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## Responsiveness of Brown Tree Snakes to Odors

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### ABSTRACT

The brown tree snake (*Boiga irregularis*) is native to the islands of Papua New Guinea and Northern Australia. An introduced population on Guam has been implicated in the decline of that island's avifauna, and the snakes regularly cause power outages on the island. Concern exists for accidental introduction on the Hawaiian Islands. Traps baited with live mice have been used in control efforts, but the logistics of maintaining live mice in the field is difficult and expensive. This study has two objectives. First, using efficacy reports of small mammal and bird feces as attractants, we set out to identify active fractions of these potential prey odor sources. If active fractions are identified, there is a possibility of encapsulating reagent grade attractant without having to process feces or urine. Second, any successful snake lure should result in the snake's entry into a trap. Four snakes, 2-4 m in length, were obtained from the Philadelphia Zoo. These snakes were attracted to warm water-extracted vole and starling feces, but tended to avoid aqueous acid bird feces extracts. Snakes were indifferent to aqueous-base bird feces extracts. Snakes avoided Big Game Repellent®, cadaverine, butanethiol, and ethanethiol. In summary, potential prey odors lost their attractiveness quickly upon fractionation, suggesting the overall odor profile is important for attractiveness. We are focusing efforts to encapsulate chemicals so that field operatives will not need to process raw feces. In general, sulfur- and amine-bearing volatiles seem to repel brown tree snakes, but these same compounds are attractive to mammalian carnivores. If proven true, commercially available predator odors may be used to treat potential hiding places around air cargo areas and electric power plants.

### KEY WORDS

*attractant, brown tree snake, Boiga irregularis, odor, repellent*

### INTRODUCTION

The brown tree snake (*Boiga irregularis*) represents a serious hazard to biological diversity on islands where it is not a native species. Since the brown tree snake's accidental introduction to Guam, the island's fauna has been devastated (Savidge 1987, Rodda and Fritts 1992), and there

is concern that other Pacific islands may be at risk to introduction of this pest predator. In particular, concern has focused on the increasing reports of brown tree snake sightings on Saipan and Hawaii. The brown tree snake also causes economic losses by causing power outages, destruction of power equipment (Fritts et al. 1987), and through its depredation of subsistence and commercial poultry operations (Fritts and McCoid 1991).

Research into methods to regulate brown tree snake populations is ongoing. Methods to fumigate enclosed spaces and cargo are being explored (Brooks and Savarie 1995, Brooks et al. 1995, Savarie et al. 1991, 1995, Toriba et al. 1992). Trapping and enclosure efforts to clear open areas of snakes remains an effective tool for local snake control (Fritts et al. 1989). Currently traps use a live-mouse lure. Although mice are effective lures, the logistics of maintaining large numbers of traps in the field is not practical or economical. Laboratory data suggest that brown tree snakes increase appetitive prey search behavior in response to chemosensory cues (Chiszar et al. 1992), and there are limited field data to suggest that brown tree snakes are attracted to prey odors when these odors are used as lures (Fritts et al. 1989). Questions remain as to what chemical cues might best serve as artificial lures, and how the efficacy of the artificial lure compares to the use of live mice.

This study summarizes pilot laboratory experiments designed to screen and test the efficacy of potential artificial lures and the serendipitous discovery that many natural prey odorants also may act as repellents.

## METHODS

### Study Subjects

Four brown tree snakes were obtained on loan from the Philadelphia Zoo and housed at the Monell Chemical Senses Center. The two females measured 2.1 and 2.7 m, snout-vent length. The two males had snout-vent lengths of 2.4 and 3.3 m. The snakes had been originally captured from the wild on Guam and were of undetermined age, but had resided at the Philadelphia Zoo for 7 years. Snakes were maintained on a diet of voles (*Microtus pennsylvanicus*). Snakes were housed individually in cages (2' × 4' × 5'). Each cage was lined with licorice root mulch. Environmental conditions were maintained for humidity (80% relative humidity), temperature (28 °C), and light cycle (12:12 light:dark).

### Test Protocol

Initial tests relied on the passive diffusion of chemical stimuli from enclosed, darkened boxes. These boxes were designed to simulate natural refugia in the wild and were not based upon current trap designs. The boxes were designed to mimic places that a brown tree snake might utilize around human habitations. Chemicals believed to be of biological interest to snakes were applied to filter paper and placed inside a 9" × 9" × 18" black acrylic box. The box contained one opening, and no visual cues were apparent from outside the box. A second box, the control, was prepared with filter paper treated with distilled water. Both boxes were placed inside a test area that was identical to the layout of the housing cages in which the snakes were maintained. The test boxes were placed at random along one side of the test arena such that a snake would be

presented with a three-choice test. Snakes could enter the odor box, control box, or choose to remain outside the boxes. Each test was 2-hr in duration. Behavior of the snakes was videotaped. Snakes were introduced into the test cage from their housing cage. We used the latency to enter the black boxes as a measure of attractiveness of the artificial lure. Thus, each test consisted of two scores: the time from the start of a test that a snake would enter the odor-containing box, and the time from the start of the test that a snake would enter the control box. The maximum time scored would be 120 min for each condition. The data reported are censored. In some tests the snake did not enter either box during the observation period, and this no-choice situation was not used in calculating mean latency times.

Latency to enter a box was considered a good practical measure of a lure's appeal, because in the field, the success of a lure would be evaluated for the likelihood of a snake entering a trap. Thus, we infer that a short latency is an index of interest to the lure by a snake, and an index of motivation for the snake to enter a darkened cavity (relative to the non-odor control). In these experiments, volatile cues were the prime modality for information transmittal.

Because of the limited number of snakes available ( $n = 4$ ) and the numerous compounds to be screened, data are limited. Therefore, the results should be viewed as a pilot study to be used in evaluating what compounds might be of future interest.

Snakes were tested no more than once every 2 weeks in an effort to avoid habituation to the experimental paradigm. Testing did not take place if there was evidence that a snake was about to shed. Shedding limited the time period for which snakes could be tested. Snakes were fed 80 g of vole every 2 weeks. Voles were placed inside a darkened test box. This was done to maintain reinforcement that boxes might contain food. Snakes were introduced into new cages at random intervals. At introduction, the pair of boxes might contain no food or odor stimulus, one of the two boxes might contain an odor stimulus, or one of the two boxes might contain a vole. The only constraint was that odor testing generally occurred at the end of the 2-week period when the hunger state of the snake was assumed to be at its highest.

## Test Stimuli

### *Whole Prey Odors*

Previous reports indicated that brown tree snakes were attracted to bird feces (Fritts et al. 1989). To test the hypothesis that components of prey odors are attractive to brown tree snakes, feces extracts were obtained from European starling (*Sturnus vulgaris*) and voles. Briefly, 100 g of dried feces was ground with mortar and pestle, mixed with 1 L of water (DID) and, allowed to stand for 24 hr at 23 °C. The aqueous phase was decanted, filtered and saved for testing. Ten milliliters of the test solution was applied through an atomizer to Whatman's filter paper (20-cm diameter) and air dried overnight.

### *Prey Odor Fractions*

Carnivorous mammals are attracted to odor stimuli containing amines and sulfur compounds (Nolte et al. 1994). These compounds typically are indicative of protein degradation products

derived from a carnivorous diet. In contrast, herbivorous mammals avoid compounds associated with digestive protein degradation. These observations suggested the possibility that the carnivorous brown tree snakes might be attracted to similar types of compounds.

Acid treatment of aqueous bird feces extract will tend to suppress volatility of base constituents (i.e., amino groups) and increase volatility of compounds containing carboxylic acids. Base treatment of aqueous bird feces extract will tend to suppress volatility of acidic functions and increase volatility of compounds containing amino and sulfur groups. We hypothesized that if the odor of prey were related to compounds characteristic of protein degradation, the base fraction should retain its attractiveness relative to the whole feces extract. Concentration of extracts prepared for testing were 0.05% weight/volume or volume/volume.

### *Simple Odors Indicative of Protein Diets*

Many odors derived from single compounds are attractive to carnivores and repellent to herbivores for the reasons cited above. Big Game Repellent, 1,5-diaminopentane, butanethiol, and ethanethiol, were obtained commercially and prepared as aqueous phase test stimuli (0.05% weight/volume or volume/volume).

Big Game Repellent is a commercial mammal repellent targeting herbivores based upon extracts of decomposing eggs. The rotten egg odor is associated with sulfides contained in the repellent. Cadaverene is a compound produced by bacteria that decompose flesh. It is generally toxic to humans and has a foul odor. Butanethiol is a sulfur- (thiol) bearing mercaptan compound that is commonly used to odorize natural gas, and it is generally associated with microbial decomposition of flesh. Ethanethiol is a sulfur- (thiol) bearing mercaptan that is commonly used to odorize natural gas. The latter three compounds were selected because anecdotal reports indicated that offal and afterbirth are attractive to brown tree snakes.

### **Statistical Analyses**

In some circumstances a particular snake received multiple presentations of the same stimulus over time. The analyses included only the first test when the snake showed a response to either the control or stimulus within the allotted 120-min test period. Latency differences between the test stimuli and the control (blank) were determined by a paired t-test.

## **RESULTS**

### **Stimuli**

#### *Whole Vole Feces Aqueous Extract*

Snakes 6 and 5 (Philadelphia Zoo notation) were tested once each. In both instances the snakes entered a box within the 120-min observation period. Snake 1 was tested three times. This snake made a choice during the allotted observation time for the first two tests, but did not enter

either the stimulus or control box the third time it was tested. Snake 4 was tested four times. It did not react for the first two tests. The snake did react within the allotted observation period for the third test, but did not react during the fourth test. Snakes entered boxes containing aqueous extracts of vole feces significantly more quickly than they entered the control boxes (Figure 1,  $P = 0.031$ ) (See figures at end of chapter).

#### *Whole Mongoose Feces Aqueous Extract*

A total of four tests pairings using water extracts of whole mongoose feces with the control were conducted. Each of the snakes was tested once. Snakes 1 and 4 did not react within the allotted 120 min. The remaining snakes entered boxes with mongoose feces odor significantly more quickly than the control (Figure 2,  $P = 0.014$ ).

#### *Whole Starling Feces Aqueous Extract*

Snake 6 was tested three times, but only showed interest in the test boxes during the first test. Snakes 1, 4 and 5 were tested once each, and each showed an interest in at least one of the test boxes. There was no statistical difference between the latencies for first entry into a box (Figure 3,  $P = 0.569$ ).

#### *Aqueous-Acid Bird Feces Extract*

Snakes 1, 4, and 6 were tested once each, and all entered at least one of the boxes within the allotted time. Snake 5 was tested once, but failed to show interest in either the stimulus or control box within the allotted time. There was no difference in latency time for entry into a box between the test odor and control ( $P = 0.309$ ), though there was a tendency for snakes 4 and 6 to avoid the box containing the test odor (Figure 4).

#### *Aqueous-Base Bird Feces Extract*

Snakes 1, 4, and 6 were tested once each. The results were equivocal (Figure 5,  $P = 0.909$ ). One snake avoided the odor, one preferred it, and the third investigated both boxes within a very short period of time.

#### *Big Game Repellent*

Snakes 1 and 6 were tested. Snakes avoided the odor stimulus box; they entered the control boxes and stayed there (Figure 6,  $P = 0.028$ ).

#### *1,5-Diaminopentane (Cadaverene)*

Snakes 1 and 4 were tested. There was a tendency for snakes to avoid the odor-bearing box and take up residence in the control boxes (Figure 7,  $P = 0.068$ ). Increased sample sizes are needed to verify this trend.

### *Butanethiol*

Snakes 4 and 6 were tested. Snakes avoided the odor-bearing box and took up residence in the control box (Figure 8,  $P = 0.008$ ). Increased sample sizes are needed to verify this trend.

### *Ethanethiol*

Snakes 4 and 6 were tested. There was a tendency, as with butanethiol, for snakes to avoid the odor-bearing box and take up residence in the control (Figure 9,  $P = 0.265$ ).

## DISCUSSION

The attractiveness of whole vole feces odors is not surprising, because snakes were maintained on a diet of voles. The snakes were familiar with vole odors and would be expected to associate the odor with a food reward. The odor of feces of a potential snake predator, i.e., mongoose, was also attractive. It may be possible that any mammal feces could serve as an attractant, providing that snakes associate mammals as prey items. In contrast, there was no evidence that starling feces was attractive to the brown tree snakes. Given field reports of the attractiveness of bird feces, the present results may simply reflect the unfamiliarity of starling feces as potential prey for the particular snakes tested.

Contrary to expectations, aqueous extracts promoting amine and sulfur volatility were not attractive to brown tree snakes. One explanation for this observation is that the extracts were based upon bird feces, a generally unfamiliar and nonattractive stimulus. A second explanation is that the concentration of the stimuli was too weak. A third possibility is that whole prey odor profiles are needed to be attractive to snakes. Though not significant, the tendency for acidic volatiles to be avoided was surprising.

Most surprising was the strong avoidance of the simple compounds. Sulfur-bearing compounds (e.g., the mercaptans) are often associated with microbial degradation of protein and would presumably be attractive to a carnivore. It is possible that endothermic mammals such as carnivores are more resistant to the toxicity of these compounds relative to ectothermic reptiles. If true, snakes may use such odor cues to avoid potentially noxious or toxic food items. Tests on the concentration effects of these compounds might prove interesting. It is conceivable that low concentration of these compounds might be attractive, whereas higher concentrations might signal toxic levels of microbial contamination.

## MANAGEMENT IMPLICATIONS

Snakes use a variety of tactics to hunt prey (e.g., active foraging, sit-and-wait-strategies). Furthermore, the sensory cues that a snake relies upon will vary depending upon which tactic is used and the phase of that particular tactic. For example, an actively foraging snake may rely on visual, vibratory, and chemical cues to locate prey, to locate their trails, or to locate areas of high prey activity. In the case of chemical cues, the composition of the cue may provide information

about the type of prey and whether the prey is still in the vicinity (i.e., trail freshness). This information may influence whether a snake continues to follow a chemical trail, investigates a site more thoroughly, follows a sit-and-wait strategy, initiates consumptive behavior, or loses interest. The difficulty in developing artificial lures is that the chemical cue is often a weak representation of the whole prey and is out of context with the natural situation. The consequence is that traps using artificial lures often have poor success relative to traps using live lures.

However, the objective in developing an artificial lure is not to exactly mimic live prey. The artificial lure strategies are to (1) develop a chemical signal that promotes investigatory behavior and/or trail-following behavior, and (2) ensure that such a chemical cue is easy to employ in the field. The tests described herein are valuable as first steps in elucidating the types and strengths of chemosensory cues needed to influence appetitive behavior of brown tree snakes. The tests focusing on compounds associated with rotting flesh are intriguing in that they indicate that an acceptable snake repellent might be achieved.

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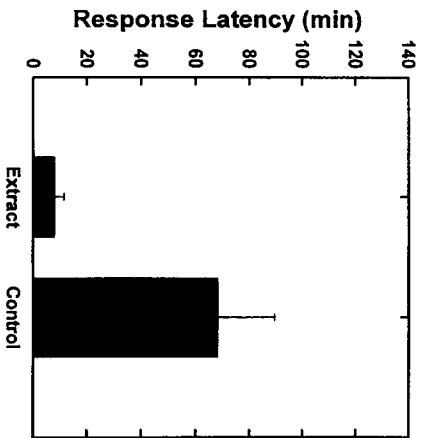


Figure 1: Response latency (RL) as a function of test condition.\*

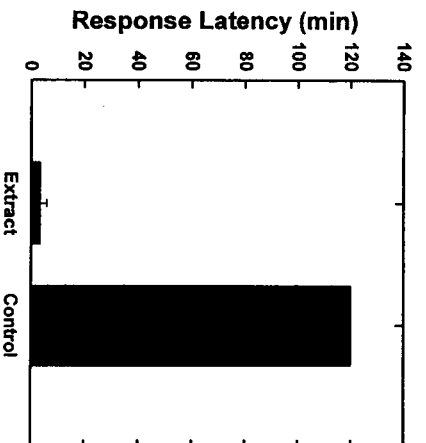


Figure 2: RL as a function of test condition for water-extracted mongoose feces.

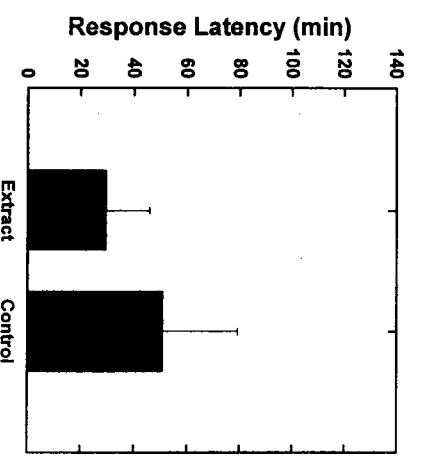


Figure 3: RL as a function of test condition for water-extracted bird feces.

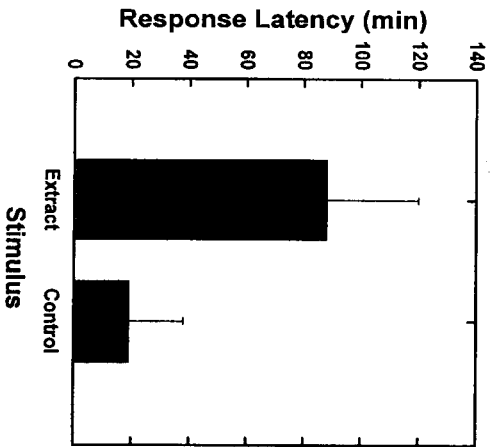


Figure 4: RL as a function of test condition for water-acid extracted bird feces.

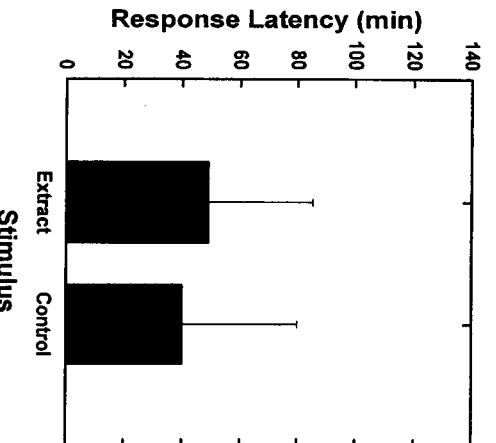


Figure 5: RL as a function of test condition for water-base extracted bird feces.

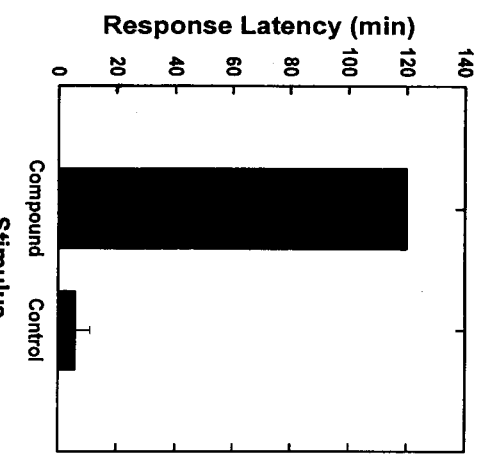


Figure 6: RL as a function of test condition for Big Game Repellent.

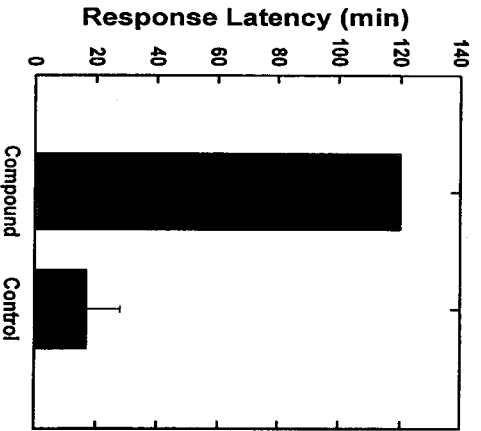


Figure 7: RL as a function of test condition for 1,5-diaminopentane.

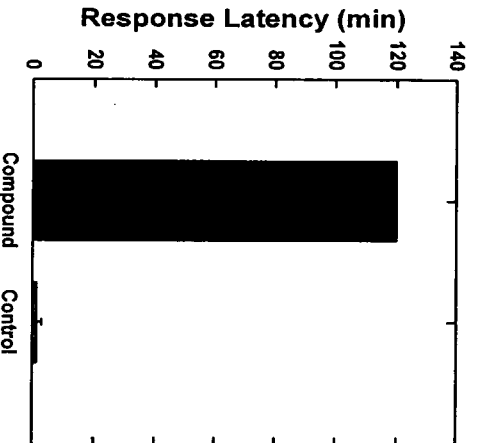


Figure 8: RL as a function of test condition for butanethiol.

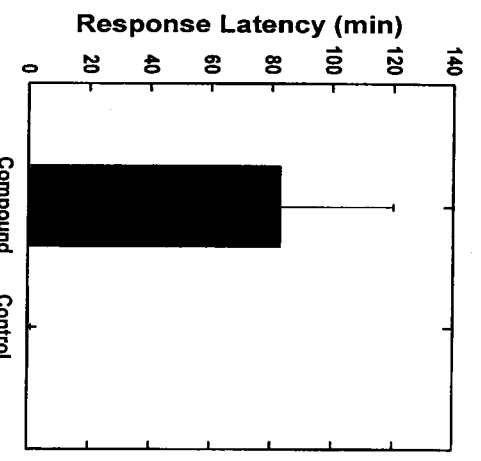


Figure 9: RL as a function of test condition for ethanethiol.

(\*i.e., the time it takes a snake to enter either the control or odor-containing test box from the start of the experiment for water-extracted volefeces.)