THE EFFECTS OF CLOACAL SECRETIONS ON BROWN TREE SNAKE BEHAVIOR

Michael J. Greene[4] and Robert T. Mason[5]

All snakes possess paired cloacal glands that release secretions through ducts along the cloacal orifice (Graves and Duvall, 1988; Mason, 1992; Price and LaPoint, 1981). As these secretions represent an obvious source of glandular, volatile, and potentially meaningful chemical information in snakes their role in the regulation of snake physiology and behavior has been examined for many years, however few studies have shown measurable effects of cloacal secretions on snake behavior (Gelbach et al., 1968; Graves and Duvall, 1988; Noble, 1937; Mason, 1992; Mason et al., 1998; Watkins et al., 1969). Garter snake (Thamnophis) cloacal secretions are composed mainly of nonvolatile lipids and proteins as well as volatile components including trimethylamine, acetic acid and propanoic acid (Weldon and Leto,. 1995; Wood et al., 1995). Blindsnake (Leptotyphlops dulcis) cloacal secretions are composed primarily of glycoproteins and free fatty acids including linoleic, palmitic and oleic acids (Blum et al., 1971).

As snakes often release cloacal secretions when disturbed and the secretions are volatile and odorous, it has been hypothesized that they act either as predator deterrents or as alarm pheromones (Graves and Duvall, 1988; Mason, 1992; Price and LaPointe, 1981). Graves and Duvall (1988) showed that exposure to cloacal secretions caused the rattlesnake, Crotalis viridis, to be more responsive to provocation. It rattled more and increased the increased heart rate, a physiological response correlated to defensiveness in this species. The blind snake, L. dulcis, is a predator of ant and termite brood that uses cloacal secretions as a defense against ant attacks and also as an ant repellent (Gelbach et al., 1968; Watkins et al., 1969). There is evidence that blindsnake cloacal secretions are attractive to conspecifics but repellent to some ophiophagous snakes (Watkins et al., 1969). An early hypothesis for the function of cloacal secretions was that they acted as the female sex pheromone in snakes (Noble, 1937; Price and LaPointe, 1981), although more recent work has shown that these pheromones are present in snake skin lipids (Greene and Mason, 1998; Mason, 1992; Mason et al., 1998).

[4] Department of Biology, University of Colorado at Denver, Denver, CO 80217 U.S.A.; michael.greene@cudenver.edu
[5] Department of Zoology, Oregon State University, Corvallis, OR 97331 U.S.A.

The aim of this paper is to discuss the effects of cloacal secretions on brown tree snake (Boiga irregularis) behavior. The brown tree snake, Boiga irregularis, is a rear-fanged colubrid native to Australia, Papua New Guinea, and the Solomon Islands (Cogger, 1992). The snake is an invasive pest species of Pacific islands where it has caused economic damage as well as a dramatic decrease in the abundance and diversity of the native fauna it preys upon (Rodda et al., 1992, 1997). The snake has most notably been a pest on Guam where it has caused economic problems such as power outages and ecological damage including the extirpation or extinction of 9 of Guam's 12 native forest bird species (Rodda et al., 1997). The snakes can reach lengths of over 3 meters and reach masses of up to approximately 1.5 kilograms (Cogger, 1992).

Female brown tree snakes release cloacal secretions to inhibit male courtship (Greene and Mason, 2003). Female brown tree snakes are particularly active in the process of courtship compared to reports for other snakes (Greene and Mason, 2000). Females display overt courtship behaviors that appear to solicit courtship from males and may represent mechanisms to asses to quality of potential mates (Greene and Mason, 2000). Also, females lift their tails, gape their cloacae, and deposit secretions during courtship to which males respond by ceasing courtship activity (Greene and Mason, 2000). These secretions are released from females' paired cloacal glands and are combined with liquid from the urogenital tract. Laboratory-based bioassays showed that female cloacal secretions inhibit male courtship, causing both a decrease in the amount of time spent courting and in the courtship intensity (Greene and Mason, 2003). The pheromone is only present in female cloacal secretions, not in male secretions. Also, the response of males to this courtship inhibition pheromone is context specific; males only responded to female cloacal secretions during courtship, not during bouts of male-male ritualized combat. The secretions had no effect on female courtship behavior. It appears that this pheromone is a mechanism for females to reject unsuitable males or to signal that they are not in a proper condition to mate (Greene and Mason, 2003).

Only freshly collected cloacal secretions were used in the courtship inhibition study (Greene and Mason, 2003), however in the process of running that study we serendipitously made the observation that brown tree snakes responded to secretions that had been aged in a sealed vial for over 30 minutes with behaviors normally seen when snakes are irritated or in a defensive situation. Brown tree snakes on Guam are known for their willingness to strike and bite when provoked, although they initially use crypsis and a decrease in locomotion as defensive behaviors (Rodda et al., 1999). Other defensive behaviors include lateral compression of the body, head hiding, forming and S-shaped coil of the anterior portion of its body that is used in lunging strikes, and flaring the quadrate bones to form a more triangular shaped head (Fritts, 1988; Johnson, 1975; Rodda et al., 1999). Prolonged provocation can lead to fleeing by the snakes, accompanied by lashing of their bodies and tails. As with other snakes, brown tree snakes commonly release cloacal secretions, often while lashing their tails, in response to disturbance.

We tested the effects of cloacal secretions that had been aged in a sealed vial on brown tree snakes. Our bioassay measured changes in locomotion, avoidance behavior and the expression of defensive behaviors in response to touch stimuli. A blank control, freshly collected cloacal secretions and aged cloacal secretions were the stimuli used in the study. An applied aim of this study was to investigate chemical cues that might be of use in controlling the brown tree snake in its introduced habitat (Mason and Greene, 2001).

We used randomly chosen snakes from our captive colony at Oregon State University; the snakes were all mature adults in breeding condition during the study (Greene and Mason, 1998, 2000, 2001, 2003). The animals were originally collected from the introduced population on Guam and were housed in our laboratory for approximately six years prior to the study under an established laboratory protocol (Greene et al., 1997). Temperatures cycled from 25°C to 30°C and relative humidity ranged between 75% and 80% in the snake room, simulating conditions on Guam. Lighting (approximately 14L:10D) was provided by overhead fluorescent lights and ambient sunlight entering the room through windows (Greene and Mason, 1997; 2000; 2003).

The experiment was conducted during scotophase when the snakes were most active. Each snake (N = 6; 3 males and 3 females) was tested with three stimuli in a random order on different nights: 1) a volatile control composed of a 2% solution of Aqua Velva cologne (JB Williams Company, White Plains, New York, U.S.), 2) freshly collected cloacal secretions, and 3) aged cloacal secretions. The dilute cologne control was chosen as it volatilizes at a rate much like cloacal secretions and the snakes do not respond to it adversely. On each night, cloacal secretions were collected by applying light pressure both anteriorly and posteriorly to a snake's cloaca and collecting the fine spray emanating from the paired cloacal glands along with the bolus of liquid from the urogenital opening into a clean, although not sterilized, 15 ml glass screw top tube. The cloacal secretions from three to five snakes not being used in the study as subjects were pooled for use in the trials. Freshly collected cloacal secretions were used in the experiment approximately 10 minutes after collection. Aged cloacal secretions were allowed to sit in the sealed vial for approximately 60 minutes at room temperature before being used in the experiment. All of the snakes used in the study were tested with all three stimuli on different nights and in a random order.

Our behavioral bioassay was designed to take advantage of the high degree of thigmotaxis displayed by the snakes and their tendency to actively explore novel environments. Snakes were tested in a clear cube-shaped Plexiglas arena, measuring 1.25m on each side. The floor of the arena was evenly divided into four quadrants with tape. Before a snake was added to the arena, a randomly chosen quadrant was treated

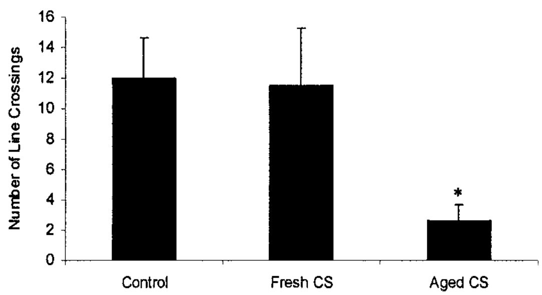

Figure 1. The number of line crossings exhibited by focal snakes during the observation period in response to the dilute cologne control, freshly collected cloacal secretions (Fresh CS) and aged cloacal secretions (Aged CS).

by rubbing approximately 2 ml of the stimulus evenly over the entire quadrant. Immediately after the stimulus was applied, a snake was placed in the center of the arena and observed for 30 minutes. In each case, the snakes quickly moved towards the walls of the arena and slowly explored the arena edges (Greene and Mason, 2003). After a trial ended, the snake was removed to its home cage and the arena was washed with soap and water, dried with paper towels and allowed to air out for 30 more minutes before used in the next trial.

During the observation period an observer, who was not aware of the treatment added to the arena, measured the number of line crossing as an indicator of activity and the amount of time the snake spent in each quadrant as an indicator of avoidance of the treated quadrant. At the end of the observation period a second person lightly touched each snake at its mid-body with the rubber handle of a snake hook two times separated by 5 seconds while the observer recorded the behaviors displayed by the snakes. The number of line crossings and the amount of time the snakes spent in the treated quadrant were compared using a Friedman ANOVA. The number of snakes responding with defensive behaviors was compared using a Cochran Q test. Aged cloacal secretions caused a significant decrease in the number of line crossings by the experimental snakes in comparison to freshly collected cloacal secretions and the dilute cologne control (Friedman ANOVA: $Fr = 9.0$; $df = 2$; $p < 0.011$; Figure 1), indicating that the snakes do not actively avoid the aged cloacal secretion stimulus. The snakes crossed a mean of 2.667 (± 1.022 SE) lines when treated with aged cloacal secretions versus 11.500 (± 3.819) for freshly collected cloacal secretions and 12.000 (± 2.646) for the control treatment. There were no significant differences in the mean times that the snakes spent in the treated quadrant of the arena during the trials between the treatments ($Fr = 1.2$; $df = 2$; $p < 0.549$; Figure 2), indicating that the snakes did not avoid treated quadrants. However, we noted that while exploring arenas treated with aged cloacal secretions snakes typically elevated their heads a few centimeters above the treated quadrant, an uncommon behavior during brown tree snake locomotion and exploration.

There were significant differences in the number of trials in which defensive behaviors were displayed after being touched by the snake hook handle between treatments (Cochran's Q-test: $Q = 7.6$; $df = 2$; $p < 0.022$). Snakes displayed defensive

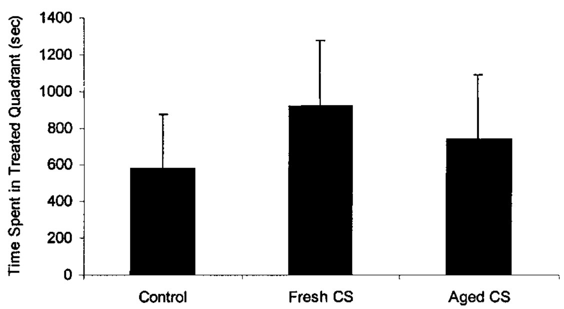

Figure 2. The mean amount of time focal snakes spent in the treated quadrant of the testing arena when treated with dilute cologne control, freshly collected cloacal secretions (Fresh CS) and aged cloacal secretions (Aged CS).

behaviors in 2 of 6 trials to the control treatment; in one case a single defensive strike at the snake hook handle and in the other case a weak bout of tail lashing (Table 1). There were no defensive behaviors displayed to the freshly collected cloacal secretion treatment. Snakes displayed defensive behaviors in 5 of 6 trials when exposed to the aged cloacal secretion treatment (Table 1). In all cases where defensive behaviors were displayed in the aged cloacal secretion treatment, the head shape of the snakes was triangular as observed during defensive displays. In four of these cases, huffing behavior was displayed in which the snakes audibly exhaled air from their lungs in a rhythmic manner. Huffing bouts lasted up to 20 seconds; we have never observed this behavior so obviously in another context. During these huffing displays, the snakes' bodies were laterally compressed. In four of the cases, the snakes displayed body lashing, in which the snakes erratically flailed their heads and tails, and in three cases snakes displayed fleeing, in which the snakes quickly moved away from the snake hook while lashing their bodies. No strikes at the snake hook were observed with the aged cloacal secretion treatment.

Aged cloacal secretions elicited behaviors in brown tree snakes normally associated with defense, including a decrease in locomotion, lateral compression of the body and fleeing. To the human nose, aged cloacal secretions smell rancid in comparison to the already malodorous freshly collected cloacal secretions, although this clearly does not indicate biological activity. However, differences in the behavioral responses of snakes to aged cloacal secretions versus fresh cloacal secretions and the control suggested that changes in the chemical composition of the cloacal secretions had occurred. The compounds that elicited a defensive response in the brown tree snakes studied were produced from chemical changes in the cloacal secretions over time. Oxidation or bacterial degradation of compounds in fresh cloacal secretions may have created the compound or compounds that elicit the defensive response in the snakes. The vials used to collect cloacal secretions were clean, but not sterilized, providing a source of microbes along with bacteria present in the cloacal secretions itself. Further work is needed to characterize the rate of production for the active compounds and to determine if these chemical changes can occur on the natural substrate, or if the reaction is limited to a sealed vial that prevents complete evaporation of the sample.

The experimental snakes did not display avoidance of the treated quadrants in the study. However, this is not unexpected as the experimental design did not test this directly as each of the three stimuli evaporated within the first few minutes of each trial.

Table 1. Behaviors elicited from snakes by touch after a 30 minute exposure to the control, freshly collected cloacal secretions, and aged cloacal secretions.

	Behaviors Displayed To:		
Snake	Control	Fresh Cloacal Secretions	Aged Cloacal Secretions
1	—	—	—
2	Strike	—	Body-lashing; Fleeing
3	—	—	Huffing; Body-lashing; Fleeing
4	Tail-lashing	—	Huffing; Body-lashing
5	—	—	Huffing; Body-lashing
6	—	—	Huffing

The snakes also tended to remain motionless in one spot for long periods of time in response to aged cloacal secretions. Responding to aged cloacal secretions with a reduction in locomotion may be somewhat mutually exclusive to avoidance of the cue as brown tree snakes do not generally flee when disturbed but instead attempt to use crypsis. However, the snakes maintained an elevated head posture only over areas of the arena treated with aged cloacal secretions, indicating an aversion to the aged cloacal secretion stimulus. This particular behavior is not normally observed during brown tree snake locomotion and exploration (MJG, personal observation).

In this study, we have documented an effect of aged cloacal secretions on brown tree snake behavior. Except for huffing behavior, the behaviors elicited from the snakes after the aged cloacal secretions treatment were similar to those observed under two other contexts: 1) after a male loses a bout of ritualized combat behavior (Greene and Mason, 1998) and 2) after a prolonged bout of defensive behavior by a captive animal, including repeated striking. After long bouts of defensive behavior the snakes respond to touch with body lashing and by quickly jerking the part of their body touched away from the stimulus (MJG, personal observation). Although the snakes did respond to the control treatment with two displays of defensive behavior, the behaviors seen under the aged cloacal secretions treatment were of a much greater duration and intensity. Although have never observed huffing behavior in the brown tree snake, we believe that it reflects a high degree of agitation. Much like the study by Graves and Duvall (1988) it appears that exposure to aged cloacal secretions made the focal snakes more likely to respond to an aversive stimulus with defensive behaviors. Our results are similar to an anecdotal report of a king snake (*Lampropeltis getulus*) responding to cloacal secretions left for over 15 minutes on a countertop with behaviors described by the authors as "extremely disturbed" (Brisbin, 1968).

In conclusion, cloacal secretions have the following behavioral effects on brown tree snakes: 1) they act as a pheromone that inhibits male courtship behavior, and 2) when aged, they act as a chemical signal and/or chemical irritant that that elicits defensive behaviors from the snakes. Our results represent a significant part of only a small handful of studies that have empirically demonstrated behavioral effects of cloacal secretions, despite the attention they have historically received in the literature (Mason, 1992). The data also demonstrate the effects of volatile chemical signals on snake behavior; the effects of non-volatile signals on snake behavior have been more commonly studied (Mason, 1992).

1. ACKNOWLEDGEMENTS

The authors would like to thank Shantel Stark for assisting with the experiments and Wes Mizuno for designing and building the experimental arena. This work was supported by a grant-in-aid award from the Sigma Xi Scientific Research Society to MJG and by grants from the U.S. Fish & Wildlife Service (USDI 14-16-0009-1577), the National Science Foundation (INT-9114567), as well as a National Science Foundation National Young Investigator Award (IBN-9357245) to RTM. This research was conducted under the authority of U.S. Fish & Wildlife Permits No. PRT-769753 and Oregon State University Institutional Animal Care and Use Committee Protocol No. LAR-932.

2. REFERENCES

Blum, M. S., Byrd, J. B., Travis, J. R., Watkins, J. F. II, and Gehlbach, F. R, 1971, Chemistry of the cloacal sac secretion of the blind snake *Leptotyphlops dulcis*, *Comp. Biochem. Phys. B* **38**:103-107.

Brisbon, I. L., 1968, Evidence for the use of post-anal musk as an alarm device in the king snake *Lampropeltis getulus*, *Herpetologica* **24**:169-170.

Cogger, H. G, 1992, *Reptiles and Amphibians of Australia*, Comstock/Cornell University Press, Cornell, New York.

Fritts, T. H., 1988, The brown tree snake, *Boiga irregularis*, a threat to Pacific Islands, *US Fish and Wildlife Biological Report 88(31)*.

Gehlbach, F. R., Watkins, J. F., and Reno H. W., 1968, Blind snake defensive behaviour elicited by ant attacks, *BioScience* **18**:784-785.

Graves, B., and Duvall, D., 1988, Evidence of an alarm pheromone from the cloacal sacs of prairie rattlesnakes, *Southwest. Nat.* **3**:339-45.

Greene, M. J., and Mason, R. T., 1998, Chemical mediation of reproduction in the brown tree snake, *Boiga irregularis*, *Ecoscience* **5**:405-409.

Greene, M. J., and Mason, R. T., 2000, The courtship, mating and combat behaviour of the brown tree snake, *Boiga irregularis*, *Herpetologica* **56**:166-175.

Greene, M. J., and Mason, R. T., 2003, Pheromonal inhibition of male courtship behaviour in the brown tree snake, *Boiga irregularis* - A mechanism for the rejection of potential mates. *Anim. Behav.* **65**:905-910.

Greene, M. J., Nichols, D. K., Hoyt, R. J., Jr., and Mason, R. T. 1997. The brown tree snake (*Boiga irregularis*) as a laboratory animal. *Lab Animal.* **26**:28-31.

Greene, M. J., Stark, S. L., and Mason, R. T., 2001, Pheromone trailing behavior of the brown tree snake, *Boiga irregularis*, *J. Chem. Ecol.* **27**:2193-2201.

Johnson, C. R., 1975, Defensive display behavior in some Australian and Papuan-New Guinean pygopodid lizards, boid, colubrid, and elapid snakes, *Zool. J. Linn. Soc-Lond.*. **56**:265-282.

Mason, R. T., 1992, Reptilian pheromones, in: *Biology of the Reptilia, Vol. 18.*, C. Gans and D. Crews eds., University of Chicago Press, Chicago, pp. 114-228.

Mason, R. T., and Greene, M. J., 2001, Invading pest species and the threat to biodiversity: Pheromonal control of Guam brown tree snakes, *Boiga irregularis*, in: *Chemical Signals in Vertebrates, IX*, A. Marchlewska-Koj, J. Lepri and D. Müller-Schwarze, eds., Kluwer Academic/Plenum Publishers, New York, pp. 361-368.

Mason, R.T., Chivers, D., Mathis, A., and Blaustein, A.R., 1998, Bioassays with reptiles and amphibians, in: *Methods in Chemical Ecology*, J. Millar and K. Haynes, eds., Chapman & Hall, New York, pp. 271-325.

Noble, G. K., 1937, The sense organs involved in the courtship of *Storeria*, *Thamnophis* and other snakes, *B. Am. Mus. Nat. Hist.* **73**:673-725.

Price, A. H., and LaPointe, J. L., 1981, Structure-functional aspects of the scent glands in *Lampropeltis getulus spendida*, *Copeia* **1981**:138-146.

Rodda, G. H., Fritts, T. H., and Chiszar, D., 1997, Disappearance of Guam's Wildlife, *BioScience* **47**:565-74.

Rodda, G. H., Fritts, T. H., and Conry, P. J., 1992, Origin and population growth of the brown tree snake, *Boiga irregularis*, on Guam, *Pac. Sci.***46**:46-57.

Rodda, G. H., Fritts, T. H, McCoid, M. J., and Campbell III, E. W., 1999, An overview of the biology of the brown tree snake (*Boiga irregularis*), a costly introduced pest on Pacific islands, in: *Problem Snake Management: the Habu and Brown tree snake*, G. H. Rodda, U. Sawai, D. Chiszar, and H. Tanaka, eds, Cornell University Press, Ithaca, New York, pp. 44-80.

Watkins, J. F. II, Gehlback, F. R., and Kroll J. C., 1969, Attractant-repellent secretions of blind snakes (*Leptotyphlops dulcis*) and their army ant prey (*Neivamyrmex nigrescens*), *Ecology* **50**:1098-1101.

Weldon, P. J., and Leto, T. L., 1995, A comparative analysis of proteins in the scent gland secretions of snakes. *J. Herp.* **29**:474-476.

Wood, W. F., Parker J. M., and Weldon, P. J., 1995, Volatile components in scent gland secretions of garter snakes (*Thamnophis* spp.), *J. Chem. Ecol.* **21**:213-219.