Foraging Cues and Thermal Environments Influence Retreat Site Selection in Fossorial Earthsnakes

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Abstract.—Retreat selection can be a complex process to understand due to the effects of multiple biotic and abiotic factors. The control of these variables through experimental studies may help to explain the factors that affect microhabitat selection. We experimentally examined whether retreat-site selection by two fossorial Mexican earthsnakes (Conopsis biserialis and C. nasus) is determined by the effects of temperature inside the retreat and/or the presence of chemical prey cues in the laboratory. We also examined whether visual prey cues or their combination with chemical prey cues have a significant effect on retreat-site selection. Overall our results show that retreat-site selection of both earthsnake species was mainly based on foraging opportunities, as indicated by the selection of retreats with presence of prey chemical cues, even when thermal conditions were not favorable. In the absence of prey cues, however, earthsnakes preferred warmer retreats. Earthsnakes showed strong selection for chemical prey cues, and no selection for visual prey cues. The addition of visual prey cues to chemical prey cues did not affect retreat selection. Therefore, resource selection by earthsnakes appeared to be strongly guided by the presence of chemical prey cues, and to a lesser extent, thermal conditions. We observed similar habitat selection in the two earthsnake species, likely due to their close phylogenetic relationship. Although several other factors might affect habitat selection by fossorial earthsnakes, it may be explained, at least partially, by the influence of physiological needs such as foraging and thermoregulation.

Key Words.—chemical prey cues; Conopsis; ecological requirements; foraging behavior; habitat selection; retreat-sites; thermal cues; visual prey cues.

Introduction

Retreat-sites are one of the most important habitat structural elements used by different groups of animals (Goldsbrough et al. 2004; Rowley et al. 2007), which use their sensory abilities to obtain site-specific information (Webb et al. 2004; Goldsbrough et al. 2006). Frequently this information allows animals to select sites that meet their ecological requirements (Schoener 1977). Overall, there are several rewards when animals select an appropriate site, for example, to reduce the risk of predation (Roberts and Liebgold 2008), appropriate breeding and nesting sites (Kolbe and Janzen 2002; Kloskowski et al. 2010) and optimal feeding sites (Beest et al. 2010). Advantageous features of suitable sites are of significance to understanding habitat selection, which may inform other ecological processes such as the distribution, abundance, and habitat relationships relevant to the conservation of many species (Boyce et al. 2003; Whittingham et al. 2005).

Retreat habitat selection in ectothermic reptiles can be strongly influenced by vegetation structure (Heatwole 1977; Martín and López 1998; Shine and Sun 2002) and by the interaction of abiotic conditions such as humidity or temperature (Reagan 1974; Krochmal and Bakken 2003). Thermoregulation requirements may explain the selection of refuges (Huey et al. 1989; Webb and Shine 1998; Shine et al. 2002a), but this decision can be strongly influenced by other factors such as risk of predation, which may incur significant costs on physiological functions, foraging time, and body condition (Martín and López 1999a,b). Additional factors involved in retreat selection include biotic signals, such as the presence of chemical or visual cues of prey (Duvall et al. 1990; Webb and Shine 1992; Clark 2004). Active or ambushing predators can accommodate their particular foraging mode to habitat characteristics in order to efficiently access prey (Shine et al. 2002b; Eskew et al. 2009). Other reptiles with fossorial lifestyles exhibit highly specific structural requirements in their retreat selection (e.g., soil types; Martín et al. 1991, 2013). The causal determinants of retreat selection are often unclear due to complex correlations among multiple variables that comparative field studies have not been able to completely clarify (Shah et al. 2004; Du et al. 2009). Therefore, it is important to implement controlled laboratory experiments, which can evaluate the specific cues for functional and operational selection.
of optimal retreat sites in isolation (Downes and Shine 1998; Downes 1999).

The highly secretive and small fossorial Two-lined Mexican Earthsnake (*Conopsis biserialis*) and Large-nosed Earthsnake (*C. nasus*; Fig. 1) are ambush foraging species that overlap in their habitat and geographic distribution in parts of central México (Goyenechea and Flores-Villela 2006). They are commonly known as earthsnakes and information about their biology and natural history is markedly limited (but see Castañeda-González et al. 2011; Raya-García et al. 2016). Frequently, these earthsnakes can be found in Pine-oak Forests surrounded by a mosaic of agricultural land and small reforested patches (Vázquez and Quintero 2005; Castañeda-González et al. 2011). They spend most of their time under rocks or fallen trunks used as shelters (Goyenechea and Flores-Villela 2006). For snakes such as *Conopsis* that might remain in the same retreat-site for long periods, selection of a suitable site becomes even more relevant for their survival, because a badly chosen site may result in starvation (Tsairi and Bouskila 2004). The diet of fossorial earthsnakes consists largely of burrowing insects (Minton and Minton 1977; Castañeda-González et al. 2011). The selection of an appropriate refuge by a sit-and-wait ambush predator is thus necessary for maintaining an adequate energy intake for survival and reproduction (Tsairi and Bouskila 2004).

Therefore, due to the importance of fossorial ectotherms to select between favorable shelters and the dearth of information available on the factors that influence habitat selection in small and cryptic species that do not lend themselves well to telemetry studies, *Conopsis* snakes present an opportunity to experimentally evaluate which retreat characteristics influence this habitat selection. Therefore, fossorial *Conopsis* snakes are an appropriate model to evaluate the effects of biotic and abiotic factors on their ability to select optimal shelters. Here, we experimentally examined the selection of retreat sites in two fossorial earthsnakes, *C. biserialis* and *C. nasus*, by simulation and control of different cues in the laboratory. Particularly, we evaluated whether earthsnakes actively select retreat-sites and if this selection is influenced by thermal conditions and/or the presence of chemical prey cues. In addition, although reduced vision is generally recognized in fossorial reptiles, we evaluated the importance of visual prey cues relative to chemical prey cues in the active selection of retreat sites.

**Materials and Methods**

*Study subjects and laboratory conditions*.—During the rainy season (July-September) of 2015, we collected 18 adult *C. biserialis* in the Ichaqueo area (19°56'86"N, 101°13'10"W; 2,257 m elevation) of México that had a mean snout-vent length (SVL) of 240 mm (+ 7 mm SE) with a range of 190–280 mm, and a mean body mass of 12.2 g (+ 0.6 g) with a range of 7.8–16.8 g. We also collected 14 adult *C. nasus* from the Chiquimitio area (19°47'15"N, 101°17'27"W, 2,020 m elevation) of México with a mean SVL of 273 mm (+ 12 mm) with a range of 210–320 mm, and a mean body mass of 17.6 (+ 1.8 g) with a range of 10.0–24.8 g. Both localities were Pine-oak Forests surrounded by agriculture plots and are located 30–40 km from the city of Morelia in the state of Michoacán, México.

We kept snakes under captive conditions in the herpetology laboratory of the Instituto de Investigaciones Sobre los Recursos Naturales of the Universidad Michoacana de San Nicolás De Hidalgo in the city of Morelia, Michoacán, México (2,000 m elevation).
We maintained individuals indoors under natural photoperiod 12:12 light-dark hours at an air temperature of about 23–24°C in a laboratory room isolated from noise and movement of people. We placed snakes individually in plastic translucent containers (35 × 25 × 15 cm) with a tight lid and small holes for air circulation. Each container had coconut fiber substrate and a bowl with water available ad libitum. We fed snakes with House Crickets (Acheta domesticus) two days per week. We suspended this feeding routine two weeks before and during each experiment. Orthopterans are known prey items in the natural diet of both Conopsis snake species (Minton and Minton 1977; Castañeda-González et al. 2011). Earthsnakes are able to recognize this prey type based on chemical cues alone and readily accepted it as food under captive conditions (Raya-García 2016). All animals were healthy and we returned them to their capture sites at the end of trials.

**Experimental design.**—We designed an experiment to evaluate the role of thermal characteristics of retreat sites, and the presence of chemical and visual cues of prey on retreat selection by both species. We tested each individual under two sets of discrete choice experiments to evaluate selection for scented retreat vs. visual prey cues and a heated retreat vs. chemical prey cues. Each set of experiments consisted of three discrete choice trials, offering snakes two retreat sites with different conditions (treatments). Each individual participated in one trial per day (24-h period) consecutively until the end of each set of experiments.

We used six large plastic containers (75 × 50 × 40 cm) containing a thin layer of coconut fiber substratum and covered with a metal mesh lid. We positioned containers in two columns of three stacked enclosures. Each container was a separate experimental arena. The temperature inside the containers was similar to room temperature (22°C). We placed two rectangular concrete bricks (25 × 12 × 5 cm) as potential retreat-sites at opposite extremes in each of the experimental containers. We placed each brick on top of six geometrically equidistant steel nuts glued on the container floor to provide a 10 mm space between the bottom of the brick and the floor of the container where snakes could hide (Du et al. 2009).

We randomly selected all trials and individuals before initiatiing an experiment. At midday (1200) one of us (ERG) placed an individual snake at the center of each container. We recorded the retreat site occupied by each individual 12 h later (2400). We then momentarily placed snakes in a bucket while the positions of retreat sites within the experimental container were switched. We returned the individual snakes to the same experimental container and recorded the selection of a retreat site again at 1200 of the next day. Swapping the retreat positions and recording a second observation for each individual-trial controlled for potential positional effects on retreat site selection.

We conducted trials between August and September (rainy season), at the time both fossorial earthsnakes are active and abundant in their temperate habitats (Goyenechea and Flores-Villela 2006). After every trial was completed, we returned snakes to their original container and washed experimental containers and bricks twice with commercial detergent, rinsed them with clean water, and placed them in the sun for drying. The cleaning of the experimental containers minimized the possibility a trial (snake and cricket scents) influenced the results of subsequent trials during the experiment.

**Experiment 1: chemical vs. visual prey cues.**—To evaluate the effects of chemical and visual prey cues on the selection of foraging sites, we presented snakes with the following options to choose: (1) scented vs. a visualized retreat; (2) visualized vs. control retreat, and (3) a scented (chemical cues only) vs. a scented and visualized retreat (both chemical and visual cues). To simulate the presence in the retreat-sites of chemical prey cues, we marked bricks with scents of around 50 live crickets purchased as live food in a pet shop. To do this, we previously placed the crickets in an additional closed container (28 × 18 × 15 cm) together with the bricks for three days before the beginning of a trial. We provided visual cues using 10 dead adult crickets euthanized by cooling and fixed with colorless enamel (trying to maintain natural body shape) and placed inside a clear plastic tube (2.54 cm in diameter and 20 cm long) with the tube ends covered tightly with circular tabs, positioned inside the container between the wall and an unscented retreat (brick). To obtain visual and chemical cues simultaneously, we placed a clear plastic tube (with visual crickets) next to a scented retreat (brick dusted with prey cues) into the experimental container. To eliminate the potential confounding effects of the presence of this plastic tube within the container, we included a same but empty control tube in the scent-only treatments.

**Experiment 2: temperature vs. chemical prey cues.**—In this experiment, we did not provide any of the retreat sites with visual prey cues. To evaluate the effects of temperature and prey chemical cues on the selection of foraging sites, we presented snakes with the following options to choose: (1) heated vs. control retreat (both retreat-sites without prey cues); (2) scented vs. control retreat, and (3) scented vs. heated retreat. To obtain a high temperature in the heated retreat, we provided a heat mat (15.2 × 27.9 cm) adhered above the brick, resulting in a temperature of 26–28°C inside the retreat, which is similar to that found under rocks in the natural
microhabitat of earthsnakes (Castañeda-González et al. 2011). Temperatures in the control retreat were 18–20°C. We simulated the scented retreat-site as described above.

**Data analyses.**—We assessed the differences in the number of observations that earthsnakes selected one or another shelter in both experiments using a binomial test assuming equal probability for each of the two shelters (Zar 1996). We used Fisher’s Exact Probability Tests to assess the differences between the first (1200–2400) and the last time (2400–1200) when retreat selection was recorded in each paired set, and between snake species. We performed all statistical analyses with the interface Rstudio in R software version 3.3.2 (R Development Core Team 2013).

**RESULTS**

There were no significant differences among treatments during the first and last time trials of retreat selection in both fossorial earthsnake species (Fisher’s Exact Tests, all \( P = 0.180 \)). Selection of retreats was not significantly different among species in any case (Fisher’s Exact Tests, all \( P = 0.590 \)). Thus, we conducted separate analyses for each earthsnake species and treatment pair.

When we offered two retreats with different prey cues (scented vs. visualized), both *C. biserialis* and *C. nasus* significantly chose the scented retreats (binomial test, \( P < 0.001 \); Fig. 2a,b). Between visualized retreats and control retreats, there was no significant preference either for the two retreats (binomial test, \( P = 0.400 \) for both cases; Fig. 2a,b). When scented retreats and scented + visualized retreats were offered, *C. biserialis* and *C. nasus* showed no significant preference between retreats (*C. biserialis*: binomial test, \( P = 0.500 \); Fig. 2a; *C. nasus*: binomial test, \( P = 0.300 \); Fig. 2b).

When we offered two retreats with a different temperature (heated vs. control), both *C. biserialis* and *C. nasus* significantly chose the heated retreats (binomial test, \( P < 0.001 \); Fig. 3a,b). Between scented retreats and control retreats, both *C. biserialis* and *C. nasus* earthsnakes significantly chose the scented retreats (*C. biserialis*: binomial test, \( P < 0.050 \); Fig. 3a; *C. nasus*: binomial test, \( P < 0.001 \); Fig. 3b). Finally, both *C. biserialis* and *C. nasus* preferred scented retreats in comparison to heated retreats (binomial test, \( P < 0.001 \) for both cases; Fig. 3a,b).

**DISCUSSION**

Both earthsnake species significantly selected retreat-sites that provide them an environment with chemical cues of prey and a high temperature, which should improve foraging and thermoregulation opportunities. When earthsnakes had to decide between prey and temperature, however, they preferred prey cues. Also, chemical cues of prey were much more important than visual cues when deciding the selection of a retreat. Moreover, these preferences were consistent in the two earthsnakes species.

Retreat-sites selection is an important aspect of the behavioral ecology of many snake species (Shine...
and Bonnet 2000; Gregory 2004; Clark 2006; Webb and Whiting 2006; Scott et al. 2013). Similar to the *Conopsis* earthsnakes, other snakes also selected retreat-sites through the use of several prey chemical cues (Roth et al. 1999; Theodoratus and Chiszar 2000; Bevelander et al. 2006). This implies, at least partially, a selection of retreats to increase their foraging opportunities. Further evidence suggests that some snake species select shelters due to their thermal properties to meet physiological requirements of thermoregulation (Huey 1991; Blouin-Demers and Weatherhead 2001; Whitaker and Shine 2002). Body temperatures of earthsnakes can be broad (7.2–31.6°C), meaning that they could be thermal generalists rather than specialists (Castañeda-González et al. 2011). In addition, they live (primarily) in fossorial environments with less elevated and more homogeneous temperatures (11.6–29.4°C) than epigean environments (Castañeda-González et al. 2011). Earthsnakes consistently chose shelters with prey scents, even if thermal conditions in the retreat-site were favorable. This could suggest less precise thermoregulation in retreat selection for earthsnakes, and particularly in ambush foragers; however, when the sites were not chemically favorable to foraging, earthsnakes always chose warmer shelters. A possible explanation is that selection of a warmer ambush site could enhance thermal performance, and thus increase the probability of successful prey capture. A warmer retreat comes at the cost of increased energy expenditure, however, which could be problematic if foraging opportunities are infrequent. Nevertheless, the choice of retreats by *Conopsis* earthsnakes based on the presence of prey chemical cues may reflect the physiological state of the individual snakes used in this experiment (e.g., Du et al. 2009). This result could be due to fasting the snakes prior to the experiment because well-fed snakes might have preferred to mainly search for sites with more appropriate thermal conditions, which allow an efficient digestion process (Huey et al. 1989; Du et al. 2009).

Furthermore, earthsnakes preferred chemical to visual cues of prey to decide retreat selection. Visual prey cues may be more important for arboreal and terrestrial snakes that feed on vertebrate prey (Shine and Sun 2002; Du et al. 2009) than for small and fossorial snakes feeding on invertebrate prey in an underground environment where visual cues would not be very useful. Neither earthsnake preferred a combination of visual and chemical cues of prey against chemical cues alone. Thus, our results did not support the premise that multiple types of cues are used to improve assessment of potential foraging opportunities.

In addition to temperature and prey chemical cues, others factors could be involved in the process of retreat-site selection by fossorial earthsnakes. The effect and interaction of structural attributes in shelters (shape, size, crevice profundity, and dimensionality) on habitat selection remain unknown in many squamate taxa, including fossorial species (Shah et al. 2004; Croak et al. 2008). The patterns of use and selection of a habitat can be complex (Shah et al. 2004). Individual or combined effects of multiple factors may influence the final choice of a microhabitat by an animal that needs to execute different activities, such as foraging, hiding from predators, and reproduction (Shah et al. 2004). Fossorial snakes like *Conopsis* carry out much of their activities in retreat-sites where they...
remain hidden for long periods of time (Goyenechea and Flores-Villela 2006). Therefore, the availability of suitable retreat-sites is of vital necessity in their highly modified habitat. We do not know how forest loss and conversion in agricultural landscapes affect the availability and quality of Conopsis retreat-sites, and whether such landscape processes could affect resource selection. Given the level of soil disturbance that occurs within the agricultural matrix (Bengtsson 2002; Brussaard et al. 2007), it is also possible that Conopsis populations become isolated in remnant forest patches, affecting their long-term viability and persistence. Our experiments suggest that chemical prey cues promote the selection of retreat sites in Conopsis. It is thus conceivable that certain agricultural practices, such as the use of pesticides, could diminish those prey cues within remnant forest patches (Lürling and Scheffer 2007). Conservation planning for Conopsis species may require a better understanding of how landscape change affects population dynamics. Field studies of habitat selection and occupancy could be used to address this knowledge gap by identifying habitat and landscape features that promote Conopsis population persistence.

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