Rosy boas (*Lichanura trivirgata*) use chemical cues to identify female mice (*Mus musculus*) with litters of dependent young

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Laboratory studies focusing on the ability of squamate reptiles to discriminate among prey chemical cues have been the foundation for many important contributions in animal behaviour and ecology. In this study, we examined the ability of rosy boas (*Lichanura trivirgata*) to discriminate among several sources of prey chemicals. Because of the high frequency of neonatal mammals in the diet of erycine boas, we focused on chemical cues from female mice (*Mus musculus*) with and without litters of dependent young. We presented chemical stimuli on cotton-tipped applicators in one set of experiments; in a second set, we presented chemical cues as trails placed in an observation arena with test subjects. The cotton swab assays did not reveal a difference in response to prey chemical cues, but in the arena-based assays we found that snakes showed an attraction to chemical cues from female mice with litters of young. This attraction could be caused by either the feeding experiences of these individuals, an innate ability to recognize chemical cues from neonatal mammals, or both.

Key words: *Charina trivirgata*, chemosensory behaviour, chemosensory bioassay, prey chemical cues

INTRODUCTION

Predators use a variety of cues to recognize and locate appropriate prey items. The particular mechanisms by which prey recognition occurs have important implications, because prey recognition can mediate interspecific interactions, community dynamics and other fundamental ecological processes (Polis & Strong, 1996). The chemosensory behaviours of squamate reptile predators have played a central role in our understanding of predation behaviour and prey recognition. Burghardt (1967) popularized the methodology of presenting prey chemical cues to snakes on cotton swabs as a means of investigating prey preferences. Since its inception, research using this methodology has found that many active-foraging snakes exhibit innate preferences for sympatric prey (Burghardt, 1970b; Dix, 1968), that innate responses have a strong genetic component (Arnold, 1981a,b) but can be modified by feeding experience (Burghardt, 1993; Waters & Burghardt, 2005), and that prey discrimination by chemical cues has a robust phylogenetic pattern (reviewed in Cooper, 1995, 2008). A recent study (Pernetta et al., 2009) has even used the cotton swab methodology to demonstrate kin discrimination in the smooth snake (*Coronella austriaca*).

Because the presentation of chemical cues on cotton swabs may not be appropriate for all species or situations (e.g. Burghardt, 1970a; Cooper, 1998), herpetologists have also developed various alternative approaches for chemical cue presentation. These studies often present chemical cues laid down as a trail on the substrate of a testing arena (e.g. Chiszar et al., 1983; Halpern & Kubie, 1983). Using this approach, ambush-foraging snakes have been found to use chemical cues to chose ambush sites or retreat sites (Bevelander et al., 2006; Clark, 2004b; Downes, 1999; Weaver & Kardong, 2009), and to discriminate among chemical trails from various potential prey in favour of naturally occurring prey items (Bevelander et al., 2006; Clark, 2004b; Weaver & Kardong, 2009).

Although both cotton swab methods and the presentation of chemical trails are popular techniques for studying squamate chemoreception, we know of no studies that have used both swab methods and arena-based methods in the same set of experiments. In this study, we use both techniques to assess the response of rosy boas (*Lichanura trivirgata*) to prey chemical cues. Rosy boas are one of only two boid species that occur naturally in the United States (Stebbins, 2003), and are found in a variety of habitats in southern California. Although they are not uncommon, humans see them only rarely because they are cryptic, secretive, relatively sedentary and often nocturnal (Diffendorfer et al., 2005; Klauber, 1931).

Despite their popularity in the pet trade, only a handful of detailed scientific investigations have focused on the behaviour or ecology of *L. trivirgata*. Diffendorfer et al. (2005) monitored the movements and habitat use of several individuals via radio telemetry, and found that rosy boas were non-territorial, usually exhibited only short movements, and had relatively small home ranges. Rodriguez-Robles et al. (1999) found that North American erycine boas, including rosy boas, preyed primarily on small mammals, often including neonates. Kurfess (1967) described mating and gestation behaviours in a captive pair of animals, Mazzarella (1974) documented growth rates of captive animals, and Ruben (1976, 1977a,b) presented a series of morphological and physiological adaptations of rosy boas that indicate they are specialized for constricting small mammals. However, we know of

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no previous studies that have investigated chemosensory behaviours in this species. Here, we examine the response of rosy boas to chemical cues from several potential prey items, including mice with dependent neonates.

MATERIALS AND METHODS

Subjects

We obtained 12 *L. trivirgata* from a local reptile breeding facility. These individuals were 6–12 months old and 22–37 cm in SVL (snout–vent length) during the course of the experiments. They were raised on a diet of neonatal (1–2 week old) mice (*Mus musculus*). Animals were housed individually in plastic cages 45 × 20 × 10 cm in size. They were provided with water ad libitum and fed a euthanased neonatal mouse once per week. To ensure that animals were hungry during experimental trials, snakes were not fed for two weeks prior to any behavioural assays.

Applicator assays

As an initial investigation into the chemosensory behaviour of these animals, we conducted tongue-flick assays following basic methods described in Cooper (1998). For these assays, we prepared chemical cues by wetting a 15 cm long cotton-tipped applicator with distilled water and rubbing it twice on the dorsum of chemical donor animals. We then opened the cage of each individual and waited 5 mins before initiating a trial to lessen any disturbance. We approached the individual with the applicator until the applicator was within 1 cm of the snout. We then counted all tongue flicks directed toward the applicator for the next 60 seconds. If a snake moved away from the applicator, we attempted to move the applicator back to within 1 cm and continued counting tongue flicks. If the snake moved away again, we left the applicator in place for the remaining trial time. If a snake bit the applicator, the latency until the bite occurred was recorded. We used the tongue-flick attack score (TFAS) method described by Cooper & Burghardt (1990) to score trials.

Each individual was tested six times, once with each of six treatments presented in random order: 1) a distilled water control, 2) a pungency control consisting of 3:1 distilled water:cologne (Old Spice Classic®), 3) a sympatric lizard (desert banded gecko, *Coleonyx variegatus*), 4) a neonatal mouse (*M. musculus*), 5) an adult female mouse with no litter, and 6) an adult female mouse that was currently nursing a litter of neonates. Each individual snake received only one test per day. Although *M. musculus* is not a standard prey item of *L. trivirgata*, we used it as a proxy for other murine rodents that are in the diet of *L. trivirgata* due to constraints on obtaining and breeding non-laboratory rodents in captivity. Four banded geckos, four mice with litters and four mice without litters were used as chemical donors, with the specific donor selected at random for each trial.

Chemical trail assays

In this experiment, we created chemical trails from the soiled bedding of small mammals. To conduct a trial, we moved a test subject from its cage into a 120 × 60 × 60 cm arena with a plastic hidebox positioned at one end. The bottom of the observation arena was lined with clean construction paper for each trial, and the arena was cleaned with a 10% bleach solution and air-dried in between trials. Snakes were allowed to acclimate to the arena for 24 hours prior to initiating a trial.

To initiate a trial, the snake was placed under the hidebox with the entrance to the hidebox blocked. We then made two scent trails from an 8 oz container filled with mouse bedding material (Paperchip Laboratory Animal Bedding™). This bedding was either clean or soiled. Soiled bedding was taken from mouse cages that had been occupied for approximately seven days by *M. musculus*. The mouse occupying the cage was either a non-pregnant female (no litter treatment) or a female nursing a litter of neonates (litter treatment). Mice with litters had neonates that were between 3–10 days old, with neonates still closed-eyed and sedentary. Females with litters had nesting material (Ancare Nestlets ™) present in their cages, which we avoided taking when bedding material was removed.

Scent trails were made by placing bedding material along a diagonal line 5 cm in width that spanned from one corner of the observation chamber to the other. Two scent trails were laid in the chamber so that they crossed in the middle of the chamber, with the hidebox at one end (Fig. 1). After we laid scent trails, snakes were released from their plastic containers. We used an infrared-light sensitive video recorder (Sony DCR-SR65®) positioned

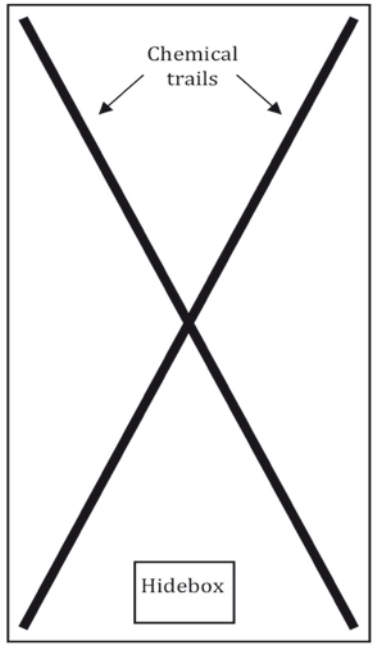

Fig. 1. Schematic diagram of the 120 cm x 60 cm x 60 cm observation chamber. Rosy boas (*Lichanura trivirgata*) were placed in the closed hidebox at the beginning of the experiment while chemical trails were placed by investigator. After chemical trails were created, the hidebox was opened and the response of the snake was videotaped from a camera placed above the observation chamber.

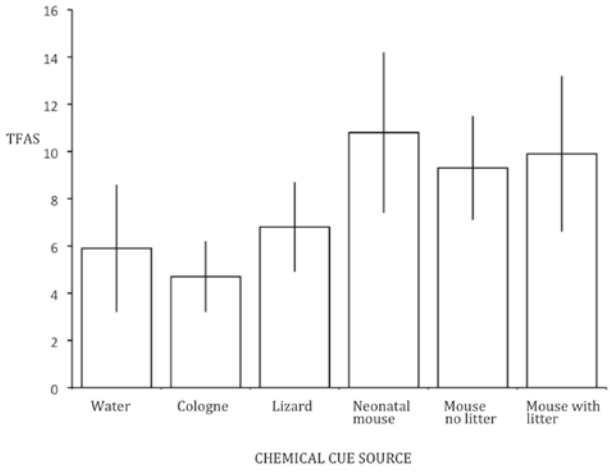

Fig. 2. Responses of rosy boas (*Lichanura trivirgata*) to chemical cues presented on cotton swabs. See text for details of chemical sources. Mean values (shown ±1 SE) of each treatment were similar.

above the chamber to record the chemosensory behaviour of the snakes for the next 12 hours. Because rosy boas are largely nocturnal, all recordings were done during the dark half of the 12L:12D light cycle.

Video recordings did not offer enough resolution for us to confidently count tongue flicks, so we used the amount of time that snakes spent with their head in contact with the chemical trails as a measure of chemosensory investigation (trail contact time, TC ±1 sec). We began recording TC as soon as the head of the snake moved onto or above the scent trail, and continued recording TC so long as the head of the snake remained in contact with that trail for at least 30 seconds. If the head of the snake did not remain in contact with the trail for 30 seconds, no time was recorded for TC. After we began recording TC, we continued recording until the head of the snake left the trail for at least 30 seconds. We chose a 30 s window for TC because this time frame generally excluded instances of snakes passing over chemical trails while exploring the arena. In addition, snakes that began following a trail would frequently move their head off the trail momentarily, but would then return to the trail in under 30 s if they were to continue following it. We recorded the total amount of TC to the nearest minute for each chemical trail.

Each snake was presented with three trials in random order. In trial 1, the two scent trails consisted of a non-soiled trail and soiled bedding from a non-litter female mouse. In trial 2, the two scent trails were a non-soiled trail and soiled bedding from a female with a litter. In trial 3, the two scent trails were bedding from a non-litter female, and bedding from a female with a litter. At least 14 days lapsed between trials for all individuals. Videotapes were scored by a single observer blind to treatment and snake identity.

Data analysis

Assumptions of normality (Shapiro–Wilk tests) and homogeneity of variances (Levene's tests) were met after a log-transformation of the data from the applicator trials. Therefore, we used a repeated measures analysis of variance (ANOVA) to compare snake responses. Because data from the chemical trail experiments were non-normal after transformation, we compared the TC time for the two trails in each experiment using a non-parametric Mann–Whitney test. We used Systat 12 software to compute statistics. All mean values are reported as ±1 standard error (SE).

RESULTS

Applicator assays

In most trials, snakes responded to cotton applicators with a few tongue-flicks, followed by movement away from the applicator. The average number of tongue flicks given to the applicators was as follows: water = 5.9±2.7, cologne = 4.7±1.5, lizard = 6.8±1.9, neonatal mouse = 10.8±3.4, female mouse without litter = 9.3±2.2, and female mouse with litter = 9.9±3.3 (Fig. 2). There were no differences in the responses of snakes to the different chemical cues (F=1.3, P=0.2).

Chemical trail assays

In most trials, snakes moved out of hideboxes and explored the test arena for several hours early in the dark phase of the light cycle before returning to hideboxes. Upon encountering experimental chemical trails, most individuals spent a prolonged period following the chemi-

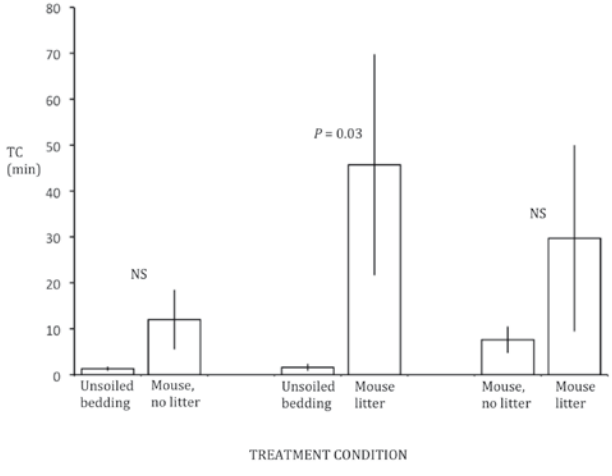

Fig. 3. The number of minutes (mean ± SE) that rosy boas (*Lichanura trivirgata*) spent investigating chemical trails (trail contact time, TC) from different prey sources. Snakes spent more time investigating chemical cues from a female mouse that was nursing a litter of neonates compared to the control trail of unsoiled bedding material. Error bars indicate SE, NS indicates no significant difference between control and experimental treatments.

cal trail to the corner of the enclosure before continuing to explore the arena. In trial one, the average TC for the non-soiled control trail was 1.3±0.5 min, and the average TC for the trail made from the mouse without a litter of neonates was 12.0±6.5 min. There was no statistically significant difference between these means (P=0.19). In trial two, TC was 1.6±0.8 minutes for the control trail and 45.7±24.1 minutes for the mouse with litter of neonates trail, a difference that was statistically significant (P=0.03). In trial three, TC was 29.7±20.3 minutes for the mouse with litter of neonates trail, and 7.6±2.9 minutes for the mouse with no litter trail, a non-significant difference (P=0.80, Fig. 3).

DISCUSSION

Our results indicate that rosy boas do exhibit measurable chemosensory responses to chemical cues from prey, but this finding was not evident from the applicator assays alone. In arena-based experiments, snakes spent more time investigating chemical trails made from bedding material soiled by a female mouse nursing a litter of neonates than the control trail (Fig. 3). They did not exhibit a significant response when the chemical trail was from a female mouse that was not nursing a litter of neonates. When litter trails and non-litter trails were presented simultaneously, snakes spent more time investigating litter-trails, but the difference was not significant.

The individuals used in these experiments had previously been fed only neonatal mice, so their increased response to the bedding from females with neonates might be a result of recognizing chemical cues from prior feeding experiences. Other species have been shown to modify their response to prey chemical cues following feeding experience with that prey (Arnold, 1978; Burghardt et al., 2000; Clark, 2004a). It is possible, however, that rosy boas exhibit an innate attraction to chemical cues from small mammals actively nursing litters, as several snake species have been found to exhibit innate attraction to chemical cues from key prey (e.g. Burhardt, 1967; Cooper & Secor, 2007). A dietary analysis of erycine boas indicated that rubber boas (*Charina bottae*) and rosy boas both prey extensively on mammals, with a high frequency of neonates present in the diet (Rodriguez-Robles et al., 1999). Rosy boas have also been observed to readily constrict multiple small mammals simultaneously, a behaviour that has been interpreted as an adaptation for feeding on rodent litters (Medina, 1959). An ability to identify chemical cues from adult females with neonates would facilitate the location of neonatal mammals. Future experiments should determine if the attraction to these chemical trails stems from previous feeding experience, or from an innate recognition of chemical cues associated with small mammal females that are nursing neonates, or a combination of both.

It could be argued that the significant response exhibited by snakes to mice with litters was a result of a difference in stimulus strength, as a litter of mice might produce a stronger chemical stimulus than a single mouse. However, our experiment used bedding material from cages, and we avoided taking nesting material from nests within those cages that contained mouse litters. It is unlikely that the neonatal, non-mobile mice exhibited significant movements outside the nests that would result in increased chemical stimuli in the bedding. Instead, we assumed that most of the chemical stimuli on the bedding came from the movement of the mobile adult female, resulting in an odor stimulus that was similar in strength between the two bedding presentations.

It is perhaps unsurprising that rosy boas did not respond strongly to chemical cues presented on cotton swabs. Both Burghardt (1970a) and Cooper (1998) indicated that the cotton swab methodology would not be appropriate for all species. Many ambush-foraging snakes use chemical cues primarily to locate appropriate ambush sites (e.g. paths or trails used by their prey). For such species, chemical cues may be best presented as trails in observational arenas. However, the arena-based methodology requires much more time and space than does the cotton-swab method. As a result, arena-based studies often have limited sample sizes. For example, Bevelander et al. (2006) and Weaver & Kardong (2009) used 11 and nine individuals, respectively, and the current study used 12 individuals. The statistical power of such sample sizes is limited, and so detecting differences requires a sensitive behavioural bioassay. Developing such bioassays, such as those presented here, will facilitate chemosensory research on species that are not amenable to the cotton swab methodology.

Overall, our results indicate that rosy boas most likely do rely on chemosensory information to identify and locate prey items, and they might recognize chemical cues from previous feeding encounters. Further research should be conducted using arena-based methods to examine how rosy boas respond to a wider range of chemical cues, and how those responses might be affected by feeding experience.

ACKNOWLEDGEMENTS

We thank the Faculty–Student Mentoring Program at San Diego State University and the College of Sciences at San Diego State University for financial support and other resources for this project. We thank V.J. Clark and D.M. Clark for helpful comments on the manuscript. All animals were housed and handled following a protocol approved by the San Diego State University Animal Care and Use Committee.

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Accepted: 18 May 2011