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Chemical Prey Discrimination of Termites in *Amphisbaena heterozonata* (Reptilia: Squamata): A Learned Trait?

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ABSTRACT.—Little is known about the diet of many amphisbaenians and even less about their prey preferences in part because of their fossorial habits. We used the tongue-flicking behavior of an amphisbaenian, *Amphisbaena heterozonata*, an apparently opportunistic feeder, to investigate its ability to discriminate among three prey items: termites (fed in captivity), tenebrionid larvae, and earthworms. We tested 16 individuals, placing each one in a glass tube, in a room lighted only with a red light to simulate fossorial conditions. Stimuli were presented on cotton swabs impregnated with the smell of a prey, using distilled water as control. Two experiments were conducted: in the first, the amphisbaenians had not eaten one week prior to starting the experiment; and in the second, they had not eaten two weeks prior to it. The number of tongue flicks per minute and latencies to the first tongue flick were recorded. The amphisbaenians made significantly more tongue flicks to termites than to the water control in both experiments. In addition, latencies were significantly shorter toward termites than toward water. The results show that *A. heterozonata* could clearly discriminate, based on chemical stimuli, between termites, one of the prey items they feed on in the field and on which they were fed in captivity, and water. Other comparisons among prey items and the water control were nonsignificant except in two cases during the second experiment. Considering that this species is most likely a generalist-opportunistic feeder, the results may indicate that the response was learned based on its year-long termite-exclusive diet.

Prey detection and predator and conspecific recognition may require the ability to identify chemical signals from the environment. Many squamates recognize prey mainly based on chemical signals (Cooper and Burghardt, 1990a,b; Schwenk, 1995; Besson et al., 2009), particularly those belonging to the Autarchoglossa clade (including amphisbaenians and snakes, Conrad, 2008) and those actively foraging for prey (Cooper, 1995a,b). López and Salvador (1992) suggested that amphisbaenians may use chemical communication extensively because of their fossorial habits, reduced eyes, and the need to locate prey underground. In amphisbaenians, tongue-flicking behavior is associated with the use of the vomeronasal organ, providing a quantifiable response to chemical stimuli. By counting the number of tongue flicks to a chemical stimulus, we can infer whether the chemical signal has been detected. Differences in tongue-flick frequencies to various stimuli may help infer whether there was discrimination between them (Burghardt, 1970, 1980; Cooper and Burghardt, 1990a,b).

Amphisbaenians feed mainly on arthropods. Some species are generalists (Cusumano and Powell, 1991; Colli and Zamboni, 1999; Bernardo-Silva et al., 2006; Gomes et al., 2009), whereas others are more selective (Cruz Neto and Abe, 1993; Webb et al., 2000; Vega, 2001; Bernardo-Silva et al., 2006). Nevertheless, there is not much information on specific diets and preferences, possibly because of their fossorial habits, except for *Blanus cinereus* from the Iberian Peninsula (López and Salvador, 1992, 1994; López and Martín, 1994;

López, 2009). In the field, the composition of stomach contents can differ from what is available in the soil (López et al., 1991), indicating that amphisbaenians can discriminate and select different types of preys.

Amphisbaena heterozonata from north-central Argentina is known to feed on termites and larval coleopterans in nature (Gallardo, 1967; Cabrera and Merlíni, 1990), and in captivity it will accept earthworms (pers. obs.). Here we investigate whether *A. heterozonata* can discriminate between prey stimuli and a control (distilled water) and whether it can discriminate among prey stimuli.

MATERIALS AND METHODS

We collected 16 adult *A. heterozonata* from localities near Iberá, Corrientes, Argentina (Galarza, Departamento de Santo Tomé; Loreto, Departamento de San Miguel; and Colonia Carlos Pellegrini, Departamento de San Martín), at sites characterized by *Eucaliptus* sp. Animals were captured approximately a year before the study took place. They were treated appropriately during the study, and once the study ended, they were kept in captivity for further observations. Those that died were fixed and deposited in the Herpetological Collection, Universidad Nacional del Nordeste, Corrientes (UNNEC). Authors complied with all applicable institutional animal care guidelines.

We kept the amphisbaenians in individual 3–5-L glass terraria, depending on the size of the animal. The substrate was a moistened mixture of earth and sand, about 10–12 cm high. Animals were kept at ambient temperature and with natural photoperiod, light entering from a large window just by the terraria. They were fed twice a week with termites (Isoptera).

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Before starting the experiments, the animals went through an acclimatization period of five days, in which they were kept in a 400×15 mm glass tube for 4 h per day, simulating fossorial conditions to get used to experimental conditions (López and Salvador, 1992, 1994). Two sets of experiments, separated by 1 week, were conducted (Experiments 1 and 2). To standardize hunger levels, the animals were not fed starting one week before the first experiment and then for another week before the second experiment. The same glass tubes were used during the acclimatization period and for the experiments, cleaning them after each trial to eliminate remaining odors.

For each experimental trial, we placed an individual in a tube, and once it had settled (approximately 10 min) a cotton swab impregnated with one of the previously assigned odors was moved slowly to within about 1.5 cm of its snout. Prey used in the experiments were collected at sites close to locations where the amphisbaenians had been found. These were termites (*Nasutitermes* sp., Isoptera), tenebrionid larvae (*Tenebrio* sp., Coleoptera), and earthworms (*Eukerria* sp., Annelida).

We counted the number of tongue flicks per minute (TF/min) and measured the latency (time in seconds from the presentation of the cotton swab to the first tongue flick). An index of tongue flicks per minute was calculated to take into account both TF and latency: $(TF/latency) \times 60$. The order of presentation of prey stimuli was randomized as well as the order of the individuals that were tested. During the experiments, the laboratory was lighted only with a red light. Each individual was tested only once in each series. Friedman two-way analyses of variance by ranks (Siegel and Castellan, 1988) were performed to test for differences between stimuli. Posthoc Multiple Comparisons were applied when results were significant.

RESULTS

A significant difference was found comparing indices of tongue-flick frequencies per minute among prey stimuli and distilled water in Experiment 1 ($F_r = 9.2$, $df = 3$, $N = 16$, $P < 0.05$; Fig. 1A). Results using Multiple Comparisons showed significantly more tongue flicks to termite than to water ($P = 0.03$, one-tailed; other comparisons were not significant). An even more significant difference was obtained in Experiment 2 ($F_r = 13.2$, $df = 3$, $N = 16$, $P < 0.01$; Fig. 1A). Results using Multiple Comparisons again showed significantly more tongue flicks to termite than to water ($P = 0.03$, one-tailed) and also more tongue flicks to tenebrionid larvae than to water ($P = 0.05$, one-tailed).

With respect to latencies, in the first experiment, no significant difference was found ($F_r = 3.0$, $df = 3$, $N = 16$, $P > 0.05$; Fig. 1B). In the second experiment, there was a significant difference among categories ($F_r = 10.1$, $df = 3$, $N = 16$, $P < 0.05$; Fig. 1B). Results using Multiple Comparisons showed significantly lower latencies toward termite than toward water ($P = 0.03$, one-tailed) and significantly lower latencies toward termite than toward tenebrionid larvae ($P = 0.05$, one-tailed).

DISCUSSION

Amphisbaena heterozonata performed significantly more tongue flicks toward the termite prey stimulus

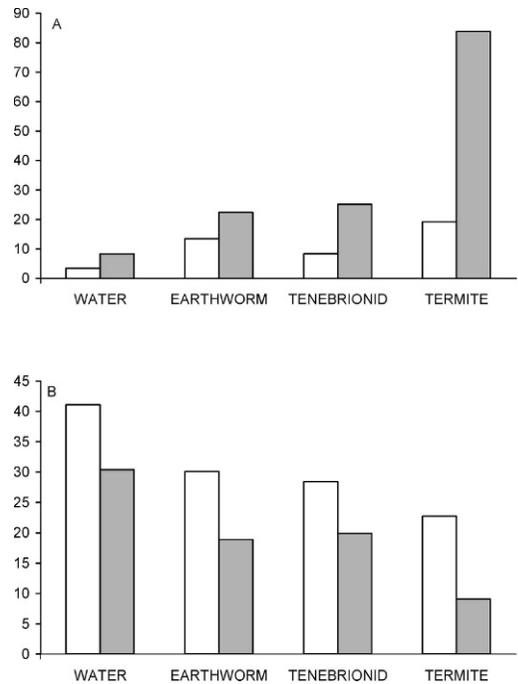


FIG. 1. (A) Indices of the average number of tongue flicks per minute (i.e., $[TF/latency] \times 60$, Y axis) of 16 *Amphisbaena heterozonata* to three prey stimuli and a control (distilled water) in Experiment 1 (white bars) and Experiment 2 (dark bars). (B) Average latency in seconds (Y axis) of 16 *A. heterozonata* to three prey stimuli and a control (distilled water) in Experiment 1 (white bars) and Experiment 2 (dark bars).

than toward the water control stimulus in both experiments but did not discriminate among prey stimuli. The other prey item that was distinguished from the water stimulus was tenebrionid larvae. Latencies were also significantly lower toward termites than toward water. The only discrimination observed among prey items was between termite and tenebrionid larvae, showing shorter latencies toward the former. López and Salvador (1994) showed that another amphisbaenian, *B. cinereus*, was able to discriminate among four prey stimuli, having a preference for earthworms. Although not many studies exist on amphisbaenian prey chemical discrimination, it is possibly not uncommon that these fossorial reptiles can distinguish among prey items.

Studies have shown that active-foraging lizards (belonging to the autarchoglossan clade) tend to rely on chemical recognition, vs. ambush-foraging lizards, which rely more on vision (Cooper, 1994, 1995a,b, 1997). For example, actively foraging anguid lizard species (Cooper, 1990; Cooper and Bradley, 2009) are capable of chemical prey discrimination, whereas ambush-foraging lizards, such as the iguanian *Liolaemus zapallarensis* (DePerno and Cooper, 1993), are not, relying on vision to identify prey (for other examples of iguanian species, see Cooper et al., 2001). The varanid *Varanus gouldii* can also identify chemical stimuli arising from prey by tongue flicking, being able to

distinguish it from odorless distilled water (Garrett and Card, 1993). Henderson et al. (1983) reported that neonate Hispaniolan Vine Snakes, *Uromacer frenatus*, could distinguish between the water control and two lizard species but not a hydrid frog.

According to Cabrera and Merlini (1990), *A. heterozonata* is most likely a generalist-opportunistic feeder, with termites and Coleoptera larvae common in their diet (Gallardo, 1967; Cabrera and Merlini, 1990), and earthworms reported for the closely related *Amphisbaena munoai* (Bernardo-Silva et al., 2006) as well as for another amphisbaenian *Blanus* (López et al., 1991). Although the clear preference of *A. heterozonata* for termites here may reflect in part their natural diet, we suggest that it is more related to the exclusive termite diet provided in captivity. Cooper (2008) points out that heritable differences are more likely among prey specialists that show strong responses to chemical cues from their preferred prey. Although we did not differentiate what may have been a hereditary from a learned response, because termites were the only prey provided, they may have become in effect their "preferred" prey. As for other differences reported here, these may show the ability to discriminate further, but since they were not as consistent as those obtained for termites, it will be important to repeat the experiments and design other tests to understand better how much learning is a factor in this context.

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