Snake ID indicate site (CRSC = Mojave Rattlesnake site SW of the hybrid zone; CRVI = Prairie Rattlesnake site NE of the hybrid zone; SCVI = within hybrid zone). Universal Transverse Mercator (UTM) coordinates are in Zone 12N. Abbreviations: HI = Hybrid Index; Rep. Stat. = Reproductive Status; npF = Non-pregnant Female, M = Male; SVL = Snout-Vent Length (mm); Mov. Calc. = Yes/no for movements statistics; HR Made = Yes/No for 95% Brownian Bridge Kernel Density Estimator; Fate: Unk = Unknown, Trans. = Transmitter, Anthro. = Anthropogenic cause of death (ex., caught in refuse, roadkill).

Snake ID	HI	Sex/ Rep. Stat.	SVL mm	Mass g	Initial Capture Date	Easting	Northing	Date of Implant	Started Tracking	Ended Tracking	Mov. Calc.	HR Made	Fate
SCVIL19	0.9733	M	710	192.8	6/22/2019	696273	3559228	6/23/2019	6/29/2019	8/20/2019	Y	Y	Survived
SCVIM20	0.9561	M	860	442.2	7/20/2020	699212	3559283	7/22/2020	8/3/2020	8/21/2020	Y	N	Unk. (Trans. failure)
SCVINN19	0.9999	M	823	365	8/17/2019	695477	3560386	8/20/2019	7/3/2020	8/12/2020	Y	N	Unk. (Trans. failure)
SCVIP20	0.9999	M	780	470.7	8/5/2020	697555	3559109	8/8/2020	8/17/2020 6/1/2021	8/22/2020 8/28/2021	Y	Y	Survived
SCVIVV19	0.9791	M	824	346.4	8/22/2019	697092	3559244	8/26/2019	6/1/2020	8/21/2020	Y	Y	Survived

Table A.8: Initial capture data for hybrid rattlesnakes (*Crotalus scutulatus* x *viridis*) implanted with radio transmitters. The first four characters of the Snake ID indicate site (CRSC = Mojave Rattlesnake site SW of the hybrid zone; CRVI = Prairie Rattlesnake site NE of the hybrid zone; SCVI = within hybrid zone). Universal Transverse Mercator (UTM) coordinates are in Zone 12N. Abbreviations: HI = Hybrid Index; Rep. Stat. = Reproductive Status; npF = Non-pregnant Female, M = Male; SVL = Snout-Vent Length (mm); Mov. Calc. = Yes/no for movements statistics; HR Made = Yes/No for 95% Brownian Bridge Kernel Density Estimator; Fate: Unk = Unknown, Trans. = Transmitter, Anthro. = Anthropogenic cause of death (ex., caught in refuse, roadkill), Mamm. = a suspected large mammal (Kit Fox or American Badger) killed the snake.

Snake ID	HI	Sex/ Rep. Stat.	SVL mm	Mass g	Initial Capture Date	Easting	Northing	Date of Implant	Started Tracking	Ended Tracking	Mov. Calc.	HR Made	Fate
CRVIB20	0.9167	M	835	346.5	5/30/2020	703223	3570475	6/1/2020	6/3/2020	8/22/2020	Y	Y	Died between seasons (Mamm.)
CRVIN20	0.9242	npF	716	245	6/23/2020	702772	3571173	6/25/2020	7/2/2020	8/16/2020	Y	Y	Died (Unk.)
SCVIA19	0.1017	M	820	337.5	5/22/2019	695962	3559455	5/25/2019	6/5/2019	8/22/2019	Y	Y	Survived
SCVIC20	0.2882	M	770	290.5	6/13/2020	695657	3558982	6/14/2020	6/19/2020	8/10/2020	Y	Y	Died (Anthro.)
SCVID19	0.6700	M	659	168.5	5/28/2019	696862	3558359	5/30/2019	6/10/2019	8/20/2019	Y	Y	Survived
SCVID20	0.7773	npF	650	214.7	6/13/2020	696545	3559292	6/18/2020	6/23/2020	8/21/2020	Y	Y	Survived
SCVID20	0.7773	pF	665	222.4	5/18/2021	696458	3559369	6/18/2020	5/23/2021	8/24/2021	N	N	Survived
SCVID21	0.4929	M	643	213	7/29/2021	686389	3559126	8/1/2021	8/5/2021	9/2/2021	Y	N	Survived
SCVIE21	0.5079	M	783	331.1	7/30/2021	696820	3558845	7/31/2021	8/5/2021	8/31/2021	Y	N	Survived
SCVIEE19	0.9329	M	745	221.3	7/29/2019	696051	3559000	8/4/2019	8/6/2019	8/22/2019	Y	N	Died between seasons (Unk.)
SCVIGG19	0.6442	M	817	427.9	8/2/2019	700062	3557278	8/6/2019	8/14/2019	8/22/2019	N	N	Unk. (Trans. failure)
SCVIH20	0.9379	npF	751	307.3	7/2/2020	697591	3559092	7/4/2020	7/8/2020	8/21/2020	Y	Y	Died between seasons (Unk.)
SCVII19	0.2192	M	714	207.4	6/5/2019	692957	3566873	6/7/2019	6/11/2019	8/21/2019	Y	Y	Survived

Table A.8 continued: Initial capture data for hybrid rattlesnakes (*Crotalus scutulatus* x *viridis*) implanted with radio transmitters. The first four characters of the Snake ID indicate site (CRSC = Mojave Rattlesnake site SW of the hybrid zone; CRVI = Prairie Rattlesnake site NE of the hybrid zone; SCVI = within hybrid zone). Universal Transverse Mercator (UTM) coordinates are in Zone 12N. Abbreviations: HI = Hybrid Index; Rep. Stat. = Reproductive Status; npF = Non-pregnant Female, M = Male; SVL = Snout-Vent Length (mm); Mov. Calc. = Yes/no for movements statistics; HR Made = Yes/No for 95% Brownian Bridge Kernel Density Estimator; Fate: Unk = Unknown, Trans. = Transmitter, Anthro. = Anthropogenic cause of death (ex., caught in refuse, roadkill), Mamm. = a suspected large mammal (Kit Fox or American Badger) killed the snake.

Snake ID	HI	Sex/ Rep. Stat.	SVL mm	Mass g	Initial Capture Date	Easting	Northing	Date of Implant	Started Tracking	Ended Tracking	Mov. Calc.	HR Made	Fate
SCVIJJ19	0.1369	M	746	242.5	8/5/2019	693944	3558167	8/11/2019	8/19/2019	8/21/2019	N	N	Unk. (Trans. failure)
SCVIKK19	0.4493	M	793	333.5	8/7/2019	693204	3564676	8/13/2019	8/20/2019	8/21/2019	N	N	Survived to 2020
SCVIOO19	0.1152	M	863	398.1	8/17/2019	695482	3560288	8/17/2019	na	na	N	N	Unk. (Trans. failure)
SCVIP19	0.5362	M	763	322.7	6/28/2019	697597	3559107	6/29/2019	7/3/2019	7/30/2019	Y	N	Died (Mamm.)
SCVIV19	0.5587	npF	738	263.3	7/5/2019	697038	3558719	7/7/2019	7/10/2019 5/30/2020	8/24/2019 8/12/2020	Y	Y	Survived
SCVIX19	0.2449	M	793	290.4	7/3/2019	693429	3562579	7/7/2019	7/18/2019	8/20/2019	Y	Y	Died between seasons (Unk.)

Table A.9: Male Utilization Distributions (UD) estimated with Minimum Convex Polygons (MCP) and Kernel Density Estimators (KDE: smoothing parameter method, *ad-hoc* [Kie 2013]). All UDs are in hectares.

Genetic Lineage	Sample Size	100% MCP	95% KDE	75% KDE	50% KDE	95% KDE	75% KDE	50% KDE
				h-reference			*ad-hoc*	
Crotalus scutulatus	13	16.8 ± 1.81	38.6 ± 5.26	18.5 ± 2.78	8.77 ± 1.43	26.3 ± 3.15	11.9 ± 1.30	5.16 ± 0.539
Crotalus viridis	14	36.6 ± 6.37	94.8 ± 26.2	43.2 ± 12.5	20.2 ± 5.94	64.7 ± 17.0	27.0 ± 7.25	11.6 ± 3.30
Crotalus scutulatus x *viridis*	6	29.1 ± 9.58	72.9 ± 27.7	35.2 ± 13.2	16.0 ± 5.75	51.6 ± 18.0	23.0 ± 7.84	10.3 ± 3.42

Table A.10: Fecal sample data. The first four characters of Snake ID indicate at which site the snake was initially captured, CRSC = Mojave Rattlesnake site (SW of the hybrid zone), CRVI = Prairie Rattlesnake site (NE of the hybrid zone), SCVI = within the hybrid zone; the following characters indicate ascension order and year (e.g., A21 = first snake captured in 2021; AA21 = 27th snake captured in 2021). HI = Hybrid Index. All females were not pregnant. Age: A = Adult, J = Juveniles. Masses are in grams. SVL = Snout-Vent Length of the snake in mm. Mammal remains were any combination of pelage, vibrissae, and teeth. Lizard remains were scales.

Snake ID	Genetic Lineage	HI	Sex	Age	Snake Mass	SVL	Date Feces Collected	Fecal Dry Mass	Mammal Remains	Lizard Remains
CRSCA21	CRSC	0.0000	F	J	99.9	533	5/21/2021	1.43	Y	N
CRSCAA21	CRSC	0.0081	F	A	219.7	679	7/16/2021	6.91	Y	N
CRSCBB21	CRSC	0.0151	M	A	667.6	900	7/16/2021	13.1	Y	N
CRSCCC21	CRSC	0.0000	M	J	64.4	470	7/30/2021	0.74	Y	Y
CRSCD21	CRSC	0.0000	M	A	333	746	6/5/2021	3.08	Y	N
CRSCD21	CRSC	0.0000	M	A	333	746	9/13/2021	2.83	Y	N
CRSCDD21	CRSC	0.0035	F	A	268.8	692	7/18/2021	7.56	Y	N
CRSCE21	CRSC	0.0000	F	J	47.2	397	6/9/2021	1.65	Y	N
CRSCEE21	CRSC	0.0000	F	J	70	474	7/19/2021	2.24	Y	N
CRSCF21	CRSC	0.0000	F	J	27.9	371	6/21/2021	0.07	N	Y
CRSCF21	CRSC	0.0000	F	J	27.9	371	6/8/2021	1.35	Y	Y
CRSCH21	CRSC	0.0000	M	J	47.9	427	6/14/2021	0.19	N	Y
CRSCL19	CRSC	0.0050	F	J	30.6	384	July 2019	0.18	Y	N
CRSCLL21	CRSC	0.0000	F	J	24.8	337	7/22/2021	0.68	N	Y
CRSCM19	CRSC	0.0000	M	A	231.4	694	8/5/2021	2.46	Y	N
CRSCMM21	CRSC	0.0219	M	A	238.7	706	7/25/2021	2.48	Y	Y
CRSCP21	CRSC	0.0000	M	A	230.7	673	6/27/2021	10.2	Y	N
CRSCQQ21	CRSC	0.0000	F	A	357.3	760	8/2/2021	6.03	Y	N
CRSCS21	CRSC	0.0000	F	J	77.3	493	7/5/2021	1.72	N	Y
CRSCV21	CRSC	0.0000	F	J	34.6	386	7/7/2021	0.99	N	Y

Table A.10 continued: Fecal sample data. The first four characters of Snake ID indicate at which site the snake was initially captured, CRSC = Mojave Rattlesnake site (SW of the hybrid zone), CRVI = Prairie Rattlesnake site (NE of the hybrid zone), SCVI = within the hybrid zone; the following characters indicate ascension order and year (e.g., A21 = first snake captured in 2021; AA21 = 27th snake captured in 2021). HI = Hybrid Index. All females were not pregnant. Age: A = Adult, J = Juveniles. Masses are in grams. SVL = Snout-Vent Length of the snake in mm. Mammal remains were any combination of pelage, vibrissae, and teeth. Lizard remains were scales.

Snake ID	Genetic Lineage	HI	Sex	Age	Snake Mass	SVL	Date Feces Collected	Fecal Dry Mass	Mammal Remains	Lizard Remains
CRSCWW21	CRSC	0.0000	M	A	566.04	951	8/7/2021	12.9	Y	N
CRSCXX21	CRSC	0.0000	F	J	69.7	494	8/9/2021	0.12	N	N
CRVIA20	CRVI	0.9957	M	A	353.6	857	8/31/2021	1.09	N	Y
CRVIA21	CRVI	0.9999	M	J	32.8	371	6/3/2021	0.27	Y	Y
CRVID20	CRVI	0.9959	M	A	222.5	761	9/29/2021	Na	N	N
CRVIDD20	CRVI	0.9999	F	J	51.4	407	8/13/2020	0.45	Y	Y
CRVIF20	CRVI	0.9973	M	A	382.1	803	June 2019	5.17	Y	N
CRVIGG20	CRVI	0.9999	M	A	504.8	888	9/5/2020	5.14	N	Y
CRVIH21	CRVI	0.9999	M	A	319.8	815	7/6/2021	0.77	N	N
CRVIHH20	CRVI	na	M	A	264.1	683	9/5/2020	1.11	N	N
CRVIK20	CRVI	0.9999	M	A	439.2	804	6/17/2020	0.50	Y	N
CRVIKK20	CRVI	0.9999	M	A	180.7	638	9/4/2020	0.92	Y	N
CRVIL20	CRVI	0.9906	M	A	371.5	795	10/29/2021	Na	N	N
CRVIL20	CRVI	0.9906	M	A	342.3	796	6/19/2020	2.95	Y	N
CRVIL20	CRVI	0.9906	M	A	371.5	795	9/9/2021	1.61	Y	N
CRVIM21	CRVI	0.9826	M	J	33.9	347	7/29/2021	0.11	Y	N
CRVIO20	CRVI	0.9999	M	A	230.3	716	6/27/2020	0.12	N	Y
CRVIP20	CRVI	0.9999	M	A	315.6	816	5/22/2021	1.56	N	N
CRVIR20	CRVI	0.9999	M	J	56.9	458	7/8/2020	1.10	Y	N
CRVIT20	CRVI	0.9999	M	A	358.7	748	7/11/2020	1.58	Y	N

Table A.10 continued: Fecal sample data. The first four characters of Snake ID indicate at which site the snake was initially captured, CRSC = Mojave Rattlesnake site (SW of the hybrid zone), CRVI = Prairie Rattlesnake site (NE of the hybrid zone), SCVI = within the hybrid zone; the following characters indicate ascension order and year (e.g., A21 = first snake captured in 2021; AA21 = 27th snake captured in 2021). HI = Hybrid Index. All females were not pregnant. Age: A = Adult, J = Juveniles. Masses are in grams. SVL = Snout-Vent Length of the snake in mm. Mammal remains were any combination of pelage, vibrissae, and teeth. Lizard remains were scales.

Snake ID	Genetic Lineage	HI	Sex	Age	Snake Mass	SVL	Date Feces Collected	Fecal Dry Mass	Mammal Remains	Lizard Remains
SCVID21	SCVI	0.4929	M	A	213	643	9/16/2021	4.84	Y	N
SCVIDD19	SCVI	0.0741	F	A	161.4	654	8/6/2019	0.95	Y	N
SCVIE20	SCVI	na	M	J	68	461	6/22/2020	0.34	N	Y
SCVIE21	SCVI	0.5079	M	A	331.1	783	7/30/2021	3.88	Y	N
SCVIEE19	SCVI	0.9329	M	A	221.3	745	August 2019	0.61	Y	N
SCVIF20	SCVI	0.3143	M	A	140.3	599	6/26/2020	1.15	Y	N
SCVIFF19	SCVI	0.8226	M	A	174.4	634	8/6/2019	3.47	Y	N
SCVIL19	CRVI	0.9733	M	A	192.8	710	8/28/2019	3.23	Y	N
SCVILL19	SCVI	0.8377	F	A	209.3	667	8/22/2019	1.24	N	Y
SCVIN20	SCVI	0.7814	M	A	194.5	686	7/24/2020	2.74	Y	N
SCVINN19	CRVI	0.9999	M	A	368.62	829	6/23/2020	3.84	Y	N
SCVIP19	SCVI	0.5362	M	A	322.7	763	June 2019	4.62	Y	N
SCVIP20	CRVI	0.9999	M	A	385.5	778	9/1/2021	4.17	Y	N
SCVIS19	CRVI	0.9999	M	J	55.9	445.6	June 2019	0.31	N	Y
SCVIT19	SCVI	0.8271	M	A	146.5	610	July 2019	1.38	Y	N
SCVITT19	SCVI	0.4536	F	J	12.9	242	8/24/2019	0.10	N	Y
SCVIU19	SCVI	0.1922	M	J	51.9	422	July 2019	0.85	Y	N
SCVIUU19	SCVI	0.2090	F	A	229.4	732	8/29/2019	2.84	Y	N
SCVIX19	SCVI	0.2449	M	A	290.4	793	8/21/2019	0.23	N	N
SCVIY19	SCVI	0.6331	M	J	57.2	443	July 2019	1.98	Y	Y

Table A.11: Stomach content data. The first four characters of Snake ID indicate at which site the snake was initially captured, CRSC = Mojave Rattlesnake site (SW of the hybrid zone), CRVI = Prairie Rattlesnake site (NE of the hybrid zone), SCVI = within the hybrid zone; the following characters indicate ascension order and year (e.g., A21 = first snake captured in 2021; AA21 = 27th snake captured in 2021). HI = Hybrid Index. All females were not pregnant. Age: A = Adult, J = Juveniles. Masses is in grams. SVL = Snout-Vent Length of the snake in mm.

Snake ID	Genetic Lineage	HI	Sex	Age	Snake Mass	SVL	Date Palpated	Content
CRSCEE21	CRSC	0.0000	F	J	70	474	7/17/2021	Merriam's Kangaroo Rat (*Dipodomys merriami*)
CRSCFF21	SCVI	0.0947	F	J	60.1	443	7/18/2021	Unknown Rodent
CRSCL19	CRSC	0.0050	F	J	30.6	384	7/27/2019	Whiptail Lizard (*Aspidoscelis* spp.)
CRVIJ20	CRVI	0.9999	M	A	325.8	772	6/16/2020	Either Merriam's or Ord's Kangaroo Rat (*Dipodomys merriami* or *D. ordii*)
SCVIA21	SCVI	0.4849	M	J	116.8	552	7/7/2021	Pocket Mouse (*Chaetodipus* spp.)
SCVID19	SCVI	0.6700	M	A	168.5	659	5/28/2019	Roundtail Horned Lizard (*Phrynosoma cornutum*)
SCVIE21	SCVI	0.5079	M	A	331.1	783	7/31/2021	Ord's Kangaroo Rat (*Dipodomys ordii*)