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The role of the vomeronasal organ of crotalines (Reptilia: Serpentes: Viperidae) in predator detection

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Most reptiles and mammals, with the exceptions of crocodilians, aquatic mammals and some primates, have a functional vomeronasal organ that detects and perceives semi-volatile chemicals in the environment. This organ is used in detection of prey and is also important for recognition of conspecifics and potential predators. We tested eight species of North American pit vipers for behavioural responses to an ophiophagous (snake-eating) predator, the common kingsnake, *Lampropeltis getula*. Kingsnakes have a substance in their skin that is recognized by crotalines, which react with a series of defensive responses including, but not limited to, avoidance, fleeing, body bridging and head hiding. The vomeronasal duct of the pit vipers was sutured closed to determine the role of this organ in detection of kingsnakes. Pit vipers with intact and sutured vomeronasal ducts were tested in a neutral cage with a kingsnake and monitored for behavioural responses. Results demonstrated that the vomeronasal organ is important in the recognition of kingsnakes by pit vipers and raises doubts that any other sense plays a major role in this behaviour.

When disturbed, venomous pit vipers (Reptilia: Serpentes: Crotalinae) usually give a threat posture consisting of coiling the body and raising the head above these coils. One group of pit vipers, the rattlesnakes, shakes the cartilaginous 'rattle' on the tip of the tail, giving an audible warning at the approach of a large, nonprey animal. Not surprisingly, the warning behaviour is not displayed when prey is encountered. Similarly, upon recognizing an ophiophagous (snake-eating) snake, a rattlesnake will cease (or never initiate) 'rattling' and will demonstrate several unique predator-avoidance behaviours (Weldon & Burghardt 1979), which usually do not include biting.

Kingsnakes, *Lampropeltis getula*, are ophiophagous snakes common throughout much of the southern and middle United States. Kingsnakes will attack juvenile or small snakes, swallowing small prey while still alive (Marchisin 1980). They attack medium-sized snakes (<1.3 m), including other kingsnakes, by grasping them in the head region with their mouth, wrapping their body around the body of the prey snake, and constricting the animal (Marchisin 1980) until it suffocates or suffers cardiac failure. The prey is then consumed whole. *Lampropeltis* are immune to crotaline venoms (Cowles 1938; Marchisin 1980); as such, a pit viper that bites a kingsnake may actually increase the kingsnake's probability of a successful attack.

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Pit vipers of less than 1.3 m usually give one or several defensive responses when confronted by a kingsnake (Gutzke et al. 1993). These include body bridging, head hiding, body inflation and retreating or avoidance (Bogert 1941; Carpenter & Gillingham 1975; Marchisin 1980). Body bridging is a manoeuvre executed from a horizontal position in which part of the midbody is raised vertically. From this position the crotaline can effectively deliver horizontal body blows to the attacking predator that are strong enough to drive the latter away (Cowles 1938; Bogert 1941; Carpenter & Gillingham 1975; Marchisin 1980). In head hiding, the animal places its head under a body coil to prevent the kingsnake from grabbing the cranial area. Body inflation, normally performed from a horizontal position, is accomplished by contracting the ventral skeletal muscles to expand the entire body, thus discouraging a potential predator by making the prey appear too large to swallow. Retreating and avoidance are coordinated reactions whereupon a crotaline encountering a kingsnake or its trail will rapidly turn and move in the opposite direction. We categorized all of the above responses as ophiophagous defence behaviour (Weldon & Burghardt 1979).

Vision appears to be unnecessary to elicit a defensive response (Klauber 1936; Bogert 1941). Crotalines presented with a cotton swab previously rubbed on the skin

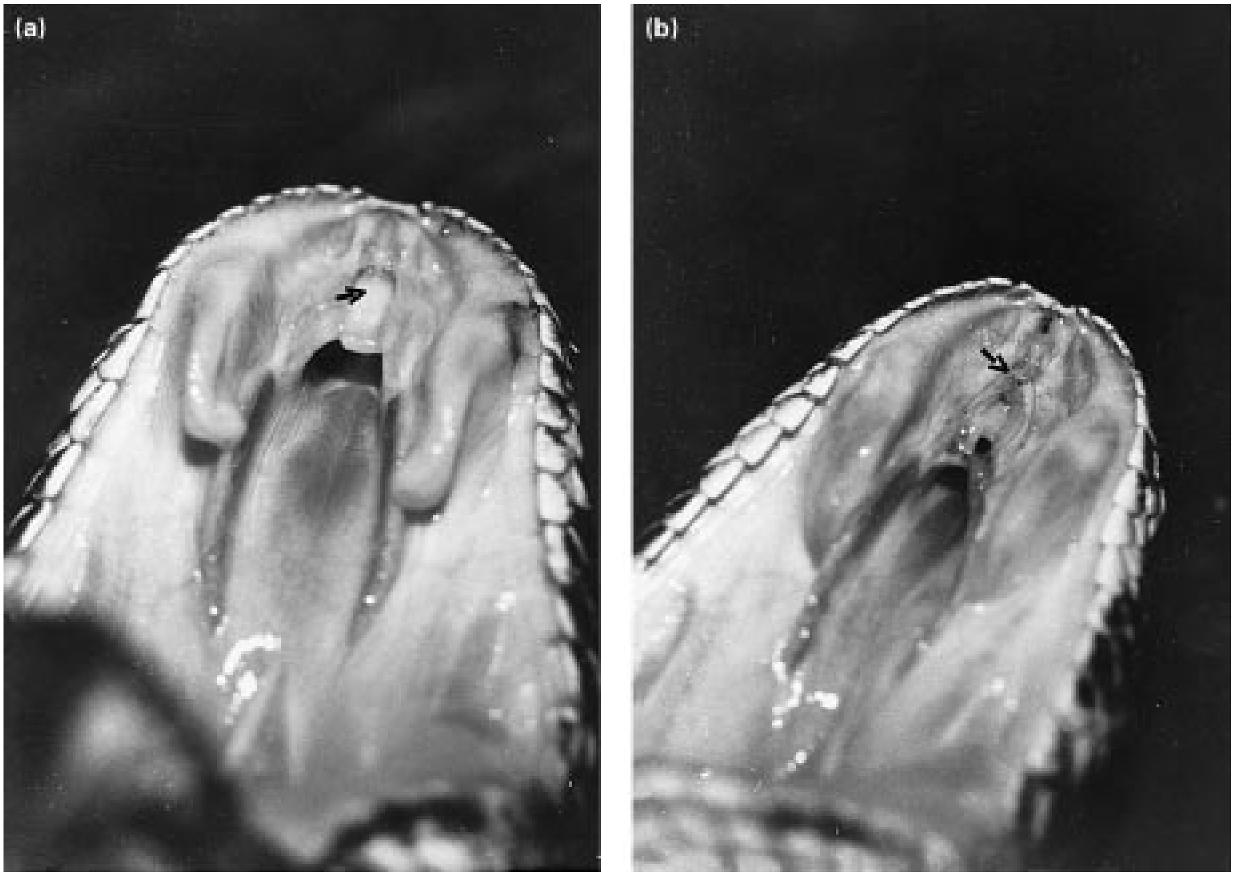

Figure 1. (a) Arrow indicates vomeronasal groove of an anaesthetized *C. atrox*. (b) Anaesthetized *C. atrox* with the vomeronasal groove sutured closed (arrow).

of a kingsnake or placed in cages recently occupied by a kingsnake will give defensive responses in the absence of a kingsnake (Bogert 1941). Blind crotalines presented with the same stimuli also display defence responses (Bogert 1941). The method by which crotaline snakes detect the kingsnake skin chemical is unknown, but the prime candidate for this ability is the vomeronasal organ, which is located in a blind sac in the proximate region of the vomeropalatine area in the roof of the mouth (Fig. 1a). Snakes use their tongues to pick up chemicals in the environment and deliver them to openings of the fenestra vomeronasalis, a pair of ducts that lead to the vomeronasal organ.

We tested the hypothesis that the defensive response is primarily mediated via the detection of skin-derived semiochemicals by the vomeronasal system. Determining how organisms sense and react to their environment is vital to understanding their ecology and life histories. More practically, this research could contribute to the development of an effective pit viper repellent.

METHODS

Animals and Housing

Subjects tested with pit vipers were speckled kingsnakes, desert kingsnakes and California kingsnakes, which are all subspecies of the common kingsnake, *Lampropeltis getula* spp. We tested the following pit vipers: the pygmy rattlesnake, *Sistrurus miliarius*; western diamondback rattlesnake, *Crotalus atrox*; canebrake rattlesnake, *C. horridus*; prairie rattlesnake, *C. viridus*; speckled rattlesnake, *C. mitchelli*; rock rattlesnake, *C. lepidus*; copperhead, *Agkistrodon contortrix*; and cottonmouth, *A. piscivorus*. The length of time in captivity for the crotalines prior to testing varied from 1 month to 8 years. The exact age of animals was not known except for neonates.

All animals were housed in plastic reptile cages, 61.0 × 30.5 × 30.5 cm, or in glass/wood cages of varying dimensions in heated (20–30°C) rooms. Cages were cleaned weekly. Snakes received a weekly diet of mice, and the kingsnake diet was supplemented with available reptiles (lizards and small snakes). Water was available ad libitum for drinking and soaking, and a rock was available for initiating shedding.

Surgical Procedures

We administered various reductions of the published dosage of anaesthesia for colubrid snakes (15 mg/kg of Brevital sodium from a 1% stock solution, Eli Lilly Corp., Indianapolis, Indiana; Wang et al. 1977) to the crotalines to determine a nonlethal level. Dosages of 50–67% of the above reported concentration of 15 mg/kg gave an acceptable anaesthetic condition in adults when administered intramuscularly in the tail (B. Graves, personal communication). The site of injection is important: equal amounts of Brevital sodium injected interperitoneally are fatal (Miller & Gutzke 1998). Lack of a righting response when the animal was placed on its back indicated that the anaesthesia had taken effect. Induction occurred approximately 10 min after the injection.

After anaesthetization, the vomeronasal groove was exposed by placing the snake on its back and opening its mouth. To disable the vomeronasal organ, we sutured the vomeronasal duct closed, which is a sufficient and reversible way of blocking chemical access to the organ (Halpern & Kubie 1980). The lower jaw was held open using a blunt probe. The vomeronasal groove was then closed by sutures using 9-0 ethilon (Ethicon Inc., Somerville, New Jersey) in a manner similar to that reported by Kubie & Halpern (1979). Each animal received two to three sutures (Fig. 1b), which prevented the tongue from introducing chemicals to the vomeronasal duct that leads to the vomeronasal organ. We allowed 1 day of recovery between anaesthesia and behavioural testing, and animals to be tested were observed to determine whether spontaneous behaviour patterns (e.g. activity, mobility, alertness) were normal.

Behavioural Observations

Tests were conducted by placing the sutured pit viper in a neutral cage and introducing a kingsnake. We recorded behavioural observations, in particular, defence responses specific to kingsnakes. The day following testing, snakes were again anaesthetized for removal of sutures. We then performed post-treatment tests for the defence responses using the recovery periods and observations noted above.

We tested pit vipers for the presence of one or more defensive behaviours. Behavioural testing occurred in a neutral cage, that is, one that had not been previously inhabited by either of the snakes being tested and which had been cleaned, washed and dried to remove possible chemical cues. We placed the pit viper in the cage first and allowed it to acclimate for approximately 15 min. To avoid the problem of habituation of the crotaline snakes (Bogert 1941; Carpenter & Gillingham 1975), we followed the procedure of Gutzke et al. (1993). Briefly, this procedure allows the kingsnake to attack, or attempt an attack, on the prey snake. The kingsnake is then removed before inflicting serious injury.

For this study, we considered body bridging, fleeing, head hiding and avoidance to be defensive responses. The lack of a defensive response was recorded if the pit viper bit more than once, attacked or ignored the predator. The test was ended after 10 min or the snake being tested failed to demonstrate a defensive response. Only those snakes giving a positive defence response in pretesting were used further. These snakes were then observed in two experimental conditions: with the vomeronasal groove sutured and after the sutures were removed. To demonstrate that any change in behaviour was not due to the sutures themselves, we then tested a subset of these two groups to determine the effects of suturing by repeating these procedures, except that the sutures were placed so as not to obstruct the vomeronasal groove.

RESULTS

Prior to experimental manipulation of the vomeronasal groove, all of the rattlesnakes (N=11) and copperheads (N=1) tested gave the defence response following introduction of the kingsnake. Six of 14 adult cottonmouths and six of 11 juvenile cottonmouths that were tested initially also displayed a defence response following introduction of the kingsnake.

All animals giving a defence response before suturing ceased to do so while the vomeronasal duct was sutured. Upon encountering a kingsnake, a sutured animal responded by tongue flicking but with little accompanying movement. After the sutures were removed, restoring use of the vomeronasal organ, the crotalines were once again able to detect the kingsnakes, reinstating the previous defence behaviour in all subjects (Table 1). In addition, animals with sutures not obstructing the vomeronasal groove continued to show a defence response (Table 1).

Results of a chi-square analysis of the combined data set allowed us to reject the null hypothesis that the vomeronasal organ does not affect the crotaline defence response specific to kingsnakes (χ^2_1=46, P<0.001). We conducted individual chi-square analyses of the response data for the two crotaline species for which we had adequate sample sizes, *C. atrox* (N=6) and *A. piscivorus* (N=6 adults, N=6 juveniles). Results of the individual chi-square analyses revealed a significant difference in the defensive responses of sutured and unsutured *C. atrox* (χ^2_1=12, P<0.001) and *A. piscivorus* (χ^2_1=10, P<0.002) following introduction of the kingsnake predator. The specific behaviours for all individuals tested are given in Table 1.

DISCUSSION

Apparently, crotaline snakes can detect and recognize a substance in the skin of kingsnakes that initiates a defensive response (Carpenter & Gillingham 1975; Weldon & Schell 1984; Gutzke et al. 1993). Generally, gustation, olfaction and vomeronasalling are the most common means of detecting external chemicals by vertebrates (Doty & Muller-Schwarze 1992). Prey trailing in garter snakes is dependent upon the vomeronasal organ (Kubie & Halpern 1979). Chemoreception by this organ also serves an active role in the male courtship behaviour of the garter snake (Kubie et al. 1978) as well as its feeding reaction (Halpern & Frumin 1979; Wilde 1938). Other studies of avoidance of predators by snakes, however, have concentrated on chemical cues and behaviour produced by the predator (Burger 1989) rather than on the organ used for predator detection by the prey.

This study demonstrates the importance of the vomeronasal organ in the ability of crotalines to detect the presence of kingsnakes. Because other methods of detection (i.e. sight, touch and olfaction) were unaltered in the

Table 1. Positive (+) and negative (−) ophiophagous defence response of eight species of pit vipers to kingsnakes before, during and after closure of the vomeronasal groove

Species	Before	Sutured	Sutures removed	Behaviours noted
Sistrusus miliarius	+	−	+	BB, HH
Crotalus mitchelli	+	−	+	HH
C. lepidus	+	−	+	BB, HH
C. horridus	+	−	+	HH, A, F
C. viridis	+	−	+	BB, HH, A, F
C. atrox				
1	+	−	+	A, F
2	+	−	+	A, F
3	+	−	+	A, F
4	+	−	+	HH, A, F
5	+	−	+	HH, A, F
6	+	−	+	HH, A, F
Agkistrodon contortrix	+	−	+	A, F
A. piscivorus (adults)				
1	+	−	+	A, F
2	+	−	+	A, F
3	+	−	+	A, F
4	+	*		A, F
5	+	*		A, F
6	+	*		A, F
A. piscivorus (newborns)				
1	+	−	+	A, F
2	+	−	+	A, F
3	+	−	*	A, F
4	+	*		A, F
5	+	*		A, F
6	+	*		A, F
Animals with sham sutures				
A. piscivorous				
1	+	+	+	
2	+	+	+	
3	+	+	+	
C. atrox				
1	+	+	+	
2	+	+	+	
3	+	+	+	
S. miliarius	+	+	+	

BB: Body bridging; HH: head hiding; A: avoidance; F: fleeing.
*Animals that did not recover from anaesthesia.

pit vipers throughout this experiment, their role in recognition of kingsnakes is unlikely. This work extends the sensory importance of the vomeronasal organ. Of future interest is the relative effectiveness of the various defensive responses of crotalines in avoiding kingsnake predation.

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