
CHEMOSENSORY RESPONSE OF THE THREATENED EASTERN INDIGO SNAKE (*DRYMARCHON COUPERI*) TO CHEMICAL AND VISUAL STIMULI OF *MUS MUSCULUS*

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Abstract.—We tested six adult Eastern Indigo Snakes (*Drymarchon couperi*) for their response to chemical and visual stimuli from prey. We recorded the rate of tongue flicking, number of tongue flicks directed at the stimuli, and the number of seconds spent investigating the stimuli. Most snake species that have been tested use both chemical and visual cues during predation; however, the relative emphasis on these cues and which cues initiate vomeronasal chemoreception varies among species. For Indigo Snakes, only visual cues of mice (*Mus musculus*) significantly elevated rates of tongue flicking and investigation of stimuli. In contrast, volatile chemical cues, even in the presence of visual cues, failed to elicit a significant response.

Key Words.—chemical cues; *Drymarchon couperi*; Eastern Indigo Snake; predatory behavior; visual cues

INTRODUCTION

Snakes in general use both chemical and visual stimuli from prey during hunting (Chiszar et al. 1981; Drummond 1985; Burghardt 1992). Which cues are used varies among species (Chiszar et al. 1981). Documenting the various cues used during predatory episodes is important for understanding the foraging ecology and conservation biology of animals. The Eastern Indigo Snake (*Drymarchon couperi*) is one of the largest snakes found in North America (Fig. 1), reaching up to 2.6 m in length (Conant and Collins 1998). Due to declining habitat, commercial pet trade, and wanton killings, *D. couperi* has been federally listed as Threatened since 1978 (U.S. Fish and Wildlife Service 1978). Although the historical range of this species consisted of the entire southeastern Coastal Plains of the United States, current populations may only extend from the Coastal Plains of southern Georgia to peninsular Florida, and from west of the lower Florida Keys to Southeastern Mississippi (U.S. Fish and Wildlife Service 1982; Diemer and Speake 1983; Moler 1985, 1992; Conant and Collins 1998). Preferred habitat of *D. couperi* varies geographically and seasonally with snakes preferring xeric upland habitats during the winter months, and mesic and hydric habitats during the warmer months (Hyslop 2007; Hyslop et al. 2009; Stevenson et al. 2009).

Indigo Snakes are diurnal active foragers, rapidly approaching and swallowing live or immobilized prey (Keegan 1944; Moulis 1976; Dodd and Barichivich 2007); however, an ambush strategy has also been witnessed (Stevenson et al. 2010). A non-constricting, non-venomous species, *D. couperi* uses its size and strength to subdue and consume its wide range of prey, including small American Alligators (*Alligator mississippiensis*), birds, fish, frogs, lizards, small mammals, turtles and their eggs, and non-venomous and venomous snakes (Keegan 1944; Groves 1960; Belson 2000; Stevenson et al. 2003, 2010). Juveniles also take invertebrates and lizards in their diet (Rossi and Lewis 1994; Stevenson et al. 2010).

Tongue flicking in squamate reptiles is associated with foraging ecology (Cooper 1994), as well as with stimuli most highly correlated with prey preference (Burghardt 1967; Burghardt and Abeshaheen 1971; Cooper 1994, 1995, 2008). Although little is known in relation to the predatory behavior of *D. couperi*, it has been shown that with garter snakes, chemical cues (both volatile and nonvolatile) and visual cues initiate vomeronasal investigation (Burghardt 1970a; Chiszar et al. 1981). In contrast, among ambush predators such as rattlesnakes (*Crotalus* spp.), visual and thermal cues are the primary initiators of this and related responses with chemical cues playing a secondary role when appropriate thermal cues are not available (Chiszar et al. 1981; Kardong 1986; Chiszar and Smith 2008). Further, interactions



FIGURE 1. An adult male *Drymarchon couperi* used in the current study. (Photographed by William E. Lamoreaux)

between visual and chemical cues have been infrequently reported, although there are some cases (Terrick et al. 1995; Hansknecht and Burghardt 2010). Currently, information is accumulating on the interactive effects of chemical and visual cues promoting vomeronasal chemoreception in some species of snakes (Terrick et al. 1995; Hansknecht and Burghardt 2010). Still, many studies examining chemoreception in snakes often consider only chemical stimuli, and fail to consider visual cues, such as prey movement, as an initiator of vomeronasal chemoreception (sometimes called priming).

In this study, we examined the response of six adult *D. couperi* to an orthogonal combination of both chemical and visual stimuli of mice (*Mus musculus*). One of our goals was to compare the response of *D. couperi* with responses of other species that have been tested under similar conditions (Chiszar et al. 1981; Saviola 2009). In addition, we wanted to understand which cues must be offered to Indigo Snakes in captivity to ensure vigorous feeding. Answering this husbandry question can lead to field experiments to determine if the same conclusions pertain to wild individuals.

MATERIALS AND METHODS

Subjects.—To test snake response to food stimuli, we used six adult *D. couperi* maintained under Federal Fish and Wildlife Permit #TE120493-0 (Permittee: William E. Lamoreaux). The snakes were captive bred, and had been in captivity for at least two years. We housed smaller snakes individually in glass terraria (91 x 76 x 46 cm) with larger snakes housed individually in glass terraria of greater volume (183 x 91 x 46 cm). All terraria contained aspen bedding, a hide box, and a water bowl. We maintained cages at 26–30° C and the

photoperiod was automatically controlled on a 12:12 light:dark cycle. We fed snakes live and euthanized mice weekly during the study period, and successive tests were always separated by at least one week. Prey used for experimental trials were also *M. musculus*. We always fed snakes several hours after tests, and snakes, therefore, were one-week food-deprived during tests.

Data collection.—We presented live mice to snakes as prey stimuli using Plexiglass™ boxes (10 x 10 x 10 cm) of four types: (1) clear without perforations, presenting only visual cues to the snake; (2) clear with perforations on each side, allowing presentation of both visual and chemical cues; (3) black with no perforations, preventing both visual and chemical cues; and (4) black with perforations, allowing presentation of chemical, but not visual cues (Chiszar et al. 1981, 2009). All perforations were circular, 2 mm in diameter, which were large enough to permit passage of chemical stimuli (Cowles and Phelan 1958) but not large enough to permit visual examination of the interior of the box. We tested each snake with each of the four conditions in random order, and a single adult male mouse, approximately 25 g, always resided in the box during tests.

Trials were always of 10 min duration. At the start of the trial, we placed a box with a live male mouse into the snake's terrarium, and measured response variables including: (1) the total number of tongue flicks for the duration of the trial; (2) total number of tongue flicks directed within 2.5 cm of the stimulus box; and (3) the number of seconds snakes spent investigating the box for the duration of the trial. Tongue flicking in squamate reptiles is an indication of vomeronasal chemoreception (Halpern 1992; Cooper 1994), which is activated by detection of volatile chemical cues by the nasal-olfactory system or by visual stimulation (Chiszar and Radcliffe 1977; Chiszar et al. 1981). Therefore, measuring the rate of tongue flicking is a useful way of measuring activation of the vomeronasal system by chemical or visual means (Cowles and Phelan 1958). We included the time snakes spent investigating because snakes would often push their snout against the box, making tongue flicking difficult to detect.

Data analysis.—We analyzed the dependent variables separately using repeated measures analyses of variance (ANOVA) treating presence vs. absence of chemical and visual cues as orthogonal factors. We pooled all interactions involving subjects in each ANOVA, giving rise to a composite error term with greater power ($df = 15$) than each of the individual interactions ($df = 5$; Hicks 1964). We used an alpha value of 0.05.

TABLE 1. Mean number of tongue flicks (those directed at the stimulus as well as any others), number of tongue flicks directed at the stimuli, and mean number of seconds spent investigating the stimulus box (\pm standard error of the mean) for the duration of the trial for the six *Drymarchon couperi*.

	Visual Cues	Chemical Cues	
		Absent	Present
Total Tongue Flicks	Absent	16 \pm 9	28 \pm 21
	Present	134 \pm 37	137 \pm 78
Tongue Flicks at Stimuli	Absent	0.8 \pm 0.8	0
	Present	36 \pm 15	101 \pm 60
Seconds at Stimuli	Absent	1.7 \pm 1.7	0
	Present	54 \pm 22	114 \pm 64

RESULTS

The mean number of tongue flicks by *D. couperi* was significantly related to visual cues of prey ($F = 12.11$, $df = 1, 15$, $P = 0.003$) but not to chemical cues ($F = 0.02$, $df = 1, 15$, $P = 0.889$) or an interaction ($F = 0.007$, $df = 1, 15$, $P = 0.934$) of visual and chemical cues (Table 1). Similarly, neither the main effect of chemical cues nor the interaction were significant in ANOVA ($F = 0.91$, $df = 1, 15$, $P = 0.355$ and $F = 0.98$, $df = 1, 15$, $P = 0.337$, respectively), but the main effect of visual cues was significant ($F = 4.60$, $df = 1, 15$, $P = 0.048$) for the mean number of tongue flicks directed at the stimuli. The same pattern was found for the mean number of seconds spent investigating the stimuli: a significant response to visual cues ($F = 6.34$, $df = 1, 15$, $P = 0.023$) but not chemical cues ($F = 0.65$, $df = 1, 15$, $P = 0.432$) or an interaction ($F = 0.75$, $df = 1, 15$, $P = 0.400$).

DISCUSSION

In the current study, *D. couperi* responded only to visual cues of the test prey *M. musculus*, and although both visual and chemical stimuli were presented during some trials, chemical cues failed to elicit any response or to enhance the response elicited by visual cues. For many snakes, predation is initiated by chemical cues (Burghardt 1992; Cooper 2007, 2008), and the critical reliance on these cues has led some researchers to examine responses exclusively to chemical stimuli (Burghardt 1970b, 1992; Chiszar et al. 1990; Cooper 2007). In this study, even in combination with visual cues, the responsiveness of *D. couperi* to chemical stimuli was surprisingly low (zero response for two of the three dependent variables). In contrast, *D. couperi* responded significantly to visual cues for all three dependent variables, indicating that initial predatory responsiveness from this species is to a large extent guided by visual cues, such as movement.

Further, because only visual stimuli significantly induced vomeronasal chemoreception in our study, it

might be hypothesized that Indigo Snakes might become more responsive to chemical cues once visual cues initiate tongue flicking. Because tongue flicking in squamates is used for detecting volatile and non-volatile molecules, thus providing information necessary for prey identification (Halpern 1992), increased tongue flicking due to visual stimulation might lead to elevated tongue flicking in response to chemical cues. Although responses were always highest in the condition presenting both chemical and visual cues, this was not significant, and visual stimuli were the only factors involved in elevating tongue flicking for our 10 min trials. It is conjecture whether longer trials might generate greater response to chemical cues after the snakes have been primed with visual cues. This hypothesis certainly should be tested.

The use of chemical stimuli by *D. couperi* should not be discounted, and until further testing, it would be premature to assume that these snakes use only a single stimulus modality during predatory episodes. Because both active foraging and ambush strategies have been documented in the field (Moulis 1976; Moler 1992; Dodd and Barichivich 2007; Stevenson et al. 2010), it may be hypothesized that *D. couperi* will respond to multiple stimuli either simultaneously or successively. Thus, initial orientation to prey may be induced by one stimulus, such as movement, and further investigation and additional behaviors such as tracking or striking prey may be triggered by chemical or other stimuli. These hypotheses currently have no support but it is reasonable to take them seriously until appropriate tests are conducted. Likewise it would be useful to learn if visual stimuli arising from prey other than *M. musculus* will be as, or perhaps more, effective in priming *D. couperi*. Also, the positioning of visual and chemical cues might have an effect on snake response. Trails, for example, might be more effective than chemical cues emerging from the plastic box, especially if the prey has disappeared into a refuge after having been seen by the predator. However, to our knowledge, the Fox Snake (*Mintonius vulpina*) is the only other snake that responds exclusively to visual cues, as all other species so far tested use chemical cues alone, or in combination with visual cues (Saviola 2009). Whether *D. couperi* will exhibit greater flexibility than *M. vulpina* remains to be assessed.

Prey records indicate that nearly 85% of *D. couperi* diet consists of anurans, young Gopher Tortoises (*Gopherus polyphemus*), rodents, and snakes (Stevenson et al. 2010). Therefore, it is possible that a diet consisting predominately of ectotherms, which may be less odoriferous than mammals, has lead *D. couperi* to respond primarily to visual cues and prey movement to initiate predatory behaviors (Herzog and Burghardt 1974; Chiszar et al. 1981; Drummond 1985; see also Cooper et al. 2000). Several suggestions, such as an

initial reintroduction to a single site compared to multiple sites, development of proper monitoring techniques, and proper rearing of snakes, have been proposed for re-establishing this listed species in its native range, as well as for protecting and conserving existing populations (Godwin et al., unpubl. report). Recovery plans stress the need to fully understand the habitat requirements, migration patterns, population ecology, as well as the predatory behavior of *D. couperi* for a successful recovery (U.S. Fish and Wildlife Service 1982; Speake, unpubl. report). To our knowledge, this is the first study to assess the sensory foundation of hunting behavior of *D. couperi*, and we believe our study can provide a basis for more detailed studies on the hunting behavior of this species. In addition, understanding the feeding strategies of *D. couperi* will be useful for establishing proper husbandry techniques that may eventually lead to successful rearing and reintroduction of neonate *D. couperi*.

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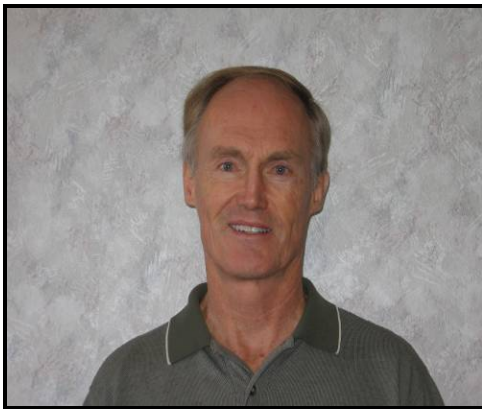
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