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Chemosensory Recognition of Its Lizard Prey by the Ambush Smooth Snake, *Coronella austriaca*

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ABSTRACT.—**The Smooth Snake, *Coronella austriaca*, is an ambush predator that waits for its main prey, the Wall Lizard, *Podarcis muralis*, inside dark rock crevices where lizards retreat. Pheromonal secretions of lizards could be used by snakes to select foraging sites but also during predatory episodes when identifying lizards under conditions of low visibility is beneficial. We used cotton applicators labeled with lizard scent to determine whether Smooth Snakes can discriminate the chemical cues of Wall Lizards. We also asked whether snakes could discriminate between male and female lizards, or detect male scents before female ones, which could indicate differential susceptibility of the sexes to predation. The greater tongue-flick rate in response to Wall Lizard scent than to deionized water or cologne indicated that *C. austriaca* is able to discriminate the chemical cues of Wall Lizards, but it did not discriminate between the sexes of lizard prey.**

Sensory capacities may reveal the effects of selective forces related to foraging behavior (Cooper, 1995). For example, ability to respond selectively to prey chemicals may be especially important to active foraging reptiles because it may help them to locate and track their prey (Cooper, 1991a). However, chemoreception is also used by sit-and-wait snakes, which move widely through the environment in search of chemical cues that indicate profitable ambush sites at which to sit and wait for prey (Roth et al., 1999; Theodoratus and Chiszar, 2000; Clark, 2004), or for tracking prey following a strike (Chiszar et al., 1983; Cooper, 1991b; Furry et al., 1991). In contrast, most ambushing reptiles fail to respond to chemical cues that are presented on cotton applicators (Chiszar and Scudder, 1980; Cooper, 1995), probably because tongue-flicking disrupts the crypticity required to ambush during a predatory episode.

Even though it is obvious that tongue-flicking could disrupt crypticity, in some cases this may not occur. For example, when ambush predators are hidden in dark places, the movement of the tongue might not be perceived by their potential prey. Furthermore, in places where visibility is limited, even ambush predators might need to rely on tongue-flicking for detecting their prey. In this context, the Smooth Snake, *Coronella austriaca*, offers an excellent model to study the importance of chemical senses for discriminating prey in sedentary foragers. This is an ambush snake that captures its lizard prey from within dark rock crevices where lizards typically take refuge (Galán, 1998) and where chemical cues might be required to identify lizard prey.

However, chemical cues play an important role in the intraspecific communication of lizards (Mason, 1992; Cooper, 1994), which is often based on precloacal and femoral gland secretions (Cooper and Vitt, 1984; Aragón et al., 2001). Males produce more femoral secretions, composed of lipids and proteins, than do females (Alberts, 1990). Male pheromones might be used to mark territories and to attract mates (Martín and López, 2000; Aragón et al., 2001). However, chemical cues may also attract predators or parasitoids because some predators have developed the ability to detect and recognize the chemical cues of prey and to use them to locate prey (Zuk and Kolluru, 1998; Kotiaho, 2001). This is a well-established result in mammals (e.g., Cushing, 1985), but in the case of reptiles, it remains little explored (but see Chiszar et al., 1997). Thus, chemical cues used in intraspecific social signaling by lizards could increase the predation risk costs of reproduction (Magnhagen, 1991; Kotiaho, 2001). In lizards, studies of predation costs of reproduction have focused on nuptial coloration of males (e.g., Martín and López, 2001) or on the decrease in sprint speed of pregnant females (e.g., Cooper et al., 1990; Schwarzkopf and Shine, 1992). We hypothesized

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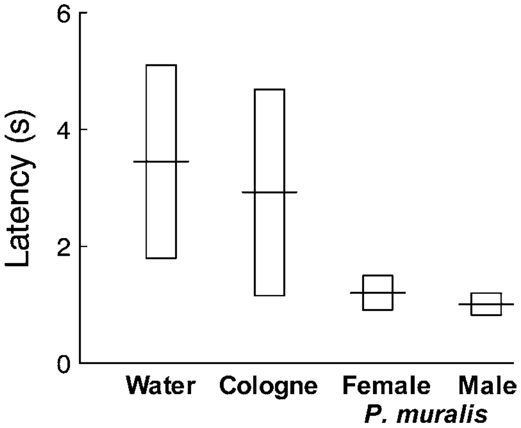

FIG. 1. Mean (± SE) of latency in seconds to the first tongue-flicks by Smooth Snakes, *Coronella austriaca*, ($N = 15$), in response to deionized water, cologne, female adult or male adult Wall Lizard, *Podarcis muralis*, stimuli, presented on cotton-tipped applicators.

that an additional cost of reproduction might arise if snake predators could recognize male lizards faster than females, because they bear or deposit more pheromone secretions than females.

We used cotton applicators in the laboratory to test the ability of Smooth Snakes to discriminate chemical cues of one of their main prey, the Wall Lizard, *Podarcis muralis* (Rugiero et al., 1995). Furthermore, we asked whether Smooth Snakes are able to discriminate between male and female lizards. If they are able to detect male scents before that of females, this could be an indicator of the differential susceptibility of the sexes to predation by this snake.

MATERIALS AND METHODS

From May through July 2001, we hand captured 15 adult Smooth Snakes (snout–vent length mean ± SE = 66 ± 2 mm) at a rock wall (120 long × 5 m high) near Cercedilla (Madrid Province, Spain). Snakes were individually housed at "El Ventorrillo" Field Station 5 km from the capture site, in outdoor glass terraria (60 × 30 × 20 cm) containing sand substratum and rocks for cover. The photoperiod and ambient temperature was that of the surrounding region. Water was provided ad libitum. To avoid using live lizards as food, we fed the snakes with domestic crickets and small bits of minced lamb. Because lamb is an artificial food, we also used multivitamin powder and kept the snakes captive for only two weeks.

We also captured 10 male and 10 female adult Wall Lizards at the same rock wall and used them as sources of prey odor stimuli. Lizards were housed separately in outdoor 60 × 40 cm PVC terraria containing sand substratum and rocks for cover. Every day they were fed mealworms (*Tenebrio molitor*) dusted with multivitamin powder for reptiles, and water was provided ad libitum. All animals were healthy during the trials and were returned to their exact capture sites at the end of the experiment.

We compared tongue-flick (TF) rate by snakes in response to stimuli arising from cotton applicators impregnated with scents of (1) male Wall Lizards; (2) female Wall Lizards; (3) cologne (pungency control); and (4) deionized water (odorless control; basic procedure follows Cooper and Burghardt, 1990). Water was used to gauge baseline TF rates in the experimental situation. We prepared stimuli by dipping the 1-cm cotton tip of a wooden applicator 150 cm long in deionized water. Other stimuli were added by rolling the moistened cotton over the surface of the cloaca and femoral pores of the lizards, or by dipping it in 50% diluted Eau Jeune cologne. A new swab was used in each trial. Every snake was exposed to each stimulus in a randomized order. One trial was conducted per day for each animal. Trials were conducted in outdoor conditions between 1700 and 1800 h, when snakes were fully active.

To begin a trial, the experimenter slowly approached the terrarium and carefully moved the cotton swab to a position 1 cm anterior to the snake's snout. The number of TFs directed to the swab and TFs not directed to the swab were recorded for 60 sec beginning with the first TF. Latency to the first TF was also recorded. Because snakes sometimes moved away from the stimulus, the swab had to be repositioned in some cases. Thus, we also recorded the time that snakes remained close (within 1 cm) to the cotton swab and analyzed the percentage of the number of TFs in relation to the actual time that snakes remained exposed to the stimulus. Therefore, directed TF rate was calculated as the number of TFs directed to stimuli divided by time exposed to the stimulus, which then was multiplied by 60 to give a per minute rate.

To examine differences in the number of TFs among conditions, we used repeated-measures one-way ANOVAs. Pairwise comparisons were made using Tukey's honestly significant difference (HSD) tests. To ensure normality (Shapiro-Wilk's test), data were log-transformed, except the rate of directed TFs, which was arcsine-transformed (Sokal and Rohlf, 1995). Tests of homogeneity of variances (Levene's test) showed that in all cases, except for latency, variances were not significantly heterogeneous after transformation (Sokal and Rohlf, 1995). Because variances of latencies to first TF were significantly heterogeneous, we used a nonparametric Friedman one-way ANOVA to compare latency to first TF among stimuli (Sokal and Rohlf, 1995).

RESULTS

All snakes responded to swabs by tongue flicking. Mean latency to first TF did not differ significantly among conditions (Friedman ANOVA: $\chi^2_{3,15} = 1.57$, $P = 0.67$; Fig. 1). Also, the total number of TFs among stimuli were not significantly different (repeated-measures one-way ANOVA: $F_{3,42} = 0.28$, $P = 0.84$; Fig. 2A). However, the number of TFs directed to swabs in relation to the time that the snakes were exposed to the stimuli differed significantly between treatments (repeated-measures one-way ANOVA: $F_{3,42} = 12.30$, $P < 0.0001$; Fig. 2B). The number of TFs directed to swabs with both male and female lizard scent was significantly greater than for the water and cologne stimuli (Tukey's tests: $P < 0.02$ in all cases). However, the responses by snakes to male and female scent were not significantly different ($P = 0.35$); nor were the responses by snakes between the water and cologne

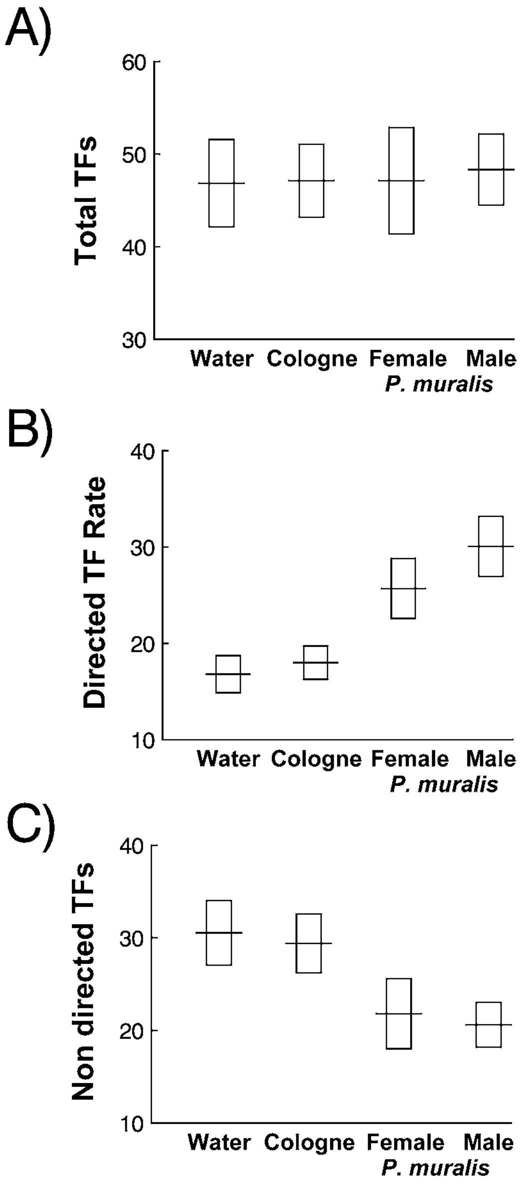

FIG. 2. Mean (± SE) of (A) total number of tongue-flicks (TF), (B) TFs directed to swabs in relation to the time exposed to the stimulus, and (C) nondirected TFs elicited by Smooth Snakes, *Coronella austriaca* ($N = 15$), in response to deionized water, cologne, female adult or male adult Wall Lizard, *Podarcis muralis*, stimuli, presented on cotton-tipped applicators.

stimuli ($P = 0.97$). Differences among treatments in the number of TFs not directed to the swab approached significance (repeated-measures one-way ANOVA: $F_{3,42} = 2.75$, $P = 0.05$; Fig. 2C). Snakes tended to perform more TFs not directed to the swab when they were confronted with water or cologne stimuli, and less nondirected TFs when presented with male or female lizard scent.

DISCUSSION

The greater tongue-flick rate in response to Wall Lizard scent presented on cotton swabs indicated that Smooth Snakes were able to discriminate the chemical cues of its main lizard prey. Numerous studies have revealed that prey chemical detection and discrimination by tongue flicking are widespread characteristics in a variety of snake genera (see references in Tanaka et al., 2001). All the snakes tested rely on lingually sampled chemical cues at some stage of foraging. In ambush snakes, chemicals are mainly used during selection of ambush sites (Duvall et al., 1990; Theodoratus and Chiszar, 2000; Clark, 2004) or during post-strike tracking of prey (Chiszar et al., 1983; Cooper, 1991b; Furry et al., 1991; Lee et al., 1992). Smooth Snakes may also use chemicals left by Wall Lizards in some crevices to select appropriate sites where they can ambush prey. However, our results suggest that prey chemical discrimination in ambush Smooth Snakes may also be used to identify potential prey during predatory episodes. For Smooth Snakes, the chemical detection of prey should be especially important because visibility is reduced inside rock crevices where these snakes ambush. Thus, chemical cues may help snakes to detect and locate lizards even without light. An alternative hypothesis may be that snakes performed tongue flicks simply because they were introduced into a novel environment to perform a trial (Chiszar et al., 1980). However, this behavior would then be the same for all treatments, and there would be no differences in TF rates.

Our results also suggest that Smooth Snakes did not discriminate between male and female lizards. This could be because, even if males produce a higher quantity of phemoral secretions than females, Smooth Snakes did not pursue their prey, before or after a strike. Smooth Snakes wait until a prey enters a refuge before tongue-flicking and attacking it. And because both male and female lizards are equally profitable prey, snakes should be able to detect both sexes. Thus, although female lizards had less secretions, these might be sufficient to allow identification of "profitable" prey by snakes. Therefore, the greater production of pheromone secretions by male Wall Lizards does not seem to increase predation risk by Smooth Snakes. It remains to be examined whether other snakes can better follow the trails of male lizards than those of females.

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