

A study of individual differences in spatial use of captive sidewinder rattlesnakes *Crotalus cerastes*

JUANITA PARDO-SANCHEZ¹, ASHLEY TAYLOR², EMILY G. WEIGEL³ & JOSEPH R. MENDELSON III^{2,3*}

¹Ecology and Evolutionary Biology, University of Michigan, Ann Arbor, Michigan, 48109, USA

²Zoo Atlanta, 800 Cherokee Ave SE, Atlanta, Georgia, 30315, USA

³School of Biological Sciences, Georgia Institute of Technology, 950 Atlantic Dr NW, Atlanta, Georgia, 30332, USA

*Corresponding author e-mail: jmendelson@zoatlanta.org

ABSTRACT – Many species of vipers are known to use ambush sites repeatedly, a phenomenon presumably informed by chemical cues from prey. Experimentally, we limited chemical cue availability and recorded site selection in five captive-born sidewinders *Crotalus cerastes* during their first active season. Snakes showed individual differences among the study subjects in spatial movement across the season and repeatedly reused specific sites in their enclosures, suggesting spatial memory and individual differences in choices of resting sites. Individual variability in spatial- and object-use and associated memories suggests attention to such should be considered in developing husbandry routines for these captive individuals.

INTRODUCTION

Multiple studies using different methodologies have demonstrated spatial cognition abilities in a variety of species of lizards, turtles and crocodiles, yet there are few studies on snakes (Wilkinson & Huber, 2012; Burghardt, 2013). Studies of spatial cognition are often conducted using active maze-based protocols (Healy & Jozet-Alves, 2010), but such scenarios are likely to be unrealistic and arguably irrelevant for stationary ambush-predators, such as viperid snakes. Nevertheless, spatial memories have been found to be somewhat similar between ambush-predator and actively foraging lizard species (Day et al., 1999). Viperid snakes are classic ambush predators, and multiple viper species often revisit specific ambush sites (Greene, 1986; Secor, 1994; Sasa et al., 2009; Reinert et al., 2011). Chemical cues from prey appear to play an important role in selection of ambush sites by snake ambush predators (Clark, 2004), but it is unclear how snakes remember and re-locate previously used sites across complex landscapes and sometimes over relatively great distances. As chemical cues are strongly relied upon in other aspects of feeding in vipers, such as post-strike prey tracking behaviour (Chiszar et al., 1983; Clark, 2006; Teshera & Clark, 2021), chemical cues may be used to identify both novel and familiar ambush sites. Positive reinforcement from prior feeding success may result in spatial memory of the sites of previous success (Clark, 2006), but we are unaware of any explicit tests of such a hypothesis in snakes.

In this study, we tracked the positions of captive neonate sidewinder rattlesnakes *Crotalus cerastes* daily for their first active season, prior to onset of brumation, to describe and compare their individual movements and use of space. Our design purposely excluded chemical cues from prey in the environment in order to examine spatial use in the situation where cues from prey would not influence site selection.

In general, our study was designed as a complement to the work of Clark et al. (2016) who emphasised the challenges of studying spatial patterns in wild juvenile snakes. We also focused on intraspecific variation in the form of individual differences in behaviours, which in non-avian reptiles has mostly been in the form of anecdotal observations and observations of anti-predator behaviours (Waters et al., 2017).

MATERIALS & METHODS

Five snakes were born to one female on 24 May 2017 and left with the attendant dam (sensu Greene et al., 2002) until their first ecdysis. By 7 June 2017 all snakes had experienced their first ecdysis and on this date all were placed in identical enclosures in a single-rack unit in an off-exhibit area at Zoo Atlanta. Enclosures were 60 x 30 cm in size with opaque walls, except for clear acrylic doors at one end (Fig. 1). Daytime temperatures were held between approximately 25–32 °C. Lights (compact fluorescent light bulbs within Zoo Med Naturalistic Terrarium Hoods) in the enclosure were on a timer to mimic natural light cycles and there was a window in the room for natural light. Temperatures were maintained by controlling ambient room temperatures, intentionally equalising any possible heat gradients across the enclosure; discrete sources of heat (e.g. heat lamp, heat tape) were not used. Clark et al. (2016) reported typical daily patterns of movement in *C. cerastes* involving refuge in burrows during the heat of the day and emergence to adopt an ambush posture during much of the evening. In the absence of both temperature gradients and potentially lethal extremes, our study is intended to represent a more simplified and controlled environment than is possible when studying wild snakes. Enclosures were fitted out identically with a 5 x 10 grid pattern printed on paper for a substrate, with two

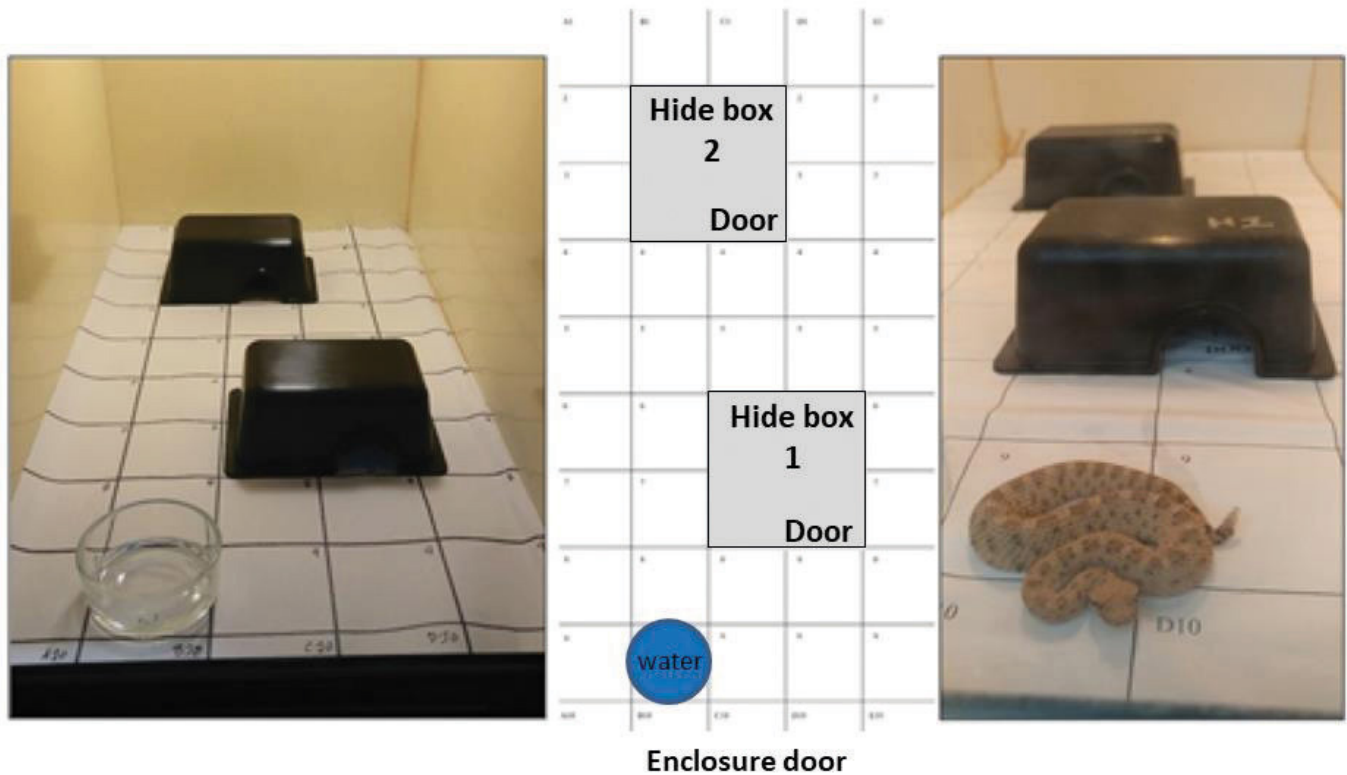


Figure 1. View of enclosure from the doorway, a schematic showing the grid-chart substrate and location of hide boxes and water bowl, and sidewinder in enclosure. Based on location of the snout, this snake is situated in quadrant C10.

identical hide boxes and a water bowl placed consistently in fixed positions through the study (Fig. 1). Each grid measured 6 cm x 6 cm.

Data collection began on 13 June 2017 (when snakes were first fed) and ran until 22 October 2017 (when cooling conditions were applied to the room for brumation). The position of each snake in its enclosure was recorded once per day at approximately (within 1 hour, typically 30 min of) 11:00 h. To standardise our data collection, we chose to determine each grid location by using the position of the tip of the snout, regardless of the direction in which it was oriented. Unless actively moving (a rare event; recorded as “no data”), snakes typically were in a tightly coiled position. The matching hide boxes covered four grids each but had a single entrance and were treated as single units of space (e.g. in Hide box 2). Snakes that were not visible (e.g. deep inside a hide box but could not ascertain which box, or under the grid paper) were scored as “not visible.” Instances of “no data” or “not visible” were not included in analyses (3–6 % of observations). Percentage of time in each grid were calculated for each individual and utilised to create heatmaps in R using the lattice package (R Core Team, 2019; Sarkar, 2008).

Feedings generally occurred once every nine days; prey consisted of thawed young mice of appropriate size. Prey were offered to all snakes at the same times and snakes were offered food regardless of their location in the enclosure. Nevertheless, not all feedings were at the same date across snakes in the study because, for example, on a given day an individual may refuse food because of their stage in a shedding cycle. Offering food directly to the snake’s location

regardless of location reduced the possibility of inadvertently training the snakes to prefer certain sites because food may function as positive reinforcement (response learning, sensu Ruprecht, 2018). Each feeding was subsequently given a number during data analysis. During ingestion, prey items inevitably contacted the paper substrate, so data was first taken on snake location, snakes were then fed, and substrates were changed (which included both replacement of the paper substrate and bleaching of the bottom of the enclosure) immediately after each feeding to remove prey cues; the same was done following defecation. After each substrate change, clean hide boxes and water bowls were replaced in their original positions relative to one another in the enclosure.

To quantify movement, we measured the displacement between each daily observation. Thus, we converted grid locations to numerical co-ordinates to obtain positions for any two consecutive days, denoting the previous position of the snake the day before (Point x_1, y_1) and its current location (Point x_2, y_2). We then calculated the displacement from the previous position during that day span using the formula:

$$\sqrt{(x_2 - x_1)^2 + (y_2 - y_1)^2}$$

We also quantified how much each snake used its enclosure by calculating Plowman’s modified spread-of-participation index for each snake (Plowman, 2003). This index provides a number from zero to one where numbers closer to one indicate that an organism is using specific areas of an enclosure preferentially.

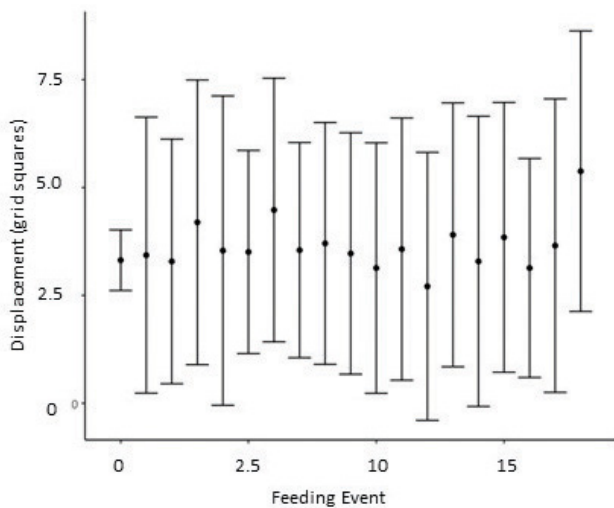


Figure 2. Mean displacement \pm standard deviation by all neonate sidewinders after each feeding event. Feeding events began as soon as a snake had eaten and ended on the observation before the next feeding.

All statistical analyses were conducted using R software (R Core Team, 2019). Our base model used displacement between observations as our dependent variable and feeding number and age in days as fixed effects. We included snake ID as a random effect to control for individuals having multiple data points. We checked the appropriateness of our model using variance inflation factors of including age in days and feeding number in the same model (examined with the car package; Fox & Weisberg, 2019), Levene’s tests of variance, normality (examined visually and through Kolmogorov-Smirnov analyses), and by graphing the residuals using the R package ggResidpanel (Goode & Rey, 2019). These analyses suggested a generalised linear mixed-effects model to be appropriate to deal with overdispersion due to zeros, which we constructed using the package glmmTMB (Brooks et al., 2017). The final model included days in age and feeding events as predictors because both independently describe age and experience.

RESULTS

Displacements (in cm) per snake varied markedly between each snake (mean \pm standard deviation; snake 1: 24.0 ± 20.5 ; snake 2: 18.7 ± 16.3 ; snake 3: 21.5 ± 20.0 ; snake 4: 20.5 ± 14.8 ; snake 5: 23.2 ± 15.7). Time and feeding number were not predictive across snakes for displacement (GLMM; $z = -1.079$, $p = 0.281$ and 1.088 and $p = 0.276$, respectively). Snake movements generally were highly variable per snake over time. Most notable was a remarkable increase in variability in displacements after the first feeding event (Fig. 2). We also ran two additional models with the variables time and feeding number as one fixed effect in each, in order to confirm that neither fixed effect masked the other. Both model results support the findings of our main model. Nevertheless, there is considerable variation, or “noise” in our data.

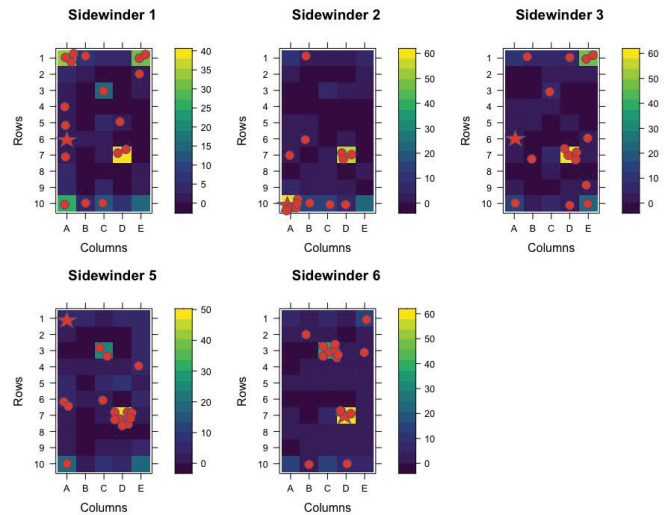


Figure 3. Heatmaps for all sidewinders representing where they spent most of their time. The entrances for the hide boxes were located at quads (3C) and (7D). Yellow represents grid where each snake was most frequently observed, followed by deepening shades of green, then deepening shades of blue.

Table 1. Modified spread-of-participation indices calculated for each snake

Snake	Modified spread-of-participation index
1	0.667
2	0.734
3	0.701
4	0.553
5	0.622

Heatmaps represent where each snake spent the most time throughout the experiment (Fig. 3). Modified spread-of-participation indices calculated for each snake indicate that snakes were using their enclosure spaces preferentially (Table 1). Most often, snakes were found in association with the hide boxes and corners of the enclosure, but much individual variation in specific locations was evident between snakes. For example, snakes 2 and 3 never used one of the hide boxes available to them, while all of the other snakes used both boxes at varying frequencies. Snakes generally spent approximately 20 % of their time in association with at least one hide box and roughly equivalent amounts of time at specific (but variable between snakes) corners of the enclosure.

DISCUSSION

After an initial period of consistent, moderate movement by all snakes at first feeding (Fig. 2), snakes were later highly variable in their displacements. We attribute this, at least in part, to a novelty effect - i.e. exploratory behaviours of individuals in new environments (Reinert & Rupert, 1999; Wolfe et al., 2018), as translocated snakes in the wild

often move greater distances than do snakes in familiar surroundings (Roe et al., 2010), which here may have required a short acclimation period, and perhaps feeding, to become manifest. Regardless, the variability of movement across snakes suggests this may be reflective of individual variation rather than a more general ontogenetic, or perhaps seasonal, trend of spatial use. Bonnet et al. (1999) found that mortality was highest among post-hatchling snakes as they dispersed, and mortality decreased as older snakes became more sedentary. Howze et al. (2021) found that wild neonatal *C. horridus* increased their dispersal differences during the three weeks after leaving their birth site, but they had no information regarding their feeding. However, both Secor (1994) and Clark et al. (2016) found that juvenile and adult sidewinders had generally similar patterns of movements, suggesting that our observations may be better explained by the novel conditions upon introduction to the enclosure rather than to ontogenetic trends.

The spatial-use heatmaps (Fig. 3) and modified spread-of-participation indices (Table 1) indicate that snakes showed individual differences in spatial use and did selectively re-use specific sites in their enclosure. However, we noted no general pattern across all snakes (e.g. all snakes preferring one of the two hide boxes, or the furthest corner from the enclosure door, etc.); such observations would have indicated a potential systematic influence bearing upon all snakes in the room. Site selection for hunting or retreat is likely to depend on a variety of factors potentially including thermoregulation, hunger, memory of past feeding events, and perhaps substrate. Our focus here was on spatial use in a purposely simplified experimental environment. Decisions concerning site use by snakes are surely multi-modal including, for example, information such as chemical cues from potential prey items (Clark, 2004). However, our design indicates that snakes do form individual spatial preferences and repeated behaviours even in the absence of such cues.

The modified spread-of-participation indices and heatmaps suggest that the snakes preferred to spend time in specific hide boxes and corners; use of such spaces generally is consistent with the thigmophilic tendencies of vipers (JRM pers. obs.). The lack of similar patterns among the five snakes is minimally an important demonstration of variability, or individual differences, that may be indicative of personality (*sensu de Vere*, 2017) in these snakes. Compared to other groups of vertebrates (e.g. felid mammals; Gartner & Weiss, 2013), personality has been poorly investigated in the non-avian reptiles; as is the case for most aspects of cognition (De Meester & Baeckens, 2021). Repeated individual differences in behaviours indicate awareness of objects and stimuli in the environment and making cognitive decisions with respect to them; this qualifies as the sentience discussed by Learmonth (2019). Waters et al. (2017) reviewed literature related to personality, much of which was anecdotal or specific to predatory and anti-predatory behaviours. However, they noted considerable evidence for individual differences in a diversity of other behaviours, such as boldness or exploratory tendencies, in lizards. As such, our results contribute to their encouragement of more studies in other non-avian reptiles.

Our evidence of individual differences and choice in these snakes suggests that their individual welfare may be influenced by factors such as object and spatial recognition. It may be appropriate to incorporate simple protocols into husbandry routines, such as returning enclosure items to their original positions after a cleaning event, for example.

Our small study suggests that future researchers should take into account the growth of individual snakes during the study period when designing their enclosures and grid patterns. By the end of our study, the snakes occupied multiple grids when at rest; however, this should not affect our data as they were scored based only on position of the tip of the snout. Nevertheless, future studies should implement larger grids as the small size of our grids created certain levels of non-independence among grids; a minor repositioning of the snake would place the tip of the snout in a different grid.

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