

PHEROMONE TRAILING BEHAVIOR OF THE BROWN TREE SNAKE, *Boiga irregularis*

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Abstract—The ability of snakes to follow pheromone trails has significant consequences for survival and reproduction. Of particular importance is the ability of snakes to locate conspecifics during the breeding season via the detection of pheromone trails. In this study, the ability of male brown tree snakes (*Boiga irregularis*), a tropical, rear-fanged colubrid, to follow pheromone trails produced by reproductively active conspecifics was tested in the laboratory by using a Y maze. Males displayed a trailing response to both female and male pheromone trails over blank controls. As males of this species display ritualized combat behavior, these responses likely represent both direct and indirect mechanisms, respectively, for the location of potential mates in the wild. Males did not, however, discriminate between male and female trails when given a choice on the Y maze.

Key Words—Trailing behavior, Y maze, mate location, brown tree snake, *Boiga irregularis*, reptile pheromones, Reptilia, Colubridae, male combat behavior, invasive pest species.

INTRODUCTION

Many species of snakes have demonstrated the ability to locate conspecifics during the breeding season by following pheromone trails passively laid on the substrate as they move through the environment (e.g., Ford, 1982; Ford and Schofield, 1984; Plummer and Mills, 1996; LeMaster et al., 2001). Trailing behavior is also used by snakes in other behavioral contexts, including migration to and from winter hibernacula, in aggregation, and in prey location (reviewed by Ford, 1986;

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Mason, 1992; Mason et al., 1998). The majority of studies concerning snake trailing behavior have been conducted with northern temperate species, with particular emphasis on the garter snakes of the genus *Thamnophis* (Ford, 1986).

The purpose of this study was to characterize the pheromone-mediated trailing behavior of the brown tree snake, *Boiga irregularis*. The brown tree snake is a rear-fanged, tropical colubrid native to Australia, Papua-New Guinea, and the Solomon Islands (Rodda et al., 1997). The species can reach snout-to-vent lengths of up to 3 m, weighs up to 2 kg, and possesses a relatively thin body typical of arboreal snakes (Rodda et al., 1997). Brown tree snakes are generalist predators that actively forage for a diet consisting of mammals, reptiles, amphibians, and birds (Savidge, 1988). Although primarily arboreal, it spends a significant proportion of its time on the ground and typically forages within the lower 3 m of vegetation (Cogger, 1992). The snake is an introduced pest species in the Pacific, notably on the island of Guam, where it is responsible for the extinctions of several native forest bird species and has caused considerable economic damage (Rodda et al., 1997).

Brown tree snakes display reproductive behaviors similar to other colubrid snake species (Greene and Mason, 2000). Males of this species display stereotyped courtship behaviors that are triggered by a sex pheromone located in female skin lipids (Greene and Mason, 1998). This pheromone has been isolated and initially characterized as a suite of nonvolatile, nonpolar skin lipid molecules (Greene and Mason, 1998). Male brown tree snakes also display ritualized combat behavior in response to other reproductively active males (Greene and Mason, 2000). In snakes, ritualized combat serves as a competition among males to gain access to females during the breeding season (Andr n, 1986; Schuett and Gillingham, 1989). This behavior appears to be stimulated by a pheromone located in male brown tree snake skin lipids (Greene and Mason, 2000).

The brown tree snake does not form large breeding aggregations, although small nonbreeding winter aggregations have been reported in its native and introduced range (Pendleton, 1947; Covacevich and Limpus, 1973). Therefore, it is likely that brown tree snakes, like other snakes, utilize pheromone trails to locate spatially distributed conspecifics during the breeding season. In this study, the ability of the brown tree snake to trail reproductively active conspecifics was tested by using a Y maze in the laboratory.

METHODS AND MATERIALS

Husbandry. The animals used were collected on Guam and housed under laboratory conditions for six years prior to being used in the experiment (Greene et al., 1997). Seven female and 10 male snakes, individually housed in Plexiglas cages designed specifically for arboreal reptiles, were used (Mason et al., 1991). Males in the colony had a mean (\pm SD) snout-to-vent length (SVL) of $162.2 \pm$

22.1 cm and a mean mass of 647.5 ± 265.7 g. Females had a mean SVL of 137.9 ± 9.9 cm and a mean mass of 385.7 ± 81.5 g. Snake cages contained branches for climbing and hide boxes attached to the roofs of the cages, in which the snakes spent the majority of their time. Room temperature cycled daily from 24°C to 30°C , and relative humidity was maintained at approximately 80% by using a room humidifier and by daily spraying of the inside of the cages with water. Light (12L : 12D) was provided by overhead fluorescent lights. Snakes were fed thawed mice or chicks every three weeks, and water was available *ad libitum* for drinking and soaking. All snakes in the captive colony were mature adults in breeding condition during the study.

Y-maze Design. To test the ability of the snakes to trail conspecifics, a Y maze was constructed of clear polyvinyl chloride tubing (PVC) with an internal diameter of 5.2 cm (Figure 1). The maze consisted of an initial stretch of tubing 96 cm long that connected to a Y junction separated by a 45° angle into two arms, each also 96 cm long. The maze was elevated at an angle of 25° from the floor to entice the snakes into the initial stretch, as they are excellent climbers and prefer to move to the highest point possible when given a choice. All hide boxes used in housing the snakes could be attached to the initial stretch by using PVC adapters. Empty, clean hide boxes were attached to the ends of the arms, also by using PVC adapters, for collection of the snakes at the conclusion of a trial. The initial stretch, Y junction, and the arms could be taken apart for cleaning between trials.

This maze design allowed for minimal handling of the snakes, thus reducing the display of defensive behaviors in this relatively aggressive species. In addition, the design allowed for easy cleaning and reassembly as well as for containment of the snakes in the maze. Importantly, by having a relatively small internal tube diameter, conspecific pheromone trails were laid down in a seminatural manner, as trail-producing snakes deposited odors on a horizontal surface (bottom of tube) and

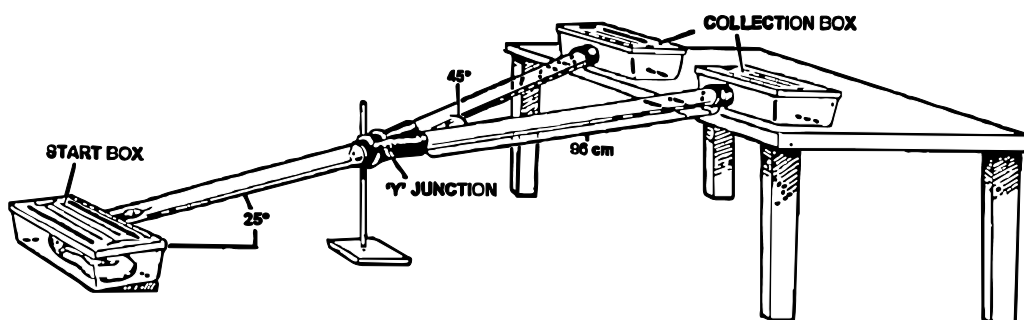


FIG. 1. Diagram of the Y-maze system used to conduct trailing experiments in this study. The Y maze was constructed of 5.2-cm-diameter, clear PVC piping. The initial stretch and arms were each 96 cm long, and the Y junction separated the maze by 45° . The maze was elevated from the floor at a 25° angle. Hide boxes were attached to the maze at the start of initial stretch and at the ends of the arms.

vertical surfaces (sides of the maze) (Ford and Low, 1983). Brown tree snakes, like other snakes species, are highly thigmotactic, preferring to travel along edges as opposed to open areas. The maze design, by having rounded edges and a small internal diameter, reduced concerns of thigmotaxis conflicting with trailing behavior (Costanzo, 1989).

General Experimental Conditions. To characterize the ability of males to trail conspecifics, four experiments were completed: (1) males trailing female pheromone trail versus blank arm ($N = 10$ trials), (2) males trailing male pheromone trail versus blank arm ($N = 9$ trials), (3) males trailing male pheromone trail versus female pheromone trail ($N = 10$ trials), and (4) blank control ($N = 10$ trials, no pheromone trails applied to either arm). Males were tested only once during each experiment. All snakes were in breeding condition when used; courtship and ritualized male combat were exhibited by all snakes during the period of time in which the study was conducted. The trailing ability of females was not tested, as females were generally inactive during this time period and would not exit the start box at the beginning of most trials.

All trials were conducted during the scotophase between 2100 and 0200 hr, when the snakes were normally active and the snake room was dark. Lighting was provided at a minimal level by a red 7 W incandescent bulb placed 2 m behind the maze so that the arms were lit evenly.

Trails were produced by allowing a randomly chosen snake of the desired sex to pass once completely through the initial stretch, Y junction, and one arm of the maze. The side of the Y junction leading to the control arm was blocked with a piece of clean cloth so that no lipids were inadvertently added to the control side. The arm treated with a conspecific pheromone trail was randomly chosen in each case. In experiment 3, in which males were given a choice between male and female pheromone trails, female trails were always applied to the maze immediately before the male trail. Both male and female pheromone trails were present in the initial stretch of the maze and the Y junction, while the arms contained only male or female trails. In experiment 3, a random male and female were chosen to produce the trail for each trial; however, males were never tested against their own trail.

To begin a trial, a hide box containing a snake was removed from its home cage and attached to the initial stretch of the maze. The snake was allowed to enter the maze of its own accord. We defined "trailing" as occurring when a snake's head entered the collection box attached to arms of the maze. During trials, an investigator, blind to the treatment, observed while hidden behind a blind.

A trial was terminated and conducted on another night if a snake exhibited defensive behavior, such as striking and tail-lashing or if a snake did not enter the maze from its hide box within 30 min after the start of the trial. Additionally, trials in which snakes did not display the trail contact response (Brown and MacLean, 1983), a set of behaviors diagnostic of snake pheromone trailing, were not used in the final statistical analyses.

TABLE 1. RESULTS OF EXPERIMENTS TESTING ABILITY OF MALE BROWN TREE SNAKES TO TRAIL CONSPECIFICS

Experiment	Condition	<i>N</i>	Trailed	Did not trail	<i>P</i> (1-tailed binomial)
1	Males trailing females	10	8	2	0.055
2	Males trailing males	9	8	1	0.020
3	Males trailing females versus males (trailed = male chose female arm)	10	5	5	1.000
4	Males trailing blank maze	10	5	5	1.000

Statistical Analyses. Statistical significance in the trailing response was determined by using a one-tailed binomial test (Sokal and Rohlf, 1995).

RESULTS

In experiment 1, eight of 10 males entered the female-treated arm of the Y maze versus the blank control arm (Table 1; $P = 0.055$). In experiment 2, eight of nine males entered the male treated arm, while only one of nine males entered the blank control arm (Table 1; $P = 0.02$). Males demonstrated no preference for female pheromone trails over male pheromone trails (experiment 3) when given a choice on the Y-maze (Table 1; $P = 1.000$). When tested on a Y maze in which both arms were left blank (experiment 4), males chose the arms of the maze equally (Table 1; $P = 1.000$).

Head-jerking behavior [a behavior only displayed in the context of courtship and male ritualized combat in this species (Greene and Mason, 2000)] was displayed in two trials (by two different males) when males were following female trails in experiment 1 and was observed in two trials (by two different males) when males were following male pheromone trails in experiment 2. Snout-probing behavior, in which the male would press his snout to the pheromone trail, was observed in most trials. While snout-probing, males would often drag their snouts backwards along the pheromone trail. In addition, all individuals that trailed displayed behaviors associated with trailing in other species, including tongue-flicks directed to the trail and periodic pauses with side to side movements of the head (Brown and MacLean, 1983).

DISCUSSION

The ability of male brown tree snakes to follow female pheromone trails most likely represents a mechanism to locate potential mates during the breeding season. Similar responses have been reported in several other species, mostly in colubrids

of the genus *Thamnophis* (Ford, 1986; Mason, 1992; Mason et al., 1998). However, few reports exist of trailing behavior in other snake taxa, including tropical species and those that display combat behavior such as the brown tree snake (Ford, 1986; Mason, 1992).

Male brown tree snakes might follow male pheromone trails in order to locate males that are near or are courting females. By initiating combat, the trailing male may be able to displace the other male and gain access to the local female, as has been suggested for other species (Shine et al., 1981). There would be little cost to this strategy, as combat would be unlikely to ensue without the presence of a female. Field reports of the courtship and combat behavior of the Swedish adder (*Vipera berus*) have described the ability of males of this species to trail both conspecific males and females (Andrén, 1986). Upon contacting and tongue-flicking the integument of a female, courtship will ensue. Conversely, upon contacting another male, males will initiate combat if a female is present. The winner of the combat bout gains access to the local female by establishing a temporary territory that it will defend from other males (Andrén, 1986).

It would be expected that, given a choice between a conspecific male and female trail, males would prefer the female trail. This choice would, at least in a natural situation, lead directly to a female and would allow the male to avoid agonistic interactions with other males. However, in this study, males did not display a significant trailing response to either of the sexes, choosing the male trail as often as the female trail. Behavioral observations and bioassays have shown that male brown tree snakes can discriminate between males and females via pheromones in other contexts, such as courtship, making it unlikely that there are no chemical differences between sexes (Greene and Mason, 1998, 2000). This apparent lack of behavioral discrimination may be an artifact of how the male and female trails were laid down in the maze. In the initial stretch of the maze, both male and female pheromone trails were present together; these trails then diverged at the Y junction. However, the male trail was always laid down on top of the female trail, which may be significant to trailing males, as has been found in vertebrate taxa, such as scent-marking rodents (e.g., Johnston et al., 1997; Ferkin, 1999). Further, under natural conditions, a similar situation might signal to a trailing male that a male and female pair are nearby. If so, following either a male or female pheromone trail may lead to the courting pair where the trailing male would have the opportunity to initiate combat and could, therefore, displace the local male. Also in this situation, the trailing male might attempt to revert to vision in order to locate the movement of the courting pair at short distances, as other species are known to do in the field (Hawley and Aleksuk, 1975; Andrén, 1986). Unfortunately, because our captive colony of brown tree snakes bred year-round, we were not able to test non-reproductively-active snakes to determine if this particular behavioral response is simply a species recognition response.

It has been hypothesized that male trailing behavior in response to females is triggered by the female sex pheromone (Mason, 1992). Snake sex pheromones are located in the skin lipids of females and are thought to be composed primarily of long-chain, high-molecular-weight molecules of relatively low volatility (Mason et al. 1989, 1990; Murata et al., 1991; Greene and Mason, 1998). Sex pheromones and other skin lipids are passively deposited on the ground as females pass through the environment, allowing males to trail females upon detection of the skin lipid trail with their vomeronasal organs. In this study, male snakes were observed displaying courtship and combat behaviors (head-nodding in both cases) in response to female and male pheromone trails, respectively. Display of these behaviors by trailing males suggests that the female sex pheromone and the male combat pheromone play a significant role in mediating trailing behavior.

Currently, brown tree snake control methods on Guam are limited to detection by trained dogs and capture by large trapping regimes. However, more attention has been given to using brown tree snake semiochemicals as repellents and attractants to aid in control of this particularly detrimental pest species (Brown Tree Snake Control Committee, 1996; Mason, 1998; Mason and Greene, 2001). Pheromone attractants would be particularly useful in increasing the efficacy of traps in the field by increasing the distances over which trap lures work. Currently, live prey is used to lure snakes into traps, a method that operates over relatively short distances. The results of this study indicate that pheromones located on the skin of male and female brown tree snakes may be effective attractants to males during the breeding season if used in a larger integrated management plan.

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