Brain Behav Evol 1993;41:234–238

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Chemical Recognition of Kingsnakes by Crotalines: Effects of Size on the Ophiophage Defensive Response

Key Words
Kingsnake
Lampropeltis
Crotalinae
Crotalus
Agkistrodon
Sistrurus
Antipredator behavior
Chemical cues
Ophiophagy

Abstract
When confronted by an ophiophagous (snake-eating) kingsnake, venomous snakes of the subfamily Crotalinae exhibit a suite of defensive responses including head hiding, thrashing, and an unusual response termed 'body bridging'. Other responses observed, such as biting and 'freezing', are more general in nature and can occur in a variety of contexts. Various crotalines of differing sizes were tested for their responses to kingsnakes *(Lampropeltis getulus)*. Responses of individuals were recorded for up to 18 months. The results indicate that, if habituation can be overcome by periodically allowing a kingsnqke to confront but not harm the crotaline, the response is dependent on the size of the crotaline, in that smaller specimens (<0.9 m) respond readily, while larger snakes (>1.0 m) tend not to respond. The size of the kingsnake apparently does not have an effect on the crotaline response. These data appear to resolve apparent conflicts in the literature regarding whether certain species respond to ophidian ophiophages. In addition, hexane extracts of kingsnake skin were fractionated using an alumina column. The various fractions obtained were tested to determine which elicited the defensive response. Activity was found only in the most non-polar fraction. Preliminary analysis by gas chromatography/mass spectrometry indicated that this fraction contained straight and branched, saturated and polyunsaturated long-chain hydrocarbons.

Introduction

Members of the subfamily Crotalinae (pit vipers) occur in Asia and throughout North, Central and South America. In the continental United States these venomous reptiles are found in every state except Alaska and Maine [Stebbins, 1979; Conant and Collins, 1991]. Interactions between these snakes and humans are not uncommon. When approached by a human (or any large vertebrate), the typical reaction of these snakes is either to retreat or to stand their ground, exhibiting what has been termed a watchful response with the head elevated above or laying

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0006–8977/93/0415–0234
$ 2.75/0

on the coils (if the person has noted the snake, this behavior allows the former to retreat). These snakes rarely move toward a large intruder to attack. In addition, the members of a subset of this group, collectively called 'rattlesnakes', will sometimes issue a warning sound with their rattles to draw attention to themselves. This sound may be mimicked by other snakes by vibrating their tails against the substrate or by producing a similar sound from their throats. While human deaths by native poisonous snake bites in the U.S. are far exceeded by deaths due to dog attacks, the public has an almost pathological fear of these animals. Many people unfamiliar with crotalines do not know that they exhibit a wide suite of behavioral responses. This paper examines published reports and presents new findings for a specific type of defensive behavior displayed by crotalines: namely, the antipredator response of these organisms when confronted by a snake-eating snake (ophidian ophiophage).

An unusual defensive behavior termed 'kingsnake defense posture', was originally described by Klauber [1927] and later by Cowles [1939] as the movements of some individual rattlesnakes (*Crotalus* spp.) when confronted by kingsnakes (*Lampropeltis* spp.) [reviewed in Weldon et al., 1991]. Many species of the latter genus are known for their ophiophagous tendencies and immunity to crotaline venom. A successful attack on another snake involves the kingsnake biting and grasping the head or neck region of the latter and coiling around the victim's body until suffocation occurs. The 'kingsnake defense posture' involves a crotaline raising one or two vertical loops of the body (body bridging) to fend off or divert the attack of the kingsnake from the head region or to aid in a startle reaction mechanism towards the kingsnake [Klauber, 1927; Cowles, 1938; Meade, 1940; Carpenter and Gillingham, 1975]. Subsequently, individuals of other crotaline genera (*Agkistrodon, Sistrurus,* and *Bothrops*) were found to body bridge in the presence of kingsnakes and other ophidian ophiophages [Carpenter and Gillingham, 1975; Weldon and Burghardt, 1979]. This defensive response was originally reported to be limited to the Crotalinae [Cowles, 1938; Meade, 1940; but see below). Interestingly, the body bridging response to kingsnakes by crotalines is expressed even when the individuals tested are not from sympatric populations [Weldon and Burghardt, 1979] and when no kingsnakes occur in the native area of the population of rattlesnake tested [Bogert, 1941]. Another unusual aspect of the defensive response is the cessation of rattling by the rattlesnake [Bogert, 1941; Carpenter and Gillingham, 1975].

The term 'ophiophage defensive response' [Weldon and Burghardt, 1979] has been coined to designate a suite of antipredator behaviors exhibited by many crotaline, a few elapid, and some non-ophiophagous colubrid (Colubridae) snakes to the presence of any ophiophagous predator [Bustard, 1969; Johnson, 1970; Carpenter and Gillingham, 1975]. These defensive responses include body bridging, escape, head hiding, biting, and 'freezing' [Cowles, 1938; Meade, 1940; Bogert, 1941; Gehlbach, 1970; Klauber, 1972; Carpenter and Gillingham, 1975; Weldon and Burghardt, 1979; Marchisin, 1980; Weldon, 1982]. In our experience, the first three behaviors appear restricted solely to defensive response, while the last two behaviors are exhibited generally in response to a variety of stimuli. Consequently, we counted only body bridging, escape, and head hiding as positive responses for our testing. Given the large number of behaviors seemingly designed to thwart an attack by ophidian ophiophages, and given the variety of responses noted for single individuals (see below), we support the use of Weldon and Burghardt's [1979] general term 'ophiophage defensive response' to include any of the antipredator responses, of which 'kingsnake defense posture' (body bridging) is just one.

The ophiophage defense response is an innate behavior. Crotaline neonates with no previous exposure to ophiophagous snakes exhibit body bridging when confronted by a kingsnake [Weldon and Burghardt, 1979; W.H.N. Gutzke, unpublished observations). However, based on published reports, the body bridging response does not apply to all species of crotalines, nor to all members of a given population [Cowles, 1938; Meade, 1940; Bogert, 1941; Kauffeld, 1943; Fitch, 1960; Carpenter and Gillingham, 1975]. In addition, the response of a single individual may vary over time [Bogert, 1941; Carpenter and Gillingham, 1975]. These apparent ambiguities have many possible causes, including but not limited to these five: (1) genetic differences among species or individuals within a single species; (2) the use of body bridging as the response criterion (rather than the more general suite of antipredator behaviors – see below); (3) habituation of individuals caused by continued exposure without an attack; (4) size of the tested animal [Weldon and Burghardt, 1979]; or (5) size of the predator to which the crotaline is exposed. In an effort to elucidate the relative contribution of each of these variables to the ophiophage defensive response, we initiated a long-term study of individuals representing three genera (*Agkistrodon, Crotalus,* and *Sistrurus*) and six species of crotaline snakes.

Table 1. Antipredator responses of individual crotaline snakes over time (P = positive, N = negative)

Species	Initial	6 mos	12 mos	18 mos	Length at last positive response (m)	Length at first negative response (m)
A. contortrix	N	N	–	–	–	1.42
A. contortrix	P	P	N	N	0.91[a]	1.13
A. piscivorous	P	P	P	–	0.88[b]	–
C. atrox	N	N	N	–	–	1.19
C. atrox	P	P	P	N	0.86[a]	1.04
C. horridus	P	P	P	–	0.77[b]	
C. viridis	P	P	P	P	0.84[b]	
S. miliarus	P	–		–	0.56[b]	

Length at last measurement prior to negative response (may be up to two months earlier).
Length at last measurement.

Materials and Methods

Venomous snakes and kingsnakes used in this study were collected from the field, donated from private collections, or loaned from zoos. All animals were maintained and cared for under guidelines established by the National Institutes of Health and the Committee on Animal Care at Memphis State University. Animals were housed individually. Kingsnakes and crotalines were housed in separate areas. Testing in the first phase of the experiment consisted of presenting the crotaline with a kingsnake in the former's home cage, in the latter's home cage, and in a neutral arena. Each venomous snake was tested in each condition twice. Individuals were tested only once daily. During testing the kingsnake was controlled using Pilstrom tongs to reduce the risk of harm to the venomous snake. A positive response was scored if the crotaline exhibited either head-hiding, body bridging, or escape behavior when presented with the kingsnake.

In the second experiment, we initiated an investigation of the chemical nature of the semiochemicals present in the skin of ophiophagous snakes that elicit antipredator behavior in crotalines. Hexane washes of the skin of nine anesthetized kingsnakes *(L. g. getulus)* were collected, care being taken to exclude the head and cloacal regions from touching the solvent. The extracts were pooled and then fractionated using an alumina (200 g, neutral, activity grade III) column. Three washes of 100 ml hexane each were passed through the column, followed by successive washes of 100 ml of 2, 4, 8, 16, 32, and 100% ethyl ether in hexane. The resulting fractions were collected and then presented in a bioassay to previously responsive individuals. Aliquots of approximately 250 µl were applied as a spot on a paper towel and presented to the experimental animal. Each fraction was tested separately. Controls consisted of dry paper towels and hexane blank. Each animal was tested only once daily. Each individual was tested with each fraction twice. The order of presentation for each animal was randomized. Positive responses were as previously described.

Results and Discussion

Data from the first experiment can be summarized as follows: Body bridging is one in a general range of antipredator behaviors exhibited by crotalines and should, therefore, not be regarded as the sole criterion for a defensive response. Individuals may exhibit body bridging on one occasion, switch to one of the other antipredator responses (e.g. head hiding, fleeing) on the following test with the same stimulus, and then exhibit body bridging again on the next test. Finally, we noted that size of the crotaline is an important determinant in whether the individual will react to the presence of a kingsnake (table 1). In contrast, the size of the kingsnake had little or no effect on a crotaline's response. In some instances, the stimulus animal was much smaller than the crotaline (approximately one-half the crotaline's size). Thus, the kingsnake could not have consumed the test animal but the latter still exhibited antipredator responses. There was variation in the size at which an individual would stop responding to kingsnakes, possibly due to differences in the girth of individuals. For five individuals who initially tested positive to kingsnakes and were maintained in the lab for long periods (up to 18 months), the three individuals who grew to exceed one meter in total length stopped responding to the stimuli, whereas those who have not reached this size (due either to age of the individual or to the adult size of the specific species) continue to respond (table 1).

Data from the second experiment can be summarized as follows: Habituation is indeed a problem in scoring an individual's response to repeated stimuli. We found that unless actually confronted by a kingsnake periodically, an individual soon loses its defensive response to the stimulus. We found that this problem is overcome by allowing a kingsnake to confront the test animal (without allowing physical damage) on a weekly basis. Fractions 1 and 2 (fractions combined because of the small amount of material we had available) were found to elicit defensive responses. Preliminary analyses indicate that these fractions contain straight- and branched-chain, saturated and polyunsaturated long-chain hydrocarbons. These compounds may initially appear to be unusual in terms of their semiochemical functions, however, studies in insects [Blomquist et al., 1987] and in other vertebrates [Nicolaides, 1965; Mykytowycz and Goodrich, 1974], as well as in colubrid snakes [Mason et al., 1989] have demonstrated semiochemical function attributable to this class of compounds. At present, we are in the process of identifying and testing specific compounds found in these fractions.

Data indicate that the antipredator defensive behavior of crotalines appears to be mediated through the vomeronasal (Jacobson's) organ [Bogert, 1941]. Removal of the tongue caused the test animal to cease its antipredator response to kingsnakes [Bogert, 1941]. Antipredator behaviors can be elicited from a responsive individual in a number of ways. These include blindfolding the test animal and then confronting it with a kingsnake [Bogert, 1941], placing the crotaline in a jar or cage that has recently housed a kingsnake [Bogert, 1941, this study], presenting a wooden rod that has been rubbed vigorously over the dorsum of a kingsnake [Bogert, 1941], presenting a cotton swab that has been moistened with methanol and rubbed on a kingsnake's skin [Weldon and Burghardt, 1979], or presenting a fresh shed skin of a kingsnake [Carpenter and Gillingham, 1975]. However, none of these tests distinguish the relative roles of the main olfactory system and the vomeronasal system in the mediation of this behavior. Thus, further experimentation is needed to address this question.

The 'kingsnake defense posture' is not limited to exposure to kingsnake or ophidian predators nor is it expressed only by crotalines. To illustrate the former, body bridging has been observed when a crotaline is confronted by the scent of a spotted skunk *(Spilogale putorius)* [Cowles, 1938]. In addition, a similar behavior has been described for individuals of three species of elapid snakes (Elapidae) when molested by a human [Bustard, 1969; Gehlbach, 1970; Johnson, 1970]. Finally, when housed with conspecifics, non-ophiophagous colubrids may exhibit a behavior similar in appearance to body bridging [W.H.N. Gutze, unpublished observations). Given the restrictive *Bauplan* of snakes, the appearance of body bridging is probably convergent rather than due to common ancestry [Weldon and Burghardt, 1979].

Beside the behavioral responses noted above, physiological responses (e.g. increased heart rate) have been noted in some crotalines when confronted by the scent of ophiophagous snakes [Cowles and Phelan, 1958]. However, this response can be elicited by the approach of any large intruder [Cowles and Phelan, 1958]. It would be interesting to examine other aspects of crotaline physiology to determine if other specific physiological responses to ophiophagous snakes are exhibited.

Acknowledgements

Animals used in this study either were collected under appropriate state collecting permits or were loaned by the Memphis Zoological Society. The authors wish to thank Drs. J.J. Bull and M. Plummer for assistance in collecting specimens. Preliminary chemical analyses were conducted in the laboratory of Dr. L. Pannell and with the assistance of Y. Murata. D. Chymiy, S. Sartain, and Dr. P. Weldon provided helpful comments on earlier versions of this paper.

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