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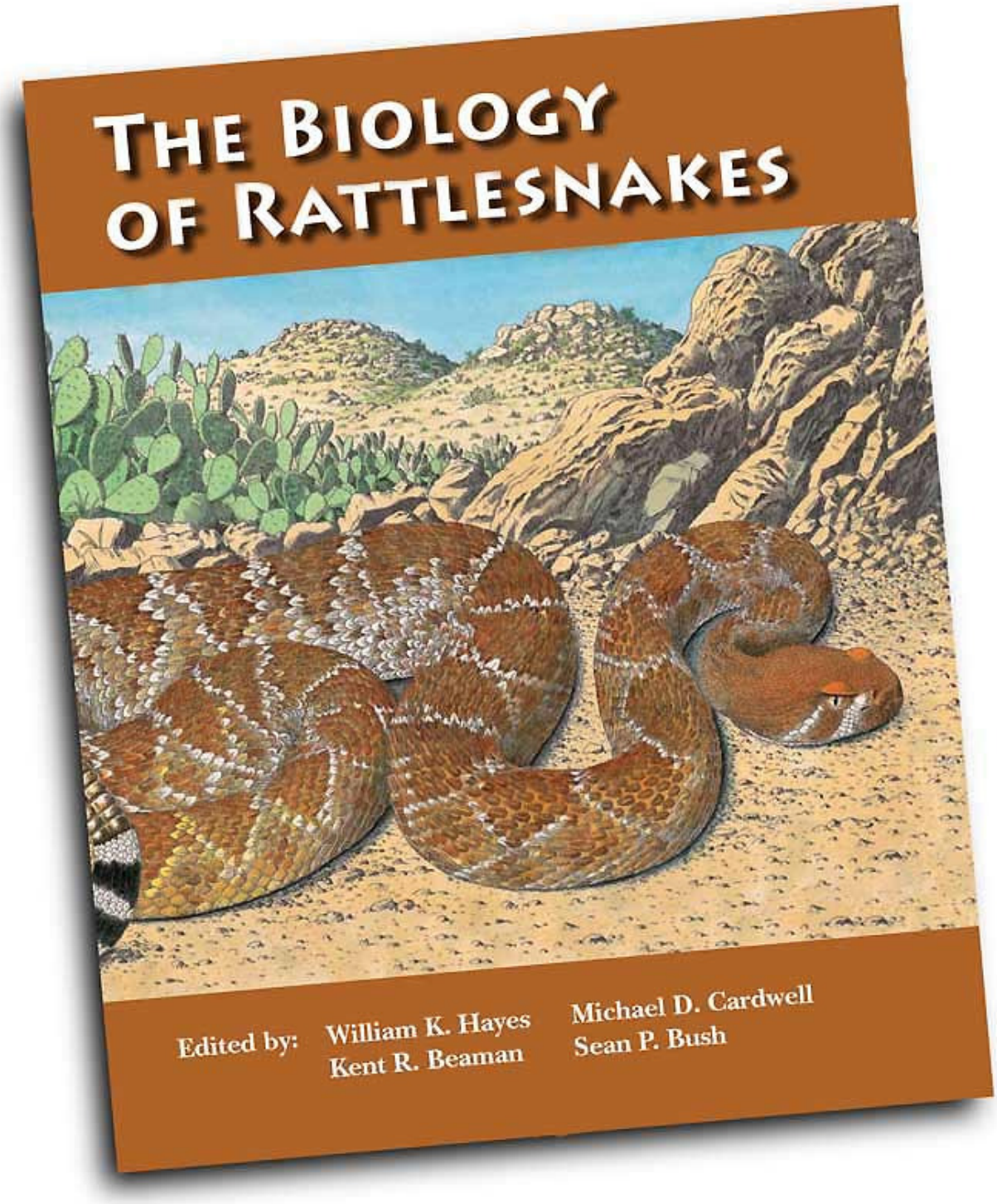

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Factors Influencing the Antipredator Behavior of Mexican Lance-Headed Rattlesnakes (*Crotalus polystictus*) toward Humans

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Abstract.—We studied antipredator behavior in a population of *Crotalus polystictus* (Mexican Lance-headed Rattlesnakes) inhabiting an agricultural area in central Mexico. We simulated an escalating predation attempt by a human in laboratory trials designed to examine the effects of age, temperature, and sex and/or reproductive condition on *C. polystictus* defensive behavior. We compared three antipredator responses (rattling, posturing, and striking) exhibited by neonates, adult males, and gravid females at two temperature levels (cool: 18–19°C; warm: 29–30°C). In *C. polystictus*, only the antipredator behavior of gravid females was influenced by temperature. Neonates rattled and struck more than gravid females but did not differ from adult males in their antipredator behavior. We did not detect heritability of antipredator behavior in *C. polystictus*, which may be due to the influence of temperature on gravid females or other factors. We discuss the adaptive significance of different antipredator behaviors in neonate, adult male, and gravid female rattlesnakes and suggest directions for future research.

Introduction

Antipredator behavior includes the suite of responses an animal exhibits toward a potential predator or perceived threat. Antipredator behavior has likely been important in the diversification and persistence of reptiles, as entire clades have evolved specialized morphological features just for this purpose (i.e., turtle shells and rattlesnake rattles; Greene, 1988).

Defensive behavior of rattlesnakes may be influenced by sex, reproductive condition, temperature, age, or other factors (Goode and Duvall, 1989; Prior and Weatherhead, 1994; May et al., 1996; Kissner et al., 1997; Glaudas et al., 2005). Female *Sistrurus* sp. are more likely to flee when disturbed than males, but sexes do not differ in striking frequency (Glaudas et al., 2005). *Sistrurus* sp. also rattle and strike more frequently at higher temperatures (Prior and Weatherhead, 1994; May et al., 1996). Only the antipredator responses of gravid females are temperature dependent in *C. v. viridis* (Goode and Duvall, 1989; Kissner et al., 1997). In the field, smaller *C. v. viridis* allow closer approaches by humans before rattling than do larger individuals (Kissner et al., 1997).

The Mexican Lance-headed Rattlesnake (*Crotalus polystictus*) is a small rattlesnake, usually less than 1 m snout-vent length, found on the southern Mexican Plateau (Campbell and Lamar, 2004). Humans have converted most of the habitat of *C. polystictus* for agricultural use (Bryson et al., 2003), resulting in close proximity of humans and snakes. In some agricultural areas, *C. polystictus* occurs in locally dense populations, where human-snake encounters are common and lead to apparently high incidences of anthropogenic mortality for *C. polystictus* and snakebites for humans (Amarello, 2005).

We studied a population of *C. polystictus* inhabiting an environment actively used for agriculture. We investigated several factors influencing the defensive responses of *C. polystictus* toward humans by comparing the antipredator behavior of neonates, adult males, and adult gravid females at different temperatures. As temperature increased, we expected that the frequency of rattling and striking would increase, as is the case for several other species of rattlesnakes (Goode and Duvall, 1989; Prior and Weatherhead, 1994; May et al., 1996). Though many people believe that smaller, younger snakes are more aggressive than larger, older individuals, previous studies have both refuted (Kissner et al., 1997) and supported (Shine et al., 2002; Roth and Johnson, 2004) this idea. Therefore, though we expected we might see differences in antipredator behavior of adults and neonates, we did not predict which would exhibit more defensive displays.

Materials and Methods

We collected *C. polystictus* at an elevation of about 2,500 m on a ranch near the town of Toluca, México. The ranch had fallow fields, cornfields, hay fields, and grazing pastures, all of which were surrounded with man-made irrigation canals. We searched for and encountered *C. polystictus* in all the aforementioned landscape types. We collected adult male ($N = 21$) and gravid female ($N = 50$) *C. polystictus* from 31 May–26 June 2004, between 0900–1300 h. We did not encounter any non-gravid adult females. We captured snakes with 61-cm tongs (Whitco Manufacturing,

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Inc., Weatherford, Texas, USA), placed individuals in cloth sacks, and transported them to cages in our testing facility nearby. We retained gravid females in captivity until parturition; seven females gave birth to the 50 neonates used in behavioral trials. We determined sex of adults by visual inspection: male *C. polystictus* are larger and more slender with longer tails; females are smaller and stout with short tails. Neonates were not sexed.

Experimental design.—We conducted 188 behavioral trials at a private residence near Toluca, México, in June 2004. Prior to behavioral trials, adult rattlesnakes were maintained in captivity at *ca*. 25°C for 1–17 d in 34.5 x 23.5 x 16.0 cm clear plastic containers with natural light cycles and free access to water (but not food). After birth, neonates were housed individually, in cages identical to those in which adults were maintained, for 1-2 d prior to behavioral trials. We tested snakes from 15–27 June, between 1400–2000 h, in cages identical to those in which they were maintained, minus the water.

We tested 67 adult (47 gravid females, 20 males) rattlesnakes at two temperature levels, warm (29–30°C) and cool (18–19°C). Though we did not have time to test all snakes twice, we randomly selected a sample of gravid females (N = 22) to be tested cool twice, once each on two consecutive days, to assess repeatability of our behavioral trials. We tested neonates at one temperature level (cool) only.

At least 24 h prior to testing, we placed rattlesnakes in either a cool or a warm room to acclimate to a new enclosure and temperature. We randomly determined whether each snake was tested at cool or warm temperatures first. We chose ecologically relevant temperature levels to approximate the range of conditions at which we found snakes in the field (substrate temperature mean ± 1 SE = 24 ± 1°C; range = 17–34°C). Following each adult's first trial at one temperature level, we moved the snake to the other room for testing the following day at the other temperature level.

To minimize disturbing snakes prior to testing, we took several precautions. We covered all snake boxes with an opaque cloth until just before a trial. In preparation for testing a given individual, we removed that individual's cage from the other snakes and placed it on the opposite side of the room. We then waited 3-5 min before beginning a trial.

We designed trials to simulate an escalating predation attempt, modified from the methods of Roth and Johnson (2004). A trial consisted of three consecutive stages, each lasting 20 sec, with 1 min between stages. In stage one, we removed the opaque cover and lid of the cage (for the duration of the trial) to visually expose the snake to the human predator. During stage two, we harassed the snake by tugging once at mid-body with a pair of 61-cm tongs, followed by gently prodding along the body from head to tail for the remainder of the 20 sec. During stage three, we grasped the snake with tongs, lifted it above the substrate for 5 sec, put it down, waited 5 sec, and then repeated the lifting procedure once more. Between stages, we left the visual field of the snake.

After completing the trial, we measured skin surface temperature with a non-contact, laser-sighted thermometer (Raynger ST60, Raytek, Santa Cruz, California, USA). Although a non-contact thermometer estimates body temperature within 1°C with minimal handling (Amarello and Smith, unpubl. data), we measured temperatures only after trial completion to remove this possible source of pre-trial disturbance from our behavioral tests.

We scored behavioral trials in a manner similar to Roth and Johnson (2004). During each stage of the trial, we measured three antipredator behaviors (rattling, posturing, and striking) and recorded a score, from zero to two. We measured rattling by either listening for the rattling sound, or for neonates and adult snakes without rattles, by watching for tail vibration. If the snake rattled for <10 sec, we recorded a score of one point; if it rattled for >10 sec, two points. We defined defensive posturing as the snake pulling its head back in an "S" curve slightly elevated from its coils. If the snake exhibited this posture for <10 sec, we recorded a score of one point; if it postured for >10 sec, two points. A strike was defined as an attempt to bite, whether contact was made or not. We recorded one point for one strike and two points for more than one strike. If a behavior was not observed during the 20 sec stage, we recorded zero points in that category.

Data analyses.—For each trial, total possible scores for each antipredator behavior ranged from zero (behavior not observed during any stage) to six. We pooled behavioral scores across stages because we were interested in responses to the entire trial, not differences between each stage. Following Roth and Johnson (2004), we summed the raw scores for all behaviors in all stages to create a total behavior score (TBS) for each trial. To examine the effects of temperature, sex, and/or reproductive condition, we calculated the change in each of the different behavioral scores and TBS from cool to warm temperatures (warm temperature score minus cool temperature score). Because we tested neonates only at cool temperatures, we only used adult trials at cool temperatures for comparisons between adults and neonates.

Behavioral scores were not normally distributed, though one variable (TBS) could be log-transformed to meet assumptions of normality. Therefore, we used both parametric (analysis of variance, ANOVA) and nonparametric tests (Wilcoxon signed rank for related samples, Wilcoxon rank sum for independent samples, Spearman rank) for analyses. All statistical analyses were performed using JMP 5.1 (SAS Institute, Inc., Cary, North Carolina, USA). We did not correct for multiple comparisons because we made *a priori* predictions that these groups would behave differently (Perneger, 1998). We considered statistical tests significant at $P \leq 0.05$ and, unless otherwise indicated, we report median and quartiles.

Table 1. Median scores (with upper and lower quartiles) for neonate and adult *Crotalus polystictus* at cool (18–19°C) and warm (29–30°C) temperatures (T). TBS = total behavioral score (sum of rattling, posturing, and striking scores).

Group (*N*)	T	Rattling	Posturing	Striking	TBS
Neonates (50)	Cool	4 (2–4)	1 (0–3)	0 (0–1)	5 (3–8)
Adult males (20)	Cool	4 (2–5)	3 (1–4)	0 (0–0)	7 (4–10)
Gravid females (47)	Cool	2 (1–4)	2 (0–3)	0 (0–0)	5 (2–7)
Adult males (20)	Warm	4 (2–5)	3 (1–4)	0 (0–0)	6 (3–8)
Gravid females (47)	Warm	3 (2–4)	2 (1–3)	0 (0–0)	5 (3–7)

Results

Repeatability (r) of total behavior scores based on an intraclass correlation coefficient (Lessells and Boag, 1987) was 0.95 (ANOVA: $N = 22$, $F_{1,20} = 36.50$). Total behavior scores of neonates and their mothers were not significantly correlated (Spearman rank analysis: $r_s = 0.22$, $P = 0.12$).

At cool temperatures, gravid females decreased antipredator behaviors but adult male behavior did not change (Table 1). Gravid females decreased their overall antipredator behavior (TBS) at cool temperatures (Wilcoxon signed-rank: df = 46, median change = 1, $S = 149.0$, $P = 0.03$), which was driven largely by a decrease in rattling (Wilcoxon signed-rank: median change = 0, $S = 128.5$, $P < 0.01$); there was no significant change in posturing or striking (Wilcoxon signed-rank, posturing: median change = 0, $S = 30.5$, $P = 0.26$; striking: median change = 0, $S = 18.0$, $P = 0.19$). Gravid females decreased their TBS and rattling more than males at cool temperatures (Wilcoxon rank sum, TBS: $Z = -1.92$, $P = 0.05$; rattling: $Z = -2.28$, $P = 0.02$) but not posturing (Wilcoxon rank sum: $Z = -1.02$, $P = 0.31$) or striking (Wilcoxon rank sum: $Z = -0.35$, $P = 0.73$). At cool temperatures, males did not change their overall antipredator behavior (TBS; Wilcoxon signed-rank: df = 19, median change = -1, $S = -28.5$, $P = 0.26$) or individual behaviors (Wilcoxon signed-rank, rattling: median change = 0, $S = -15.5$, $P = 0.31$; posturing: median change = 0, $S = -15.0$, $P = 0.39$; striking: median change = 0, $S = 0$, $P = 1$). At warm temperatures, gravid females and adult males behaved similarly (Wilcoxon rank sum, TBS: $Z = 0.50$, $P = 0.62$; rattling: $Z = 0.51$, $P = 0.61$; posturing: $Z = 0.83$, $P = 0.41$; striking: $Z = -0.29$, $P = 0.77$).

Gravid females behaved differently than neonate and adult male rattlesnakes at cool temperatures (Table 1; Fig. 1). Adult males and neonates behaved similarly in all aspects of their antipredator behavior (Wilcoxon rank sum, TBS: $Z = 1.23$, $P = 0.22$; rattling: $Z = 1.22$, $P = 0.22$; posturing: $Z = 1.89$, $P = 0.06$; striking: $Z = -1.30$, $P = 0.20$). Neonates rattled and struck more than gravid females (Wilcoxon rank sum, rattling: $Z = -2.27$, $P = 0.02$; striking: $Z = -1.98$, $P = 0.05$), but did not differ in posturing (Wilcoxon rank sum: $Z = -0.02$, $P = 0.99$) or TBS (Wilcoxon rank sum: $Z = -1.51$, $P = 0.13$). Adult males rattled more than gravid females (Wilcoxon rank sum: $Z = 2.61$, $P = 0.01$), and thus had a higher TBS (Wilcoxon rank sum: $Z = 2.31$, $P = 0.02$). Gravid female and adult male rattlesnakes did not differ in posturing (Wilcoxon rank sum: $Z = 1.86$, $P = 0.06$) or striking (Wilcoxon rank sum: $Z = 0.23$, $P = 0.82$) at cool temperatures.

Discussion

Components of the antipredator behavior of *C. polystictus* are influenced by temperature, sex, and/or reproductive condition in the laboratory. Gravid females reduced their defensive response at lower temperatures relative to their score at higher temperatures. Temperature had no detectable effect on behavioral scores of adult males. Neonates behaved similarly to adult males in all aspects of their antipredator behavior, but differed from gravid females in rattling and striking.

We only observed temperature-dependent changes in antipredator behavior of gravid females, consistent with published research on *C. v. viridis* (Goode and Duvall, 1989; Kissner et al., 1997). Although we did not test neonates at warm temperatures, their antipredator behavior at cool temperatures was similar to that of adult males, so it is doubtful that differences between gravid females and neonates were due to either size or age. Like Kissner et al. (1997), but unlike Goode and Duvall (1989), we found that gravid females decreased their antipredator responses

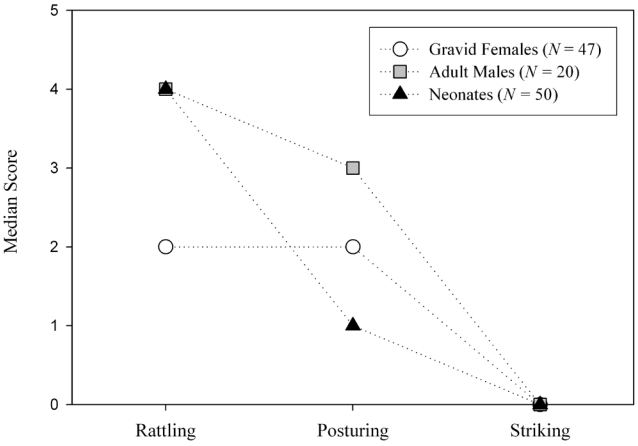

Figure 1. At cool temperatures (18–19°C), gravid female *Crotalus polystictus* rattled less than neonates (Wilcoxon rank sum: $Z = -2.27$, $P = 0.02$) and adult males (Wilcoxon rank sum: $Z = 2.61$, $P = 0.01$). Very few snakes struck during behavioral trials.

at lower temperatures. Changes in temperature may have had an effect on gravid female rattlesnakes because lower temperatures, coupled with hormonal changes or increases in body mass associated with pregnancy, can decrease the ability of females to perform antipredator behaviors (Seigel et al., 1987; Brodie, 1989a; Kissner et al., 1997).

In our study, variation in overall antipredator behavior (TBS) of *C. polystictus* was principally due to differences in rattling. Rattling is an effective defensive behavior, as many animals avoid its sound (Klauber, 1997). However, posturing is only effective if a predator is close enough to see the behavior, and striking puts a rattlesnake into close contact with the predator and may actually increase the chance of potential injury to the snake (Gibbons and Dorcas, 2002). *Crotalus polystictus* exhibited plasticity with rattling frequency only, and rarely struck, despite increased harassment. Neonates, who had not yet encountered predators in the field, were less hesitant than gravid females to resort to striking.

While adaptive explanations are intriguing, the observed patterns of antipredator behavior could arise as by-products of other factors. Differences in past experiences (Glaudas, 2004) or circulating hormone levels among individuals could explain much of the observed variation in behavior among groups. Manipulative experiments could illuminate the respective importance of adaptation or other factors on antipredator behavior. However, due to the potentially lethal consequences of encounters with predators, it seems plausible that, if genetically based, antipredator behavior would be a target of natural selection.

In the future, we would like to compare the population of *C. polystictus* we studied to other populations of *C. polystictus* as well as other species of rattlesnakes using the same methods. Populations of *C. polystictus* in natural areas experiencing little anthropogenic mortality could provide information on whether hunting pressure from humans has influenced the antipredator behavior of the *C. polystictus* we report on here. It is difficult to make direct comparisons among the few published studies of antipredator behavior in rattlesnakes and their close relatives, due to differences in methodology (Mori and Burghardt, 2004). Therefore, identical behavioral trials on different species of rattlesnakes would allow more rigorous investigation of the effects of phylogeny and environment on antipredator behaviors, and offer insight into the evolutionary forces that shape these complex traits.

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