

CHARACTERIZATION OF THE DEFENSIVE EFFICACY OF THE STERNAL  
SECRETION OF *EURYCOTIS FLORIDANA* (WALKER)  
(DICTYOPTERA: BLATTIDAE)

---

A Thesis

Presented to

The Faculty of the Department of Biology  
The College of William and Mary in Virginia

In Partial Fulfillment

Of the Requirements for the Degree of  
Master of Arts

---

By

Matthew William Turnbull

1999

APPROVAL SHEET

This thesis is submitted in partial fulfillment of  
the requirements for the degree of

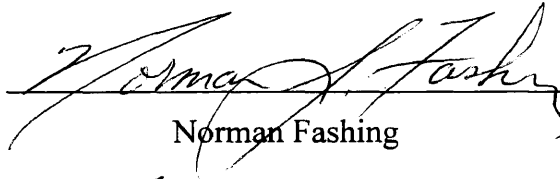
Master of Arts



---

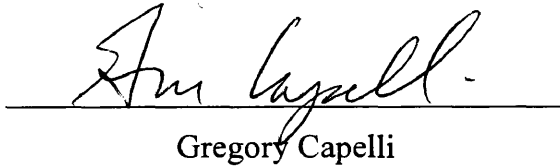
Matthew Turnbull

Approved, March 1999



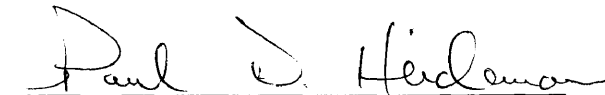
---

Norman Fashing



---

Gregory Capelli



---

Paul Heideman

## DEDICATION

This work has been done in memory of the author's best friend, his mother,  
Patricia Louise Turnbull.

## TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENTS	v
LIST OF TABLES	vi
LIST OF FIGURES	vii
ABSTRACT	ix
INTRODUCTION	2
CHAPTER I. VERTEBRATE DETERRENCE	16
CHAPTER II. INVERTEBRATE DETERRENCE	50
CHAPTER III. ROACH ORIENTATION DURING DISCHARGE	69
CHAPTER IV. BIOSYNTHESIS OF THE SECRETION	75
DISCUSSION	80
BIBLIOGRAPHY	83
VITA	90

## ACKNOWLEDGEMENTS

I wish to thank my major advisor, Dr. Norm Fashing, for the advice dispensed in mass quantities and, perhaps most of all, the (apparently) infinite patience necessary to stomach me as long as he has known me, and to wade through a morass of verbose writing. I also wish to thank Drs. Paul Heideman, Greg Capelli, and Kirt Moody for their advice and editing, and Dr. Patty Zwollo for introducing me to an altogether different realm of science. Finally, to remedy an oft-overlooked source of aid and inspiration: many thanks to my fellow graduate students, particularly the other old-timers.

## LIST OF TABLES

Table		Page
1.	Insects known to possess 2-(E)-hexenal	5
2.	Categories for Data Analysis of Mouse-Roach Assay	23
3.	Mean proportions of time per behavior per temporal class	28
4.	Multiple comparisons of proportional grooming data	29
5.	Descriptive statistics of interval between contacts	33
6.	Multiple comparisons of interval between contacts	34
7.	Number and proportion of contact types per temporal class	35
8.	Multiple comparisons of contact type per temporal class	36
9.	Number and proportion of contact initiator per temporal class	39
10.	Relation of contact location to spray event	42
11.	Proportion of ants remaining at honey at $t = 5s$	54
12.	Multiple comparisons of proportion of ants remaining at $t = 5s$	55

## LIST OF FIGURES

Figure		Page
1.	Ventral view of sternal gland	10
2.	Ventral view of sternal gland, sternites cut away	11
3.	Ventral view of sternal gland, intersegmental muscles visible	12
4.	Forepaw-mouth grooming in <i>P. leucopus</i>	19
5.	Head grooming in <i>P. leucopus</i>	20
6.	Body grooming in <i>P. leucopus</i>	21
7.	Method for determining orientation data	26
8.	Division of grooming activities per temporal class	30
9.	Mean interval between contacts per temporal class	32
10.	Proportion of contact type per temporal class	37
11.	Proportion of contact initiator per temporal class	40
12.	Typical fear-induced evasion of roach by mouse	41
13.	Contact location effect on secretion induction	43
14.	Effects of 2-(E)-hexenal on biological systems	47
15.	Setup of ant repellency assay	51
16.	Mean proportion of ants remaining at honey at $t = 5s$	56
17.	Ants at honey at $t = 5s$ of water presentation	58
18.	Ants at honey at $t = 5s$ of secretion presentation	59
19.	Mean proportion of ants remaining at honey over 30s	60
20.	<i>P. americana</i> before secretion application	61

## LIST OF FIGURES CONTINUED

Figure		Page
21.	<i>P. americana</i> following secretion application	62
22.	Number of roaches reacting positively to compound application	63
23.	Typical discharge pattern of <i>E. floridana</i> secretion	71
24.	Body orientation of <i>E. floridana</i> following an attack	73
25.	Proposed model for the biosynthesis of secretion	76

## ABSTRACT

The chemistry of the abdominal gland secretion of the cockroach *Eurycotis floridana* has been well established. A series of assays therefore were conducted to test hypotheses about the biological function of the secretion. In assays investigating the spray's effects on two vertebrate models, *Peromyscus leucopus* and *Bufo marinus*, a discharge was found to induce behaviors indicative of fear and discomfort. In invertebrates, the secretion was found to significantly alter feeding patterns in an ant, *Monomorium pharaonis*, and induced grooming responses indicative of irritancy in two roaches, *Periplaneta americana* and *E. floridana*. The compounds present in the secretion may alter predator behavior through both contact and airborne action. The secretion was also found to be autotoxic to *E. floridana*, which has implications in previously reported conspecific interactions. *Eurycotis floridana* is capable of significant accuracy in delivery of the secretion. Finally, a model for the biosynthesis of the secretion is proposed.

CHARACTERIZATION OF THE DEFENSIVE EFFICACY OF THE STERNAL  
SECRETION OF *EURYCOTIS FLORIDANA* (WALKER)  
(DICTYOPTERA: BLATTIDAE)

## **Introduction**

### **Chemical Communication**

The chemical communication of animals has been honed to the greatest degree in insects. In no other group are such a wide range of semiochemicals seen, nor such a suite of combinations and fine-tuning of messages. Although social insects (e.g., Isoptera and certain Hymenoptera) are the unquestioned masters of the chemical message, it has been hypothesized that their wide range of communicants has evolved from the chemical defenses used by other insects (Blum and Brand 1972) and not from some other evolutionary line.

Semiochemicals may be separated into pheromones and allelochemicals. Pheromones effect changes in conspecifics and are divided further by the timing of their effects: releasers provoke immediate changes, while the effects of primers are exhibited more gradually. Examples of releasers include alarm signals, spacing signals, and conspecific identifiers; examples of primers are pheromonal stimulators of development and cohort emergence.

Chemicals that effect behavioral changes interspecifically are known as allelochemicals; they are classified by the beneficiary of the signal. Compounds that benefit the sender, known as allomones, provide assistance by performing a multitude of tasks. Although the most common function of allomones in insects is to provide defense, they may also act in an offensive fashion by attracting and subduing prey (for review see Blum 1996). Signals that benefit the interspecific receiver (and in turn produce a cost on the sender) are known as kairomones. As it is thought that the cost to the sender would be a large evolutionary load, it has been hypothesized that many kairomones evolved as

pheromones or allomones which were then co-opted by a third party to the detriment of the sender (Blum 1974). An example of a kairomone is the chemical signal present in the frass of the larvae of various Lepidopterans; parasitoid wasps frequently use such compounds to find egg hosts (Quicke 1997).

### **Allomones**

Although naturalists have been describing occurrences of chemical defenses for years, it is only over the past several decades that biochemical and molecular techniques have enabled the explanation of many of those observations. While the bombardier beetle has long been known through behavioral studies to be protected by a powerful defensive spray, the chemical composition of the spray has been determined only recently. However, the relative effort on these questions has reversed, and most current studies ignore the behavioral aspect of chemical defense, focusing instead on the allomonal chemistry.

Some defensive compounds, as in millipedes and some true bugs, are released onto the body when the animal is provoked, thereby providing a contact deterrent (Eisner 1970, Eisner et al. 1967). Perhaps the most astounding example of this are the reflex bleeders. When roughly handled, these insects force hemolymph to ooze out from between their joints. The blood of these insects contains toxins which are often distasteful or sticky. In the extreme case of the blood of blister beetles, the cantharidin present is capable of causing blistering in mammals on contact, even in very small amounts.

Many insects have taken the issue of deliverance of defensive chemicals to a higher level by acquiring the ability to forcibly discharge their compounds as a spray.

The benefits of this ability are immediately apparent: the lack of physical contact means that the insect is less likely to take collateral damage while defending itself, and there is often a greater area of effect. There are many groups capable of this, including true bugs, termites, and roaches (for review see Eisner and Meinwald 1966, Roth and Eisner 1962).

Two mechanisms are responsible for the procurement of allomones. Many insects obtain the hydrocarbon components of their allomones from their diet. For example, the larvae of some Nymphalidae (Bowers and Stamp 1997) and Arctiidae (von Nickisch-Rosenegk and Wink 1993) (Lepidoptera) obtain glycosides from leaves and then alter and sequester them as defensive agents. Insects also may synthesize components *de novo*. Little is known about the biosynthesis of these compounds (Prestwich and Blomquist 1987), though current work in the field of pheromone biosynthesis holds promise due to the similarity of hydrocarbons across insect groups (Blum 1996).

The constituents of allomones tend to be conserved, in that the same chemicals are found across many orders (for review see Blum 1981, Jacobson 1966). Acids, aldehydes, and quinones, usually represented by a handful of compounds, tend to be present in the majority of secretions. An example of this is (E)-2-hexenal, an  $\alpha,\beta$ -unsaturated aldehyde found across several orders of insects (**Table 1**) (for reviews see Blum 1981, Eisner 1970, Jacobson 1966). The conservation of these defensive compounds leads to the hypothesis that they perform similar functions, even in distantly related groups of organisms (e.g., (E)-2-hexenal is known to act in a defensive capacity against potential vertebrate and invertebrate predators in several of the organisms which produce it (Benn et al. 1977, Blum 1965, Blum 1981)). The commonality of chemical constituents across groups, although chaotic in incidence, supports the hypothesis that

Order	Arthropod
Blattaria	<i>Desmozosteria scripta</i> <i>Drymaplaneta spp.</i> <i>Eurycotis spp.</i> <i>Euzosteria nobilis</i> <i>Megazosteria patula</i> <i>Pelmatosilpha coriacea</i> <i>Polyzosteria spp.</i> <i>Platyzosteria spp.</i> <i>Zonioploca spp.</i>
Coleoptera	<i>Eleodes beameri</i>
Hemiptera	<i>Acanthocephala spp.</i> <i>Acanthocoris sordidus</i> <i>Alydus spp.</i> <i>Archimerus alternatus</i> <i>Biprorulus bibax</i> <i>Bronchymena quadripustulata</i> <i>Chrysocoris stollii</i> <i>Cimex lectularius</i> <i>Dolychoris baccarum</i> <i>Eurygaster sp.</i> <i>Euthochtha galeator</i> <i>Gelastocoris oculatus</i> <i>Leptoglossus spp.</i> <i>Libyaspis angolensis</i> <i>Megalotomus quinquespinosus</i> <i>Musgraveia sulciventris</i> <i>Nezara viridula</i> <i>Oncopeltus fasciatus</i> <i>Palomena viridissima</i> <i>Piezodorus teretipes</i> <i>Poecilometis strigatus</i> <i>Scaptocoris divergens</i> <i>Scotinophora lurida</i> <i>Tessaratomyia aethiops</i>
Hymenoptera	<i>Crematogaster spp.</i>

**Table 1. Insects Known to Possess (E)-2-hexenal (From Blum 1981).**

allomones were the evolutionary antecedents of pheromones (Blum 1974). The use of common defensive chemicals as pheromones by insects, particularly as alarm pheromones, supports this hypothesis. (E)-2-hexenal functions as a disturbance signal in both the cockroach *Eurycotis floridana* (Farine et al. 1997) and ants of the genus *Crematogaster* (Blum et al. 1969, Crewe et al. 1972).

### **Semiochemicals of Cockroaches**

Cockroaches are known to manufacture and manipulate a large array of chemical compounds (for review see Blum 1981). Acids, aldehydes, and other hydrocarbons, in addition to lipid and amino acid derivatives, are all seen in cockroach semiochemicals. Tergal gland secretions are widespread in males of many species and often function in calling of females and mating (Roth and Alsop 1978). Interestingly, though, chemical defenses in the Dictyoptera are limited to the families Blattidae and Blaberidae (Wallbank and Waterhouse 1970).

The glands involved in the synthesis and storage of blattid allomones have been classified primarily by location on the abdomen (Roth and Alsop 1978). Type I glands are eversible, lateral glands which extrude from pleural regions when the roach is disturbed. Type II glands are ventral, located along the midline of the sternite. Type III glands, found only in the subfamily Blattinae, are paired, opening between the tergites. Type IV glands are paired, located in the trachea. Type V glands are found at the posterior of cockroaches, and produce a sticky, proteinaceous secretion which acts as a mechanical disrupter in small invertebrates (Roth and Alsop 1978). Type II, III, and IV glands are capable of spraying their secretions at a distance, whereas glands of type I and type V rely upon contact with the cockroach's body for deterrence.

Type II glands have been found in all Blattidae examined (Roth and Alsop 1978). The subfamilies Blattinae and Polyzosteriinae perhaps have been studied the most extensively; the former due to the inclusion of the American roach (*Periplaneta americana*) and the Oriental roach (*Blatta orientalis*), both pest species. The Polyzosteriinae do not include cockroaches of true pest value, however, and have been studied more from a basic interest than an economic. The secretory glands of the Polyzosteriinae are well-conserved among the genera, as comparative studies between *Pelmatosilpha*, *Platyzosteria*, and *Eurycotis* (Wallbank and Waterhouse 1970, Brossut and Sreng 1980) have demonstrated. The composition of the secretions also is well conserved, with (E)-2-hexenal being a primary component in the secretion of many of the Polyzosteriinae (Wallbank and Waterhouse 1970, Brossut 1983).

These characteristics are found in *Eurycotis floridana*, which possesses a functional Type II gland as an adult, as well as the Type V in the juveniles (Abed et al. 1994). The mating behavior of the cockroach has been well studied (David-Henriet et al. 1994, 1995, Farine et al. 1993, 1994, 1996), as well as the chemistry of the sternal secretion (Farine et al. 1997). However, the purported defensive role of the sternal secretion has not been adequately examined; the presence of (E)-2-hexenal (Roth et al. 1956) and a review of observational data (Eisner et al. 1959) have been cited as the only evidence for that function.

### **Biology of *Eurycotis floridana***

*Eurycotis floridana* is found primarily in the Caribbean and southeastern U.S.A. (Brenner 1988, Brenner and Pierce 1991, Hebard 1917). Typically found under wood piles and ground cover around dwellings (Brenner 1988), it may become a pest in the late

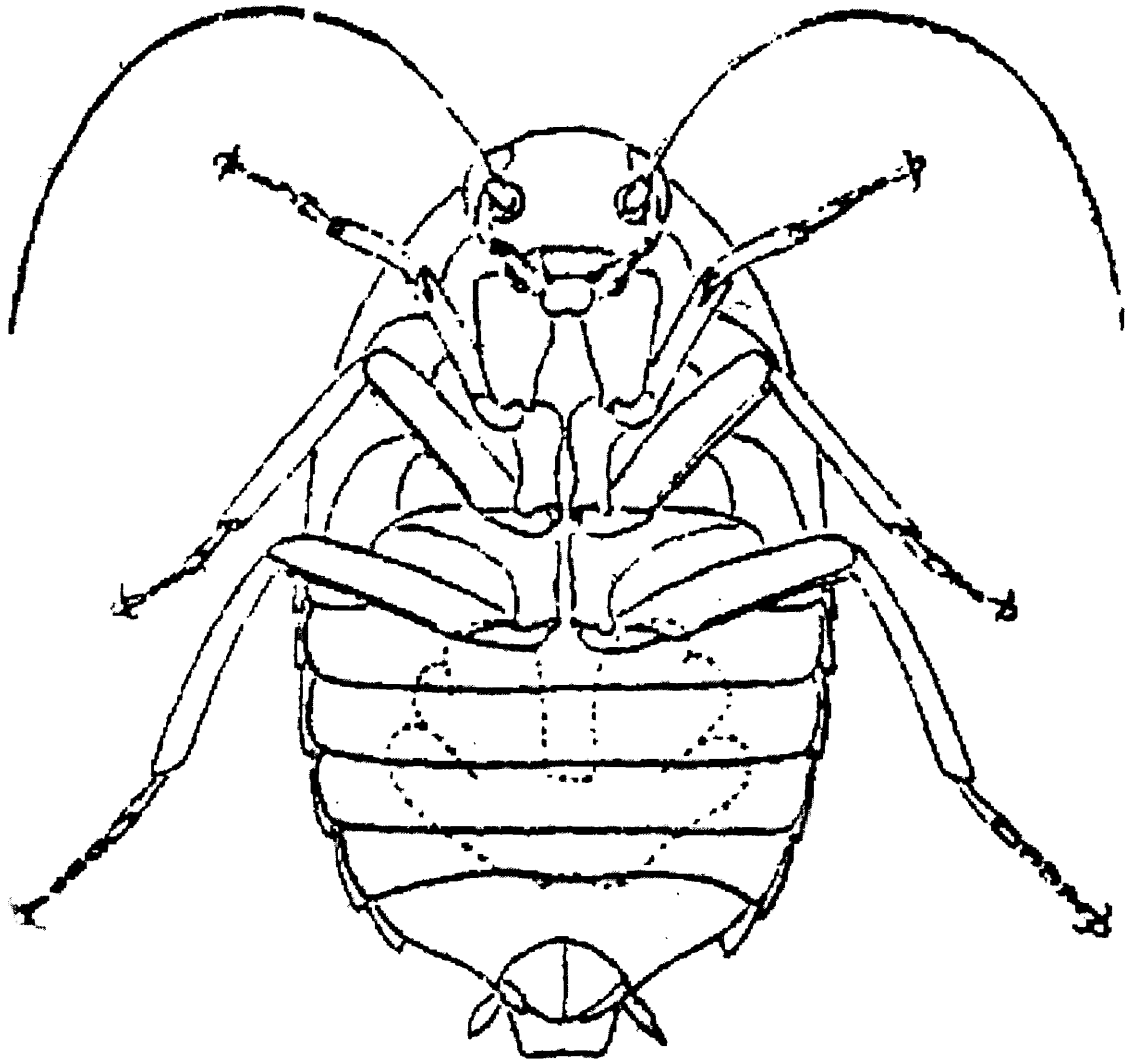
summer to fall as it moves into the dwellings (Brenner and Pierce 1991). Adults are flightless, possessing only vestigial wing-pads. The species lacks the aposematic coloration of several of its relatives: both males and females are uniformly glossy brown to dark red with yellow occasionally visible at pleural membranes. Juveniles are very similar in appearance to adults, but often are marked by the presence of lightly colored lateral thoracic bands. Although no sexual size dimorphism is exhibited by adults, the sexes may be differentiated by their supra-anal plate. The plate of the female is semicircular, caused by the convergence of the plate's lateral margins, whereas that of the male is less rounded (see description of Hebard 1917).

*Eurycotis floridana* has been observed to exhibit male agonistic behaviors (David-Henriet et al. 1995) associated with an hierarchical system (Bell et al. 1979). Tergal glands, limited to adult males, have been observed to have key roles in the calling of females (Farine et al. 1996), inciting agonistic behavior (David-Henriet et al. 1995), and possibly in recognition of hierarchical status of males (Farine et al. 1996). Farine et al. (1997) noted that males occasionally discharge their sternal glandular secretion at one another and that they are less responsive to low concentrations than are females; they postulated that this could be due to the use of the secretion in agonistic encounters. David-Henriet et al. (1995) have included this spraying in describing the sequence of events involved in an agonistic male-male encounter, noting that emission of sternal secretion by the dominant male is frequently seen following release of the tergal gland compounds and blows to the head and abdomen of the weaker male.

## Gland Morphology

The Type II abdominal secretory gland (**Figs. 1-3**) of *E. floridana* is composed of 2 large lobes lateral to the ventral nerve cord. When fully distended with secretion, the gland stretches to the 3<sup>rd</sup> sternite and exhibits 2 to 3 lesser lobes per lateral half. The duct, consisting of a tightly opposed semicircular invagination at the median of the 6<sup>th</sup> and 7<sup>th</sup> ventral sternites, lacks sphincter muscles (Stay 1957). This possibly explains the presence of secretion on the cuticle surrounding the duct noted by Farine et al. (1994). There is no innervation of the gland, but there is significant tracheal ramification arising from the median ventral tracheal trunk (Stay 1957). Release of the secretion appears to be controlled primarily by two pairs of muscles that connect the 6<sup>th</sup> and 7<sup>th</sup> sternites. Their contraction separates the apodemes at the gland's opening, opening the duct; contraction of a third pair of muscles reduces the volume of the abdomen, forcing the release of the secretion out of the ostiole (Stay 1957, Waterhouse and Wallbank 1967). Furthermore, an increase in hemocoel pressure also appears to cause release of secretion (Stay 1957), whether by manipulation of trachea (Eisner 1970) or through a general abdominal contraction (perhaps during handling by a predator). The large capacity of the gland relative to body size (Stay 1957), in conjunction with the small size of the duct, could account for the great spray distances that have been reported (Eisner et al. 1959).

The wall of the gland is composed of two cuticular layers and two cellular layers (Stay 1957). The outer cuticular and inner cuticular layers appear to aid structural integrity; the inner layer also provides an unbroken connection with the epidermal cuticle. The inner cellular layer consists of small, squamous cells suggested to be involved in chitin secretion and the formation of the secretory ducts (Konček 1924 in



**Figure 1.** Ventral view of *Eurycotis floridana*. Outline of the sternal gland is stippled (From Alsop 1970).

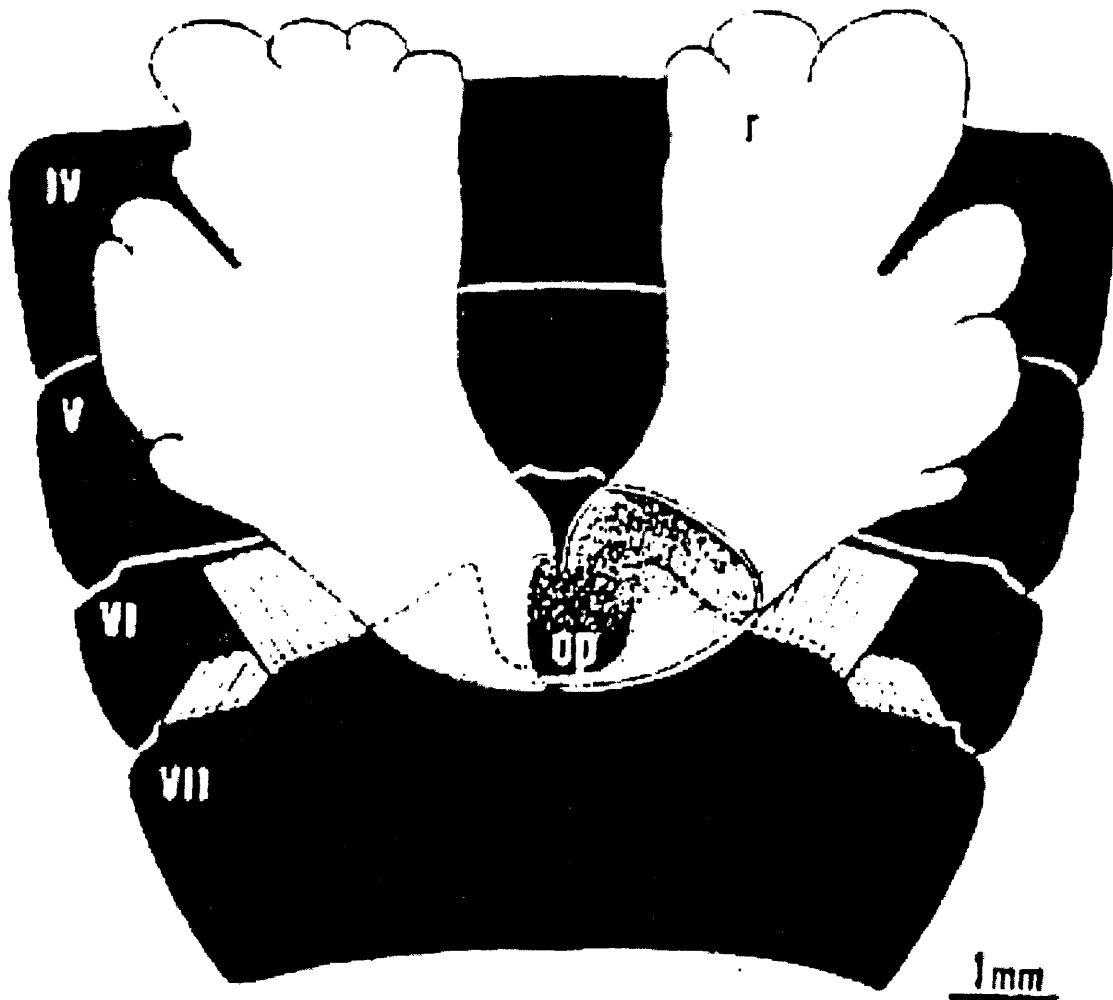


Figure 2. Ventral view of *Eurycotis floridana* sternal gland. Cutaway shows opening of gland (*op*) between VI and VII sternites. Note multiple lobes of full reservoir (*r*) (From Alsop 1970).

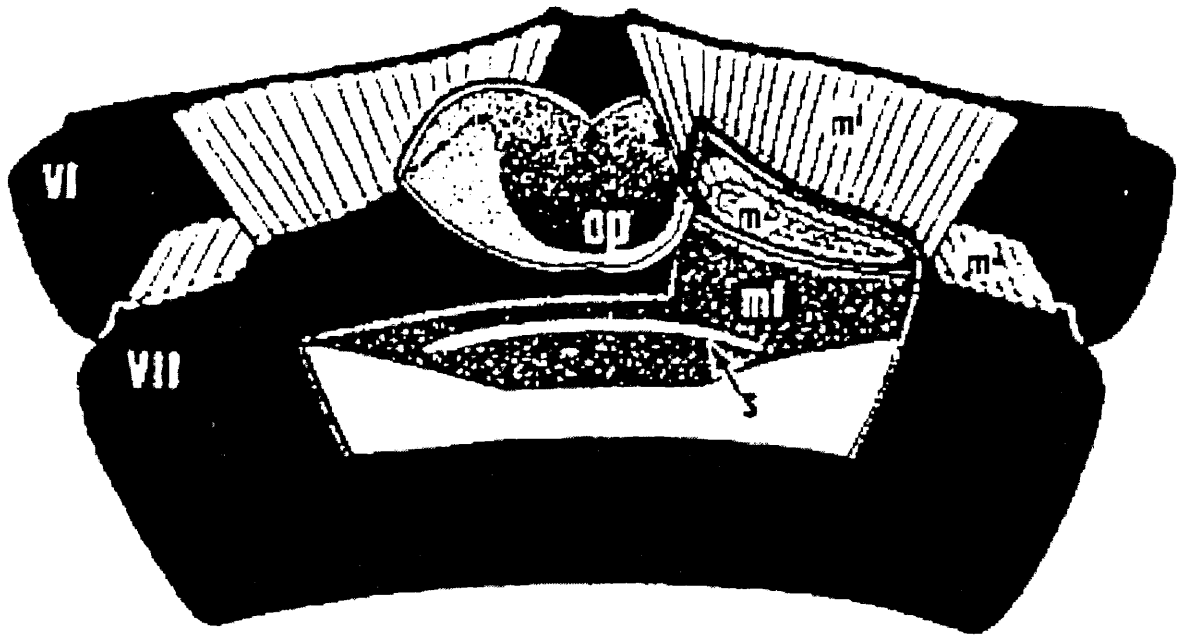


Figure 3. Ventral view of *Eurycotis floridana* sternal gland. Note positions of intersegmental muscles ( $m^1$ ,  $m^2$ ,  $m^3$ ) and the sclerite ( $s$ ) which aids in stiffening the ostiole (From Alsop 1970).

Stay 1957). The outer cellular layer consists of large, columnar secretory cells which open into the gland reservoir. Cuticular tubules pass through the secretory cells, transporting the secretion from multiple cells' secretory organelles to the reservoir (Stay 1957).

### **Ecology of *Eurycotis floridana***

Little is known concerning the ecology of *E. floridana*. Like many cockroaches, the cryptic nature of *E. floridana* makes it difficult to study. Its dark color, along with the lack of general ecological observations in undisturbed habitat, indicates that the roach is nocturnal, only emerging into the open during the day when it is sufficiently disturbed. Laboratory observations also support the probable nocturnal nature of this species. Biotic associations of the cockroach are even less well known. Although wasp and helminthic parasites are known, neither vertebrates nor invertebrates have been observed feeding on *E. floridana* in the wild (for review of the biotic associations of cockroaches see Roth and Willis 1960). This is not uncommon among chemically defended insects, as the predators that a secretion deters in nature are rarely known. This is likely to be due to the efficacy of these compounds in addition to the lack of research effort. Due to this kind of ambiguity, researchers investigating the chemical ecology of terrestrial insects have availed themselves of generalist predators, both invertebrate (most commonly ants of various species) and vertebrate (ground birds, lizards, and occasionally frogs). Investigators also have tended to assume a defensive function for the secretions based on the presence of certain compounds for which the defensive characteristics are known (e.g., (E)-2-hexenal, quinones, and various acids). The result is the cataloguing of supposed defensive compounds based upon assumptions and indirect evidence. Although

these lists may be largely correct, there are probably instances in which such assumptions are false. This trend has persisted in the study of *E. floridana*, in which an observational study by Eisner et al. (1959) is cited as the primary evidence of the defensive capacity of the sternal secretion of the cockroach. The presence of (E)-2-hexenal (Roth et al. 1956) also has been considered evidence that the secretion is defensive in function.

### **Rationale for Study**

The first objective of this study was to corroborate the speculative report of Eisner et al. (1959) concerning the function of the allomonal efficacy of the sternal secretion. The chemistry has been characterized recently (Farine et al. 1997), and nearly every article published on *E. floridana* since that of Eisner et al. (1959) has cited that paper as the seminal work on the chemical ecology of the cockroach. Although the credibility of Eisner is impeccable, it seems improper to rely so much on a pilot study. Therefore, one goal of this study was to test hypotheses about when and how the secretion is used, and whether it does provide a significant deterrence to potential predators. A second goal was motivated by a dearth of statistically-testable quantitative laboratory protocols. To this end, a model was proposed and tested using *E. floridana* as the insect prey, and *Peromyscus leucopus* and *Bufo marinus* as model vertebrate predators. Additionally, *Monomorium pharaonis* and *Periplaneta americana* were used as model invertebrates. Although the use of these species does not necessarily overcome the problem of use of inappropriate predators, a case may be made for the two vertebrates being potential predators in the wild due to range overlap and predatorial habits, in particular *B. marinus* (Roth and Willis 1960).

Finally, this thesis was written with the aim of investigating the biosynthesis of the secretion of *E. floridana* using the primary literature in the field of chemical ecology. Little is known about the de novo biosynthesis of allomonal compounds in insects, even concerning a chemical as prevalent as (E)-2-hexenal. To this end, a literature search was conducted on the production of that compound. The effects of (E)-2-hexenal at both the molecular and cytological level also are discussed, as little is known of its bioactivity in those secretions, even though its presence has been verified in a wide array of defensive compounds (Blum 1981).

## Chapter I

### Efficacy of the Sternal Secretion of *E. floridana* (Dictyoptera: Polyzosteriinae) (Walker) Toward Vertebrates

#### Introduction

No natural predators of *E. floridana* have been observed (Roth and Willis 1960), although hypotheses concerning potential natural predators may be made based on the cockroach's range and behavior. The cane-toad (*Bufo marinus*) has been observed feeding on other cockroach species in the range of *E. floridana* (Roth and Willis 1960, Brossut 1983), as have lizards (e.g., *Anolis spp.*) (Blum 1965, Roth and Willis 1960). Several insectivorous ground birds also are candidates as potential predators. The cockroach's nocturnal nature would make certain nocturnal ground dwelling mammals such as opossum and mice, both of which exhibit range overlap with *E. floridana*, potential predators.

Although Eisner et al. (1959) reported that *E. floridana* secretion appears to be an effective defense against both invertebrates and vertebrates, their study is best considered a pilot study since it lacked important controls. The present study tests whether the secretion produced by adult *E. floridana* functions effectively as a vertebrate deterrent through experiments with model species.

#### Materials and Methods

##### Mouse-Roach Assay

*Eurycotis floridana* were maintained in laboratory cultures and provided water and cat food ad lib. Individuals used in assays were not sexed because preliminary data, using a protocol similar to Barcay and Bennett (1991), suggested that adult males and females do not differ significantly in movement duration and rate. Data also show that

there is no sexual size dimorphism in the adults: males from the colony average 40.0 mm in length, females average 40.5 mm. Additionally, there is no expected difference in spray efficacy; Stay (1957), in an histological study of the adult gland, did not note a size difference in the gland, and Farine et al. (1997) noted the compositional similarity of the secretion in the 2 sexes.

The white-footed mouse, *Peromyscus leucopus*, was used as a model vertebrate predator for 2 primary reasons. Firstly, the mouse's range overlaps that of the cockroach, thereby creating the potential for natural encounters. Secondly, behaviors associated with fear and discomfort have been well catalogued in mice and rats, making such rodents excellent experimental animals for testing the efficacy of an anti-predator spray.

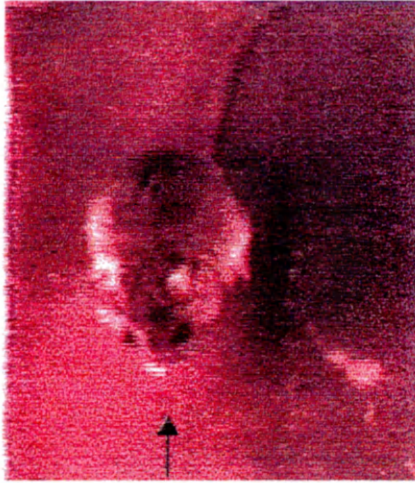
Twenty naive adult male *Peromyscus leucopus* were obtained from a colony at the College Landing Biological Laboratory (Williamsburg, Va.) and maintained on a 16:8 (L:D) h photoperiod, at a temperature of approximately 25°C. Rat chow (Southern States, Williamsburg, Va.) was provided ad lib to the mice during the acclimation and recovery periods, and water was provided ad lib at all times except during the actual assay. Two nights prior to use in a trial, each mouse was presented with large, live crickets (*Acheta domestica*) rather than rat chow. Crickets were used to give the mice experience in attacking and feeding on moving organisms. The first night, 10 crickets were presented – approximately 25%, by dry weight, of a mouse's normal daily intake (Sullivan 1997). The night immediately prior to the assay, the presentation consisted of 4 crickets.

The arena for the experiment consisted of a clear insect terrarium (17 cm w X 31 cm l X 17 cm h) with a clear Plexiglas lid. The inside walls of the aquarium were coated

with fluon (Dupont) from 1 cm to 7 cm above the floor to prevent climbing by the roach. The floor of the aquarium was lined with litmus blue paper (VWR, WLS65285-C); the roach secretion produces a brilliant pink upon reaction with the paper. The arena was placed under a video camera and four 15 Watt overhead fluorescent bulbs, covered with 3 layers of red wrapping cellophane, were used for illumination. All trials were performed during the dark cycle and recorded on videotape for later analysis.

Prior to presentation with an adult *E. floridana*, each mouse was transferred from its holding pen to the arena and allowed a 5-minute acclimation period. The mouse's activities then were videotaped for 10 minutes before a roach was placed in the arena. A single trial consisted of a 10 minute presentation of 1 adult *E. floridana* to 1 mouse. Each mouse was tested only once.

For each trial, various mouse grooming behaviors indicative of behavioral state (Dell'Omo and Alleva 1994, Petruzzi et al. 1995) were noted. Body part groomed – forepaw-mouth, head, body (**Figs. 4-6**) – were recorded, as well as the initiation time and duration of each behavior. Unusual movement data also were recorded: comatose behaviors (similar to sleeping, but only noted following a secretion event during pilot studies), rolling behaviors (as if wiping its body on the ground), and any changes in walking patterns (listing to 1 side, disoriented running patterns, and spastic movements). The rolling behaviors were combined with any disorientation noted in walking patterns, as differentiation between these behaviors was often difficult. Also, observations of physical interactions between the mouse and the roach were recorded, including the time of initiation, type of contact, the initiator of the contact, and the location of the contact on the roach.



**Figure 4. Forepaw-mouth grooming activity in *P. leucopus*.**



**Figure 5. Head grooming behavior in *P. leucopus*.**



**Figure 6. Body grooming behavior in *P. leucopus*.**

Data from 15 trials (5 of the 20 trials did not result in a secretion event) were analyzed using SPSS 8.0 (SPSS Inc. 1998). All grooming data were divided into 5 temporal classes (**Table 2**). The pre-roach presentation class was the initial 10 minutes of videotaping; this class acted as a control to provide baseline data on each mouse's grooming activities. The pre-spray class represented the time between roach presentation and the first secretion event, while the pre-hit class represented any time between an inaccurate secretion event and any contact with the secretion. The final 2 classes represented the 60 seconds immediately following contact with the secretion (the hit class) and any time greater than the 60 seconds following contact with the secretion (post-hit class).

The total time spent exhibiting all the behaviors ( $T_t$ ) and the time each individual behavior was exhibited ( $T_b$ ) were determined for each temporal class; this yielded the proportion of time spent per behavior type, relative to the total time exhibiting all 5 behaviors ( $pT_b = T_b/T_t$ ), for each temporal class. The proportion of each behavior was then analyzed for change across temporal classes, as this was thought to best represent the effect of the secretion on mouse behavior. Analysis was performed via Kruskal-Wallis test, as the data were determined to be heteroscedatic. Multiple comparisons were then performed using the Nemenyi post-hoc test (Zar 1996).

Analysis of contact data was performed using the 4 latter temporal classes defined above. The interval between mouse-roach contacts was analyzed using the Kruskal-Wallis test followed by the Nemenyi post-hoc test, again to determine changes in mouse behavior between temporal classes. Type of contact, initiator of contact, and induction of discharge relative to contact location on the roach were analyzed with a test of

Data Type	Temporal Class and Definition	Data Collected / Analyzed
<b>Mouse Behavior Data</b>	<ul style="list-style-type: none"> <li>• <b>pre-roach:</b> 10 minute control period before roach introduction</li> <li>• <b>pre-spray:</b> period between roach introduction and first spray event</li> <li>• <b>pre-hit:</b> period following discharge that missed hitting mouse</li> <li>• <b>hit:</b> 60 seconds immediately following mouse being hit by roach secretion</li> <li>• <b>post-hit:</b> period greater than 60 seconds following mouse being hit by secretion</li> </ul>	<ul style="list-style-type: none"> <li>• time of initiation of behavior</li> <li>• type of behavior exhibited</li> <li>• duration of behavior</li> </ul>
<b>Mouse-Roach Contact Data</b>	<ul style="list-style-type: none"> <li>• <b>pre-spray:</b> as above</li> <li>• <b>pre-hit:</b> as above</li> <li>• <b>hit:</b> as above</li> <li>• <b>post-hit:</b> as above</li> </ul>	<ul style="list-style-type: none"> <li>• interval between contacts</li> <li>• type of contact</li> <li>• initiator of contact</li> <li>• location of contact on roach</li> </ul>

**Table 2. Categories for Data Analysis of Mouse – Roach Assay.**

independence. Multiple comparisons between contact type were performed using a test of independence and Sidák's adjusted critical values (Rohlf 1995, Sokal and Rohlf 1995).

To ensure that the trends noted over time were not simply caused by lack of interest or movement over time, rate of grooming activities and rate of contact were fitted to regression equations.

### **Toad-Secretion Assay**

A topical irritancy assay was performed to test the efficacy of the spray as an anti-predator agent on *Bufo marinus*. The cane-toad was felt to be a representative predator due to its terrestrial nature, range overlap, and observations that it eats roaches across that range (Roth and Willis 1960). Additionally, it has been well studied both physiologically and ecologically.

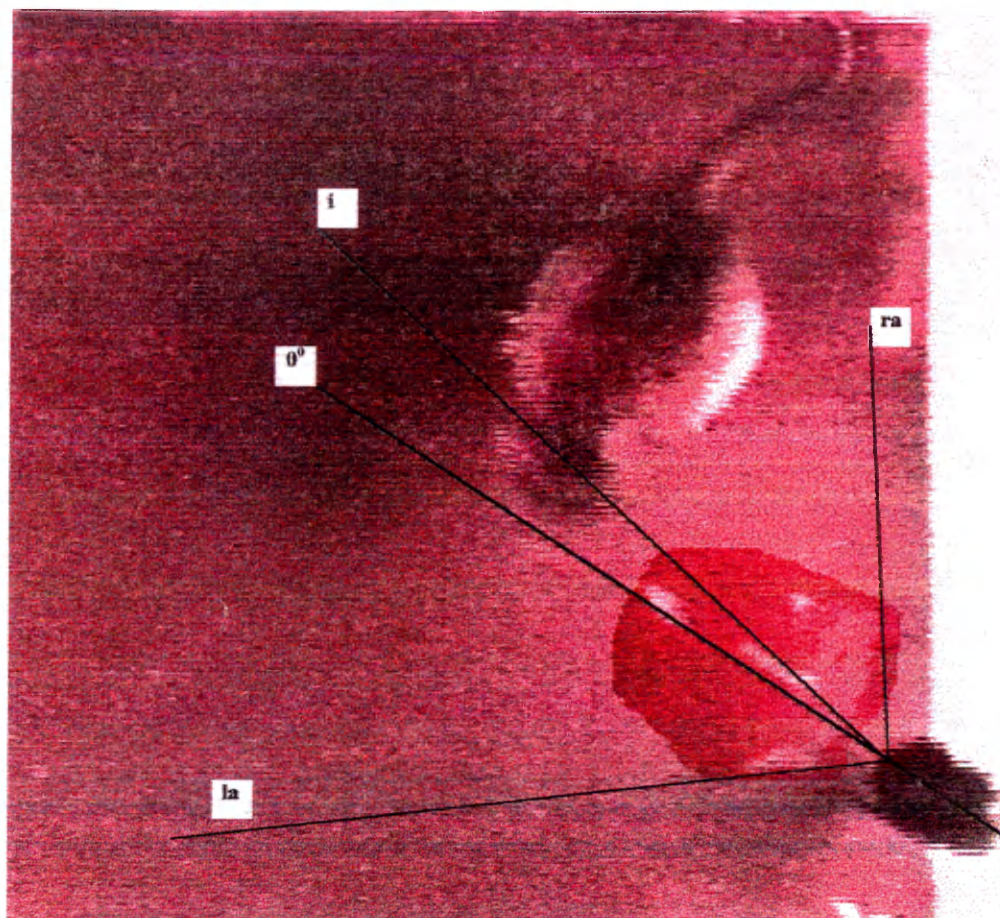
Five naive adult female toads (Carolina Biological) were used, each tested individually. The test chamber used for the assay was the same as that described above. A toad was placed in the chamber and allowed 5 minutes to acclimate. A camel-hair brush was used to paint distilled water along the upper ridge of its mouth and around its nostrils. Care was taken to ensure that the brush did not contact the eyes. After 5 minutes, a second camel-hair brush was used to paint freshly-obtained *E. floridana* secretion onto the same areas. Secretion was obtained by subjecting adult *E. floridana* to agitation of the abdomen and metathoracic legs, and was used quickly (<30 seconds) to minimize loss of volatile components. A toad was scored as responding positively to a solution if it performed a vigorous rubbing motion with its front legs across its eyes and mouth. Any behavior implying discomfort in the toad also was noted.

### Observations on Accuracy

To ascertain how accurately *E. floridana* aims its secretion in relation to offending stimuli, data from 32 secretion events from 12 trials were analyzed. The protocol used was similar to that described above (i.e., an adult roach was presented to a hungry *P. leucopus* in a fluon-coated aquarium). The trials were videotaped for later analysis.

To standardize observations of angle and distance, all measurements of degree were made according to the orientation of the roach's body (**Fig. 7**), with the line from the posterior end of the abdomen to the front of the abdomen considered  $0^\circ$ . As the roach frequently bent its body during and following a secretion event, this vector was extrapolated from the midpoint of the abdomen to the posterior when necessary. The posterior tip of the abdomen was utilized as the origin in all cases. Once angle =  $0^\circ$  and the origin were determined, a measurement was made determining the orientation of the mouse's contact. The side of the angle running through the tip of the mouse's nose was considered an accurate describer of angle of attack incidence, as the mouse's nose was the most frequently seen type of contact and the spray was found to be most effective when sprayed in the face. The mean angle of secretion coverage was determined from the two widest secretion marking. Therefore, the mean angle was a conservative estimate of the accuracy, as the area of effect usually was considerably larger.

The angle data were determined to be non-normal. Spearman's rank correlation therefore was used to analyze the data to determine whether the angle of incidence and the mean angle were correlated. Five secretion observations were subsequently discarded because the roach was attempting to climb the chamber wall when discharge



**Figure 7. Method of determining distance of discharge and angles of orientation. la – left leg of coverage angle; ra – right leg of coverage angle;  $0^0$  – origin of angle; i – incidence of attack (note – mouse has not moved into final attack position; darker red area represents false image inserted for purpose of demonstration of method).**

occurred. As the walls of the tank were coated with fluon, the roach was unable to leave the floor, thereby preventing it from accurately spraying behind itself due to its vertical orientation.

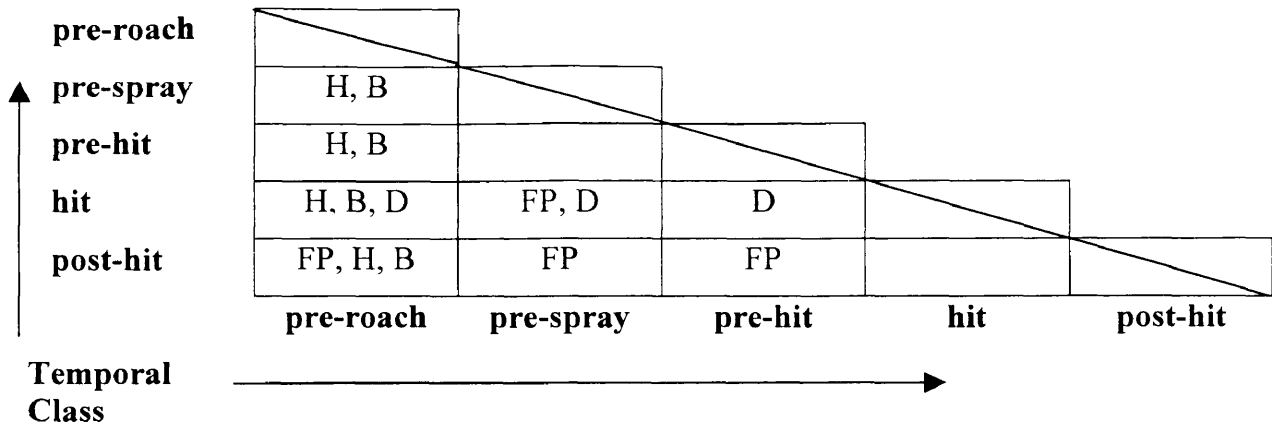
The spray distances attained in the 32 observations also were determined. However, in 23 of the spray events, the roach was either vertically oriented as it attempted to climb the tank wall, or the secretion hit the wall or the mouse. Therefore, only 9 of the secretion events were actually considered to be indicative of the distance attainable. All measurements of distance originated at the posterior tip of the abdomen, and the distance to the furthest visible mark recorded.

## Results

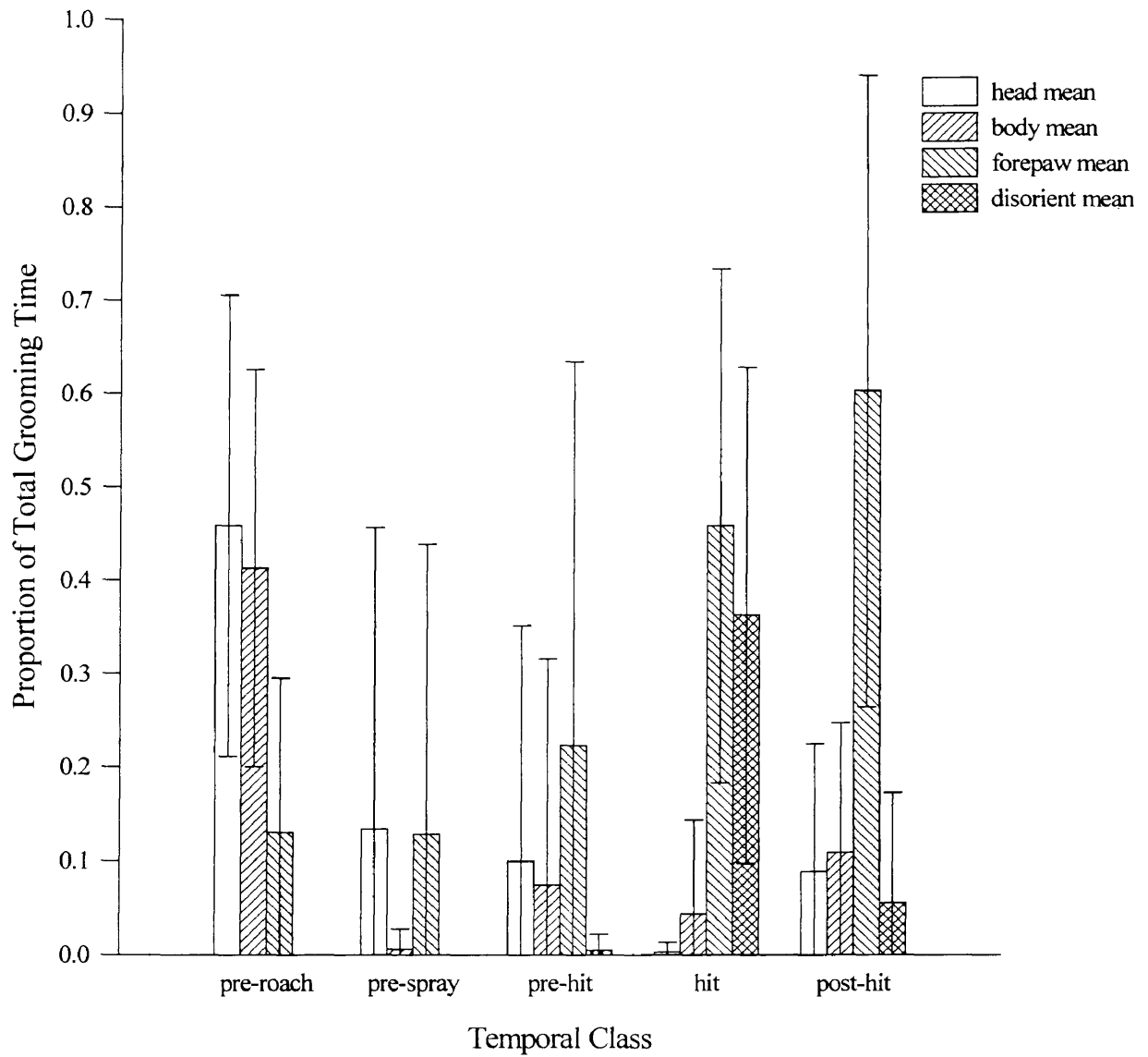
Means of the proportional grooming data, analyzed across temporal classes (i.e., in comparison to whether the roach had sprayed its secretion and whether the secretion contacted the mouse), are presented in **Table 3**. Although mean ranks, rather than population means, were used in statistical analysis, the means are indicative of the trends present in mouse behaviors. Nemenyi's post-hoc test for multiple comparisons indicated significant differences in grooming behaviors ( $\alpha = 0.05$ ) (**Table 4**), especially between the post-hit temporal class and the other temporal classes. The hit class grooming differed as well, though not as often. The 2 baseline grooming behaviors, head and body grooming, were both found to occur most often during the 10 minute control period. Not surprisingly, the two behaviors hypothesized to be indicative of discomfort, disoriented walking and forepaw grooming, were found to occur most frequently in the hit and the post-hit periods, respectively (**Fig. 8**). Only 1 mouse exhibited the comatose behavior, and then for only 10 seconds of the 1 minute of hit class grooming behaviors.

	<b>pre-roach</b>	<b>pre-spray</b>	<b>pre-hit</b>	<b>hit</b>	<b>post-hit</b>
<b>forepaw-mouth</b>	0.130	0.128	0.222	0.458	0.603
<b>head</b>	0.458	0.133	0.099	0.003	0.088
<b>body</b>	0.412	0.006	0.074	0.043	0.109
<b>disorientation</b>	0.000	0.000	0.004	0.362	0.056

**Table 3. Mean proportions of time exhibiting behaviors in each temporal class (n = 15 for each case).**



**Table 4. Results of Nemenyi's post-hoc test for multiple comparisons of proportional grooming data. Abbreviation indicates temporal classes are significantly different at 0.05 for behavior noted; H – head, B – body, FP – forepaw, D – disorientation**



**Figure 8. Division of behavioral activities by temporal class (bars represent standard deviation).**

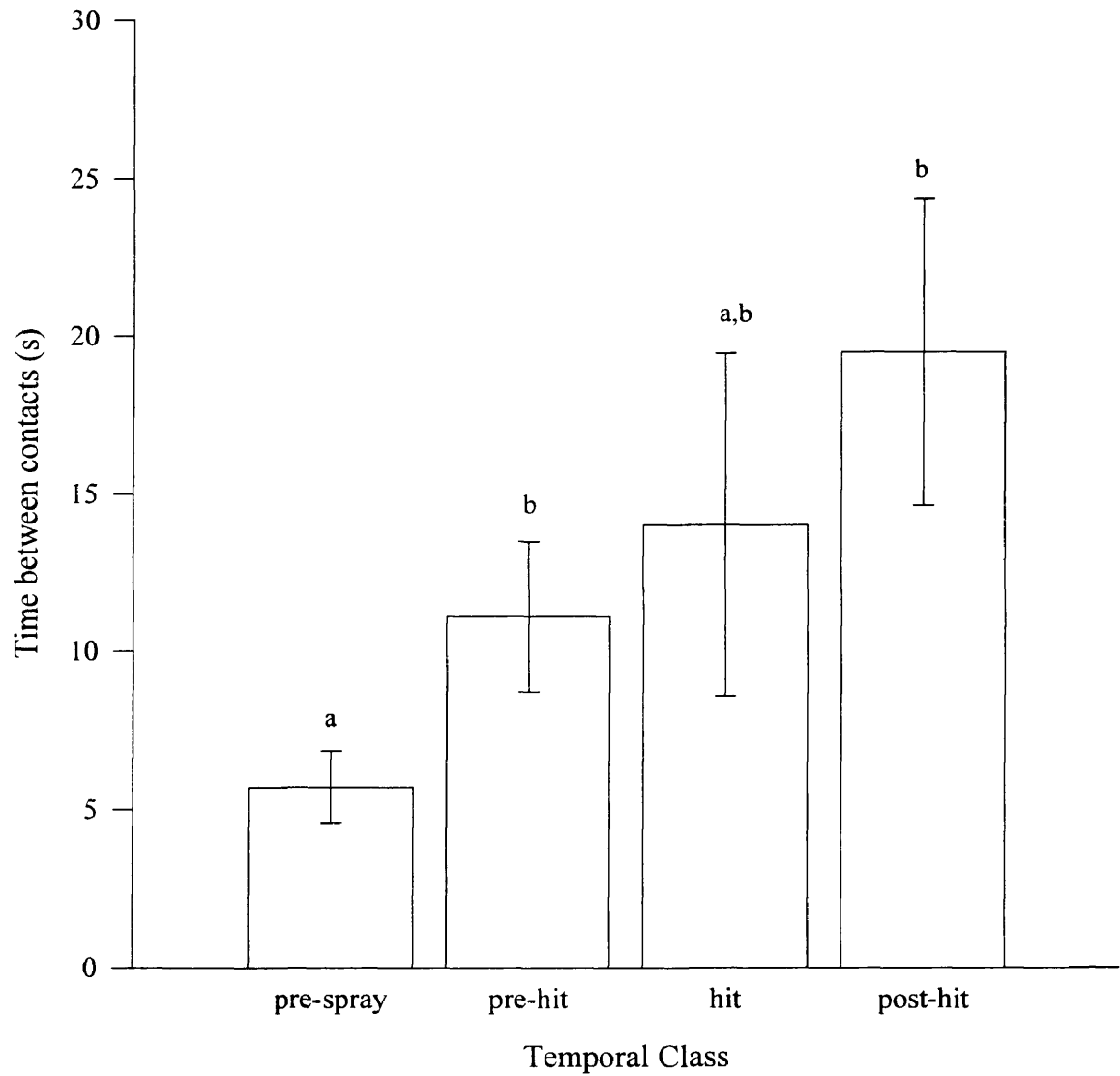
To determine whether the trend observed was due primarily to the influence of the secretion rather than initiation time, the proportion of time spent grooming per minute was fitted to a regression. Although quadratic regression ( $Y = -0.276X^2 + 2.4697X + 7.4833$ ) was significant ( $p = 0.043$ ), an  $r^2$  of 0.042 indicated that little of the variation was explained by the passage of time.

The mean interval between contacts within temporal classes followed the pattern expected (i.e., pre-spray had the shortest interval, then pre-hit, then hit, then post-hit) (**Fig. 9**), although the mean ranks did not follow that trend (**Table 5**). The result was a significant difference ( $\alpha = 0.05$ ) between the pre-spray and pre-hit temporal classes, as well as between the pre-spray and post-hit class, but the differences between the hit class and the others were not significant (**Table 6**).

To ensure that changes in contact were not related primarily to the progression of time, the intervals between contacts were plotted as a function of initiation time. Quadratic regression ( $Y = 0.00001X^2 + 0.0299X + 5.6504$ ) reveals a significant increase in interval over time ( $p < 0.005$ ), but the low  $r^2$  value (0.0501) indicates the low level of influence of initiation time over interval between contacts.

There was an increased proportion of fore-paw slaps following secretion events (**Table 7**); the test of independence demonstrated this difference to be highly significant ( $p < 0.0005$ ) (**Table 8; Fig. 10**). Multiple comparisons using Sidák's adjusted critical value ( $X^2_{0.05,6,3} = 11.693$ ) revealed the post-hit temporal class to be significantly different from both the pre-spray ( $G_H = 11.903$ ) and the pre-hit ( $G_H = 17.368$ ) classes.

A test of independence on the contact-initiator data revealed that the difference between temporal classes was not significant (Chi-square = 6.502,  $p = 0.090$ ). The



**Figure 9. Mean interval between contacts (bars represent 95% C.I.; columns with same letter not significantly different at  $\alpha = 0.05$ )**

<b>Temporal Class</b>	<b>N</b>	<b>Mean (s)</b>	<b>Mean Rank</b>
<b>pre-spray</b>	108	5.69	209.23
<b>pre-hit</b>	112	11.09	276.40
<b>hit</b>	60	14.00	260.77
<b>post-hit</b>	237	19.49	273.01
<b>Total</b>	517		

**Table 5. Descriptive statistics of interval between contacts per temporal class.**

<b>comparison</b>	<b>SE</b>	<b>Q</b>
<b>pre-spray : pre-hit</b>	20.085	3.344*
<b>pre-spray : hit</b>	23.980	2.149
<b>pre-spray : post-hit</b>	17.290	3.689*
<b>pre-hit : hit</b>	23.826	0.656
<b>pre-hit : post-hit</b>	17.077	0.198
<b>hit : post-hit</b>	21.523	0.569

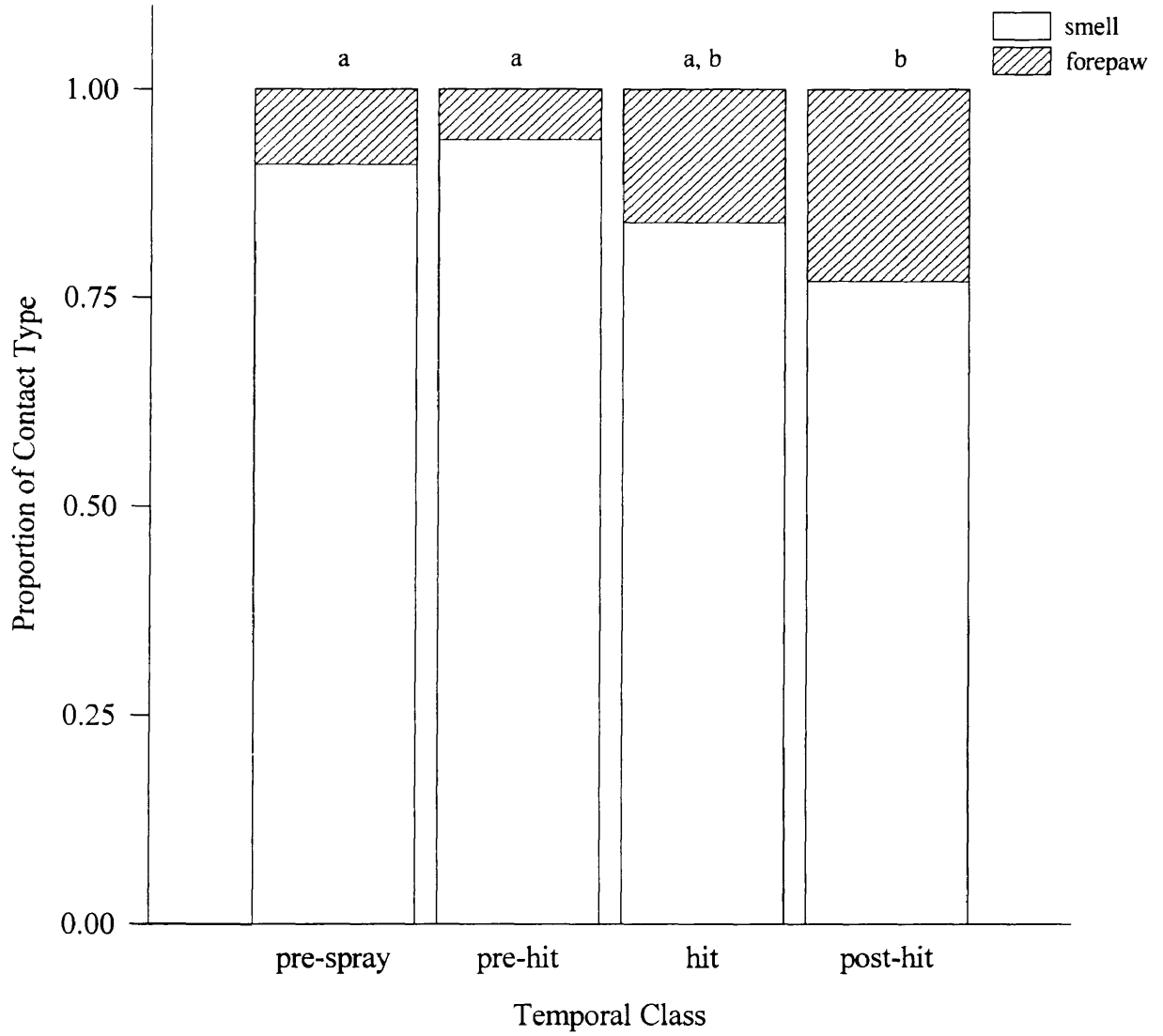
**Table 6. Results of Nemenyi's post-hoc test for multiple comparisons of interval between contacts per temporal class ( $Q_{0.05,4} = 2.639$ ; \* - data significantly different at  $p < 0.05$ )**

<b>Temporal Class</b>	<b>Contact Type</b>		
	$n_{\text{Smell}}$	$p_i \text{ smell}$	$n_{\text{Forepaw}}$
<b>pre-spray</b>	111	0.91	11
<b>pre-hit</b>	105	0.94	7
<b>hit</b>	51	0.84	10
<b>post-hit</b>	182	0.77	55
<b>Total</b>	449	0.84	83

**Table 7. Number and proportion of contact types per temporal class.**

<b>Comparison</b>	<b>G<sub>H</sub></b>
pre-spray : pre-hit	0.636
pre-spray : hit	2.080
pre-spray : post-hit	11.903*
pre-hit : hit	4.357
pre-hit : post-hit	17.368*
hit : post-hit	1.389

**Table 8. Results of the simultaneous test procedure for multiple comparisons of contact type data (Sidák's adjusted  $X^2_{0.05,6,3}$  for unplanned multiple comparisons = 11.693; \* - data significantly different at  $\alpha = 0.05$ ).**



**Figure 10. Proportion of contact type per temporal class (columns with same letter not significantly different at  $\alpha = 0.05$ ).**

means do exhibit a reduction in mouse-initiated contacts over time, as expected (**Table 9; Fig. 11**). This appears to be due, at least in part, to the overall reduction in contact rate, as the mouse often fled from the roach (**Fig. 12**) following secretion events.

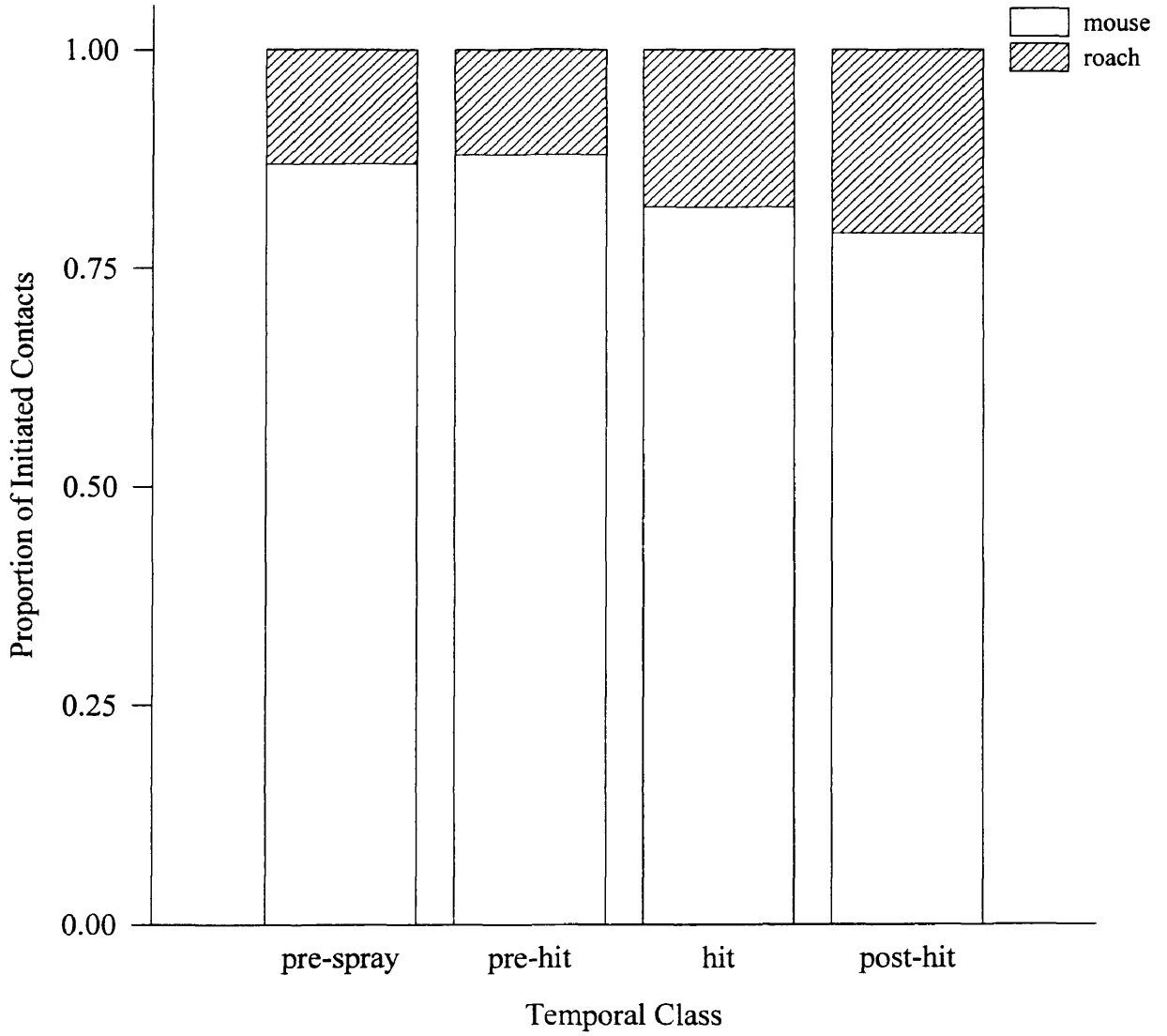
The location of contact was found to significantly influence whether the roach released its secretion. As expected, contact with the posterior of *E. floridana* resulted in a significantly greater incidence of spraying than contact with the head, thorax, or side of the abdomen (**Table 10; Fig. 13**).

All 5 of the toads reacted adversely to the secretion by wiping their faces vigorously, whereas 0 reacted adversely to the water application. In addition, the toads often responded to the secretion by tightly closing their eyes. In most cases they moved to a corner of the testing chamber, closed their eyes, and rubbed their heads against the walls for 3 minutes or longer.

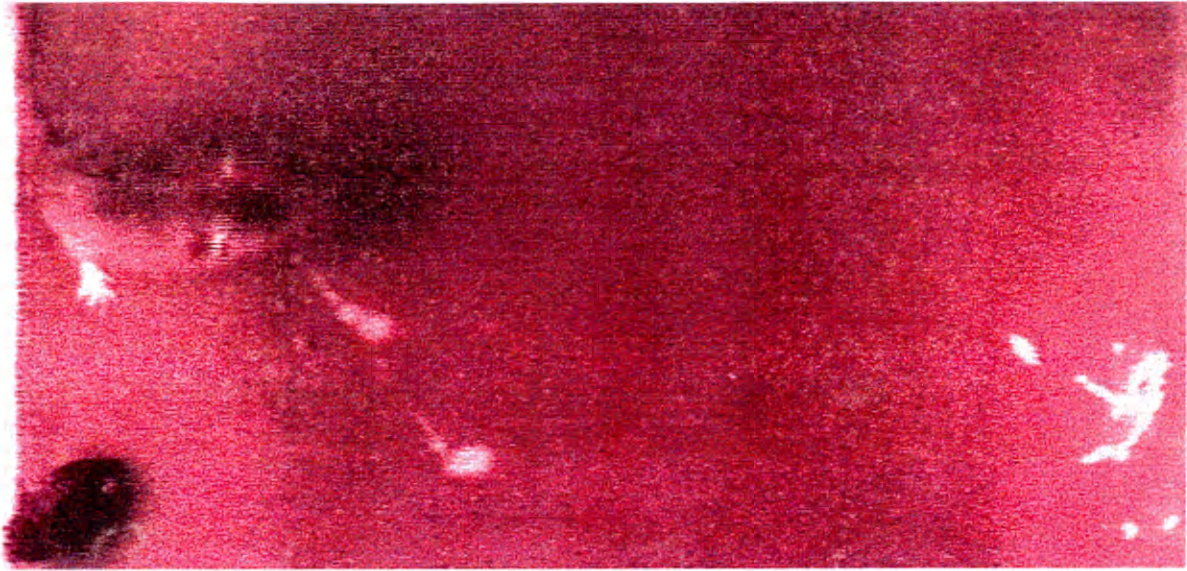
The roach was found to be capable of significant accuracy in spraying its secretion: the mean angle of coverage was found to be positively correlated with the angle of stimulus incidence (Spearman's  $r = 0.706$ ;  $p < 0.001$ ). However, the use of the mean angle of secretion coverage must be considered conservative, as the secretion frequently contacted the mouse when the angle of attack differed greatly from the mean angle. In the observations made ( $n = 27$ ), the mouse was hit by the secretion 19 times. Furthermore, in the mouse-presentation assay described above, 25 of 44 spray events hit the mouse in the face-head region. The maximum spray distance observed in these trials was 210 mm. In a separate assay, performed on a flat table, the maximum spray distance never exceeded 200 mm ( $n = 10$ ).

<b>Temporal Class</b>	<b>Initiator</b>		
	$n_{\text{Mouse}}$	$p_i \text{ mouse}$	$n_{\text{Roach}}$
<b>pre-spray</b>	106	0.87	16
<b>pre-hit</b>	99	0.88	13
<b>hit</b>	50	0.82	11
<b>post-hit</b>	187	0.79	50
<b>Total</b>	432	0.83	90

**Table 9. Number and proportion of contact initiator per temporal class.**



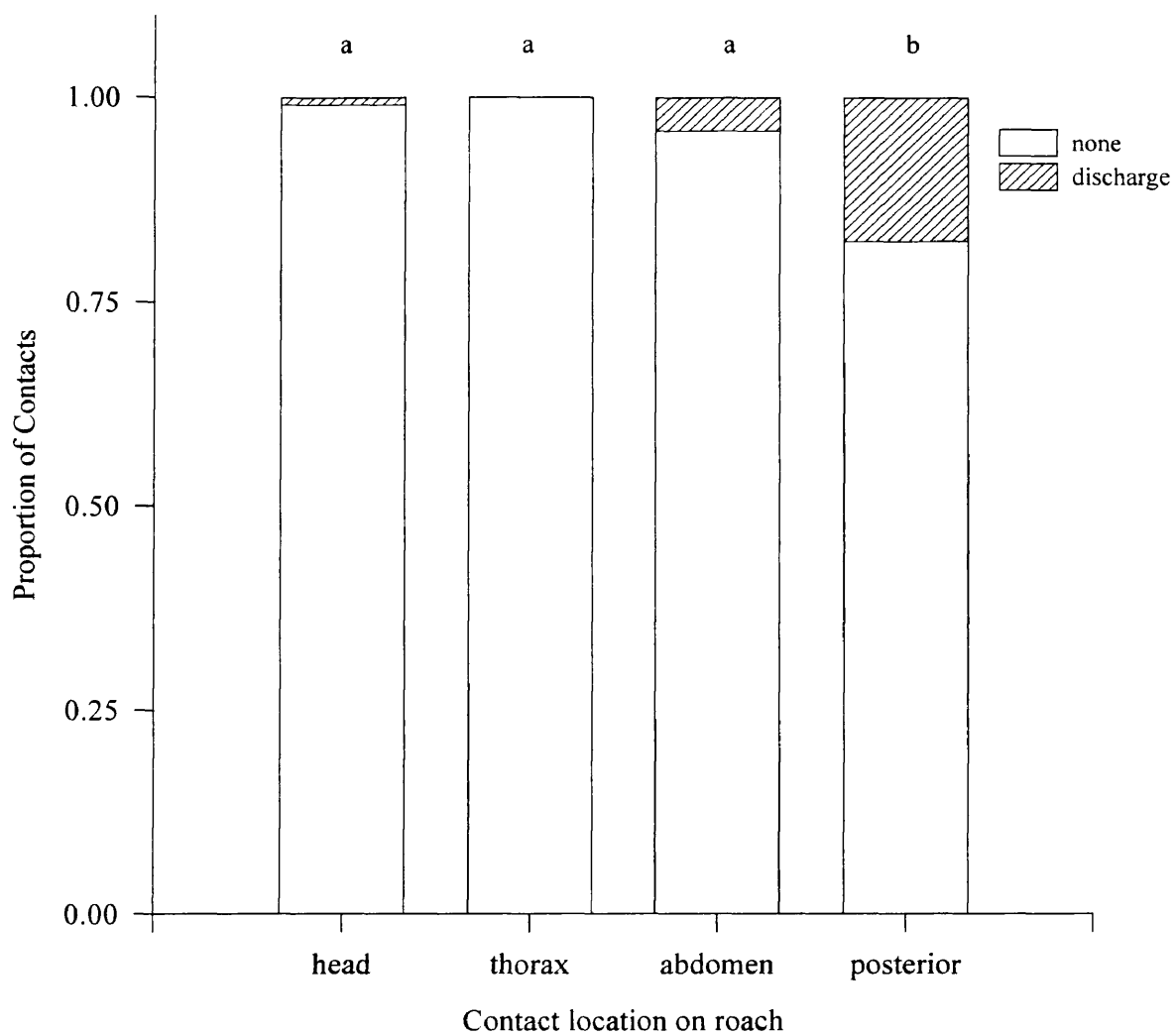
**Figure 11. Proportion of contact initiator by temporal class.**



**Figure 12. Typical fear-induced evasion of roach by mouse, following contact with the sternal secretion.**

<b>Location</b>	<b>no discharge</b>	<b>discharge</b>
<b>head</b>	119 / 112.8	1 / 7.2
<b>thorax</b>	141 / 132.5	0 / 8.5
<b>lateral abdomen</b>	118 / 115.6	5 / 7.4
<b>posterior</b>	122 / 139.1	26 / 8.9
<b>Total</b>	500	32

**Table 10. Relation of location of contact to induction of spray event (obs / exp)  
(p = 0.00)**



**Figure 13. Proportion of contact events leading to secretion, by contact location (columns with same letter not significantly different at  $\alpha = 0.05$ )**

## Discussion

Although the chemical composition of many supposed defensive secretions has been determined by researchers, the biology of many of the insect species producing the secretions has been ignored. As the predators are not known in many instances, the actual efficacy of the chemical compounds as defensive tools often is inferred based on the classes of chemicals that are present. Therefore, most research has been correlative work rather than actual controlled experimentation. This has been the case with *E. floridana*. The experiments that were run were performed to redress this.

The data provide evidence that supports the conclusions of Eisner et al. (1957): the secretion of *E. floridana* provides an effective vertebrate deterrent. Many of the symptoms induced by the spray are detrimental to the attacking vertebrate; in particular, the disorientation-effected vulnerability would impose costs. Additionally, the disorientation and discomfort felt by the predator would provide the roach with time and opportunity to escape. The accuracy and distance attainable increases the efficacy of the secretion. Although the observed spray distances of approximately 200 mm are more similar to those reported by Roth et al. (1956) than to the yard reported by Eisner et al. (1959), it is still better than reliance on a passive contact defense. Moreover, an inaccurate spray is not necessarily a wasted one; some aversion is caused by the odor. This makes it tempting to hypothesize that *E. floridana* is able to utilize its secretion as an aposematic warning (for discussion see Eisner and Grant 1981), especially as Farine et al. (1994) have reported the presence of secretion on the epicuticle surrounding the duct of unprovoked adults. The fact that the secretion is aversive to both mice and toads, with

fairly disparate physiological systems and receptors, increases the possibility that the secretion would be effective in warding off a potential predator.

It is interesting to note that, although none of the mice ate the roaches presented in these trials, naive and experienced mice have been observed to eat adult *E. floridana* in the lab ( $n = 6$ ), even after being hit by the secretion ( $n = 3$ ). It is also interesting that much variation was observed in sensitivity of the roaches to disturbance: many times a secretion was only incited after repeated disturbance, while only 1 contact was required in other instances.

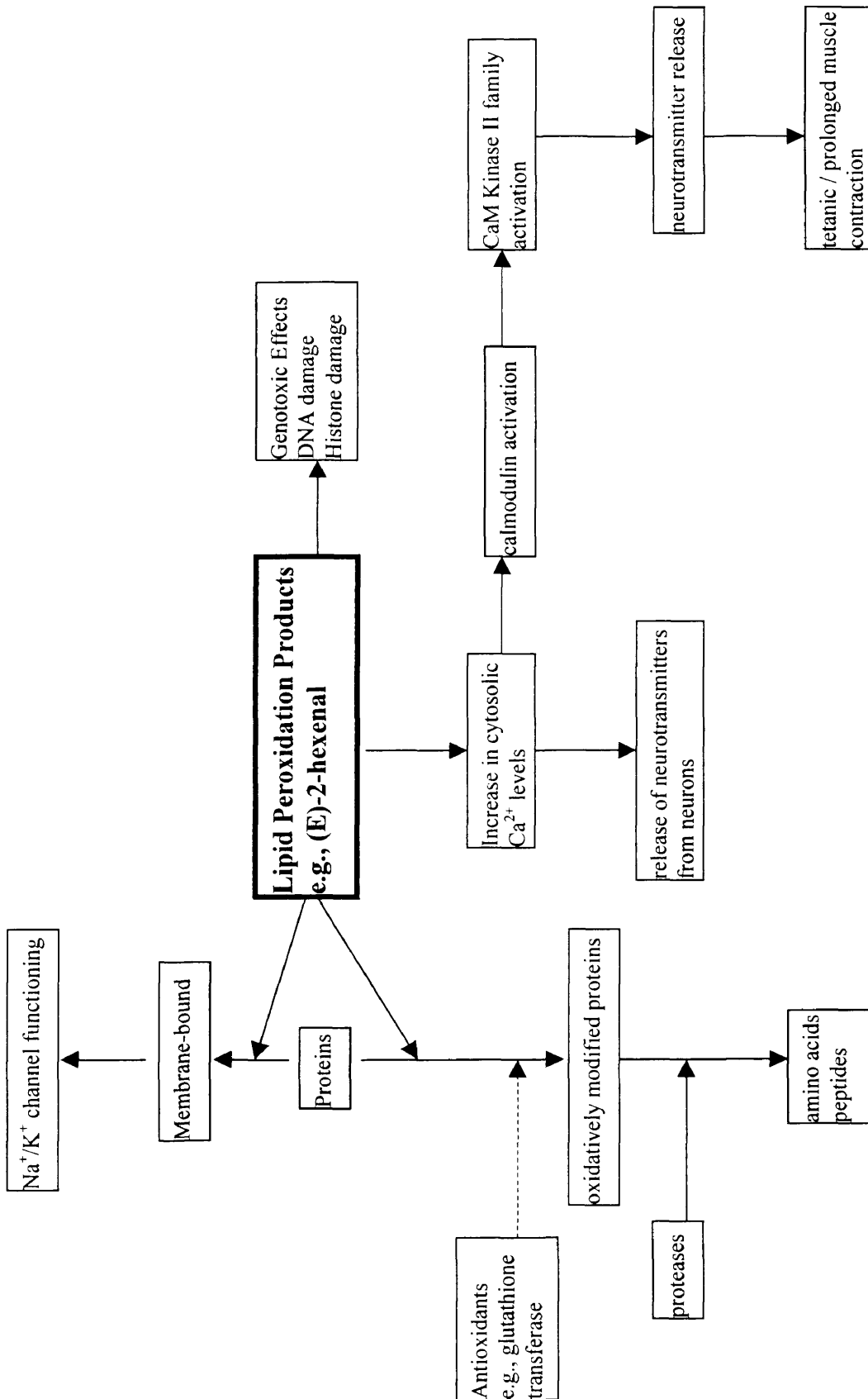
The regression analyses demonstrate that the passage of time affects both the amount of grooming and the time between contacts, however, the effect of time is quite small relative to the presence of the secretion. Therefore, the observed changes in mouse behavior are probably due to the effects of the secretion, rather than experimental artifacts.

The high concentration of (E)-2-hexenal in the organic layer of the secretion (greater than 90%; Farine et al. 1997) would appear to implicate it as the major bioactive molecule of the secretion. Furthermore, the alkenal is very widespread in both insects (Blum 1981) and plants (Karban and Baldwin.1995, Kasu et al. 1995) and is known to be very damaging to organic tissues. Following cellular uptake, (E)-2-hexenal has been shown to increase cytosolic  $\text{Ca}^{2+}$  levels by inhibiting extramitochondrial  $\text{Ca}^{2+}$  sequestration (Griffin and Segall 1989), which has cytotoxic consequences (Nicotera et al. 1988, Thor et al. 1984). Higher  $\text{Ca}^{2+}$  levels also would affect the activities of several proteins and enzymes. The seizures seen in vertebrates could easily be explained by

activation of  $\text{Ca}^{2+}$ /calmodulin mediated protein kinases, including the CaM kinase II family, which affect neurotransmitter release (Cooper 1997).

The seizure-like symptoms, along with a general lack of coordination, could also be explained by the action of the alkenal on membrane-bound receptor molecules, particularly at synapses. Aldehydes (including hexanal, a saturated aldehyde also found in the secretion of *E. floridana* (Farine et al. 1997)) are capable of interfering with gap junction intercellular communication (Seliverstov 1984). One hypothesis put forth is that the aldehydes bind to receptor proteins, thereby changing their conformation and thus altering the ability of the neuron to hyperpolarize (Blum 1978, de Haan et al. 1994). This effect, although obtained with human smooth muscles, also should be applicable at the synaptic junctions of insects. **Figure 14** summarizes some effects of the alkenal.

As there are 40 compounds present in the organic phase of the secretion alone (Farine et al. 1997), it would be unwise to assume that all of the reactions observed in the mice and toads are due to (E)-2-hexenal. It is likely that several of the molecules are by-products of the biosynthesis of (E)-2-hexenal (Wallbank and Waterhouse 1970), and are relatively inactive biologically. However, many others probably fulfill one or more roles as accessory molecules by aiding in spread of the secretion, reducing its volatility and thereby increasing its half-life, or by causing tissue damage. Hexanal and hexenol, lipid peroxidation products also present in the secretion, both fall into the latter category (Berlett and Stadtman 1997). The presence of so many compounds in a defensive solution no doubt increases the efficacy of the solution by acting on many different physiological systems, thus increasing the range of predatory species deterred. The large range of compounds also provides the possibility for a kind of sensory white noise. The



**Figure 14. Effects of the alkenal (E)-2-hexenal on biological systems.**

many airborne and contacting chemicals may overload the predator's sensory system by stimulating the transmission of many uncoded signals into the central nervous system, effectively creating a chemical smokescreen under which the roach may flee (Blum 1981). In all likelihood the chemicals also act on nociceptors, at neuromuscular junctions, and on other physiological systems in addition to the afferent nervous system.

Although both vertebrates tested were very disconcerted by contact with the secretion, it is not improbable that some predators might not be. The roach *Pelmatosilpha coriacea* has been shown to be edible to the lizard *Anolis cristatellus* (Blum 1965), even though (E)-2-hexenal is a primary component of its secretion. It appears, however, that the secretion produced and borne in the ventral abdominal gland of *E. floridana* provides an effective defense against potential vertebrate predators. Although the use of *P. leucopus* and *B. marinus* is not predicated on field observations of predation by those two animals, they are effective model organisms. Their distributions overlap that of *E. floridana*, and their willingness, when naive, to antagonize and eat adult *E. floridana* creates effective experimental organisms. The strong reactions exhibited by both organisms to the secretion, in response to both contact and olfaction, demonstrate its effectiveness as a deterrent. The wide range of symptoms exhibited are detrimental to a potential predator. Short-term symptoms such as disorientation, discomfort, and the possibility of emesis due to eating of the gland could all affect learning in the predator, particularly in vertebrates (e.g., *P. leucopus* and *B. marinus*). Also, the long-term effects of a chemical as potent as (E)-2-hexenal should not be ignored. Although the genotoxic (Goelzer et al. 1996) and cytotoxic effects of (E)-2-hexenal are unlikely to cause much damage over the course of one, or even several,

contacts, continued intake would require specialized handling to avoid long-term detrimental effects.

## Chapter II

### Effectiveness of *E. floridana* Secretion Toward Invertebrates.

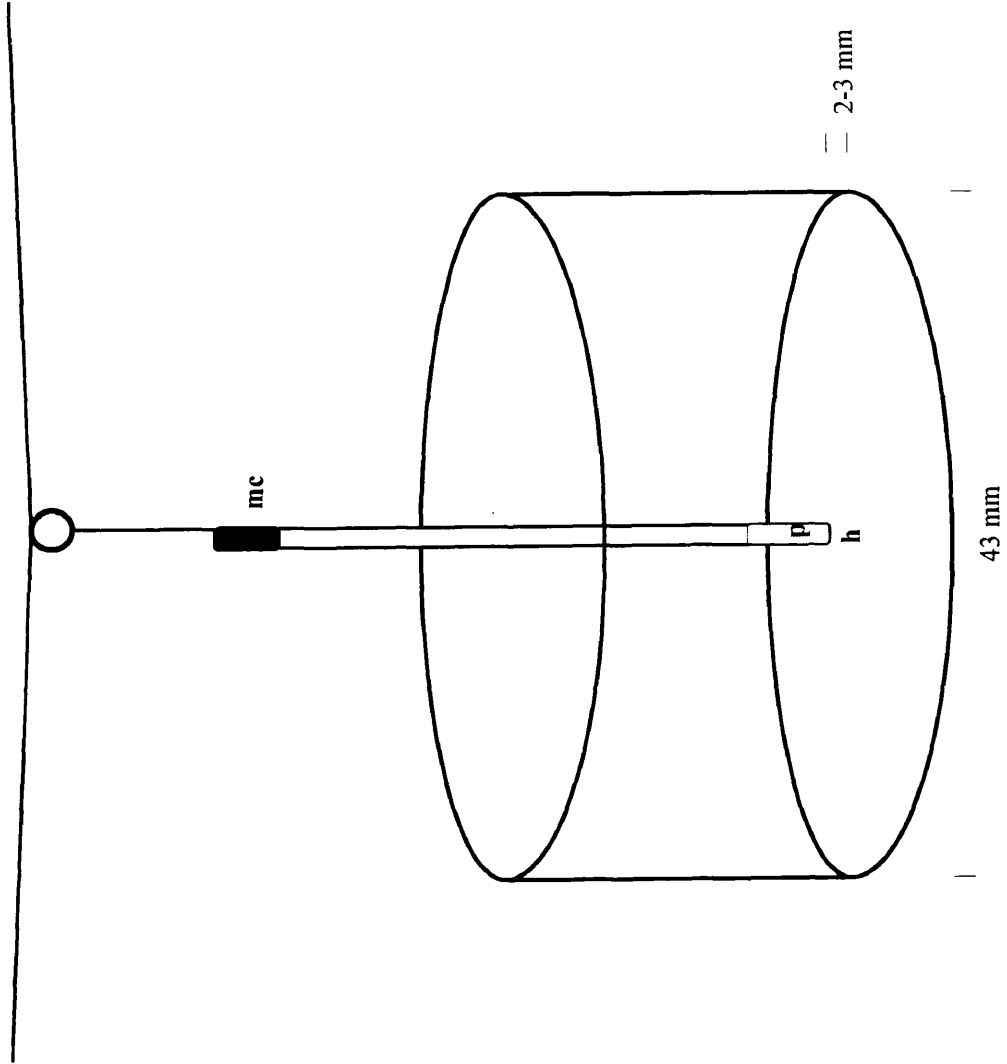
#### Introduction

Although the natural vertebrate and invertebrate predators of *E. floridana* are unknown, it is probable that ants are likely to comprise a large part of the invertebrate predatory pressure exerted on the roach. Several species of ants overlapping in distribution with *E. floridana* (e.g., *Solenopsis* spp., *Iridomyrmex* spp., and *Camponotus* spp.) are known to be extremely aggressive toward insects. To determine whether the secretion of *E. floridana* functions as an invertebrate deterrent, 2 assays were performed. The first, using ants, investigated whether the secretion is an effective olfactory deterrent. The second investigated whether the secretion is a functional contact deterrent.

#### Materials and Methods

##### Ant Repellency Assay

The arena used for this assay (**Fig. 15**) consisted of a small ( $r = 43$  mm) watch-bowl placed under a video camera. The floor of the arena consisted of a clean piece of white poster-board with a small droplet of honey placed directly at the center of the circle. Walls were coated with fluon to prevent climbing by the ants. Distilled water and secretion were presented to the ants using capillary tubes (Fisher 02-668-68) suspended perpendicularly over the honey. This was accomplished by adhering string to 1 end of the microcapillary tube (bore diameter = 1.1 – 1.2 mm) with modeling clay. The other end of the string was tied to a hook, which acted as a hanger on a zip-line over the arena. This setup allowed quick and precise compound presentations directly over the honey, at a uniform height of 2 mm. Each capillary tube's height and placement over the honey



**Figure 15.** Setup for *M. pharaonis* repellency assay (h – honey; mc – modeling clay; p – presentation compound).

were verified at the onset of each trial to ensure uniform presentation between and within trials.

Approximately 15 pharaoh ants (*Monomorium pharaonis*) were placed into the arena in each trial. The ants were given 5 to 7 minutes to become acclimated to the arena and begin feeding at the honey. The perimeter of the circle of honey appeared to be the limiting factor on the number of ants that were present: the ants that left frequently were observed to be replaced by other ants, and often returned to feed again.

Following the acclimation period, the capillary tubes were presented. Presentation order always consisted of the following: (1) an empty tube, (2) 10  $\mu$ l of distilled water, (3) 10  $\mu$ l of secretion. Each presentation was 30 seconds in duration, followed by a 2 minute reacclimation period before presentation of the next tube. The trials were recorded on video tape for later analysis.

The number of ants feeding at the honey (an ant was characterized as feeding if it was oriented toward the honey, within feeding range) was recorded at 5 second intervals to 30 seconds for each trial. The proportion of ants feeding at each interval,  $t_i$ , was then determined compared to the number present at the time of initial presentation,  $t_0$  (i.e.,  $p_i = n_{t_i} / n_{t_0}$ ).

As data were found to be heteroscedatic, the Kruskal-Wallis test was used, and multiple comparisons were performed via Nemenyi's post-hoc test. Since the data demonstrated little change in proportion of ants feeding following  $t_5$ , only the  $t_0$  to  $t_5$  interval was used in the final analysis.

### **Topical Irritancy Assay**

A modification of the method used by Peschke and Eisner (1987) was used to determine the efficacy of the secretion as a topical irritant of insects. Adult American roaches (*Periplaneta americana*), adult *E. floridana*, and juvenile *E. floridana* were decapitated by razor blade. The wounds were sealed immediately with dental wax and the roaches given a 90-minute acclimation period to adjust to their new state. There appeared to be little loss of viability over the acclimation period, as the roaches were still active 48 hours following decapitation.

Each trial consisted of the application of distilled water to both mesothoracic legs, a 30 second observation period, a 5 minute interval, and then application of *E. floridana* secretion to the mesothoracic legs. The two compounds were gently applied using camel-hair brushes. A roach was scored as having reacted positively if either leg was brought forward to where the head would be in an intact roach. In an intact roach, this attempted grooming behavior is indicative of an attempt to remove an irritant (Eisner 1961).

Data were analyzed in Minitab 12.0 (Minitab Inc. 1998) using a test of independence.

### **Results and Discussion**

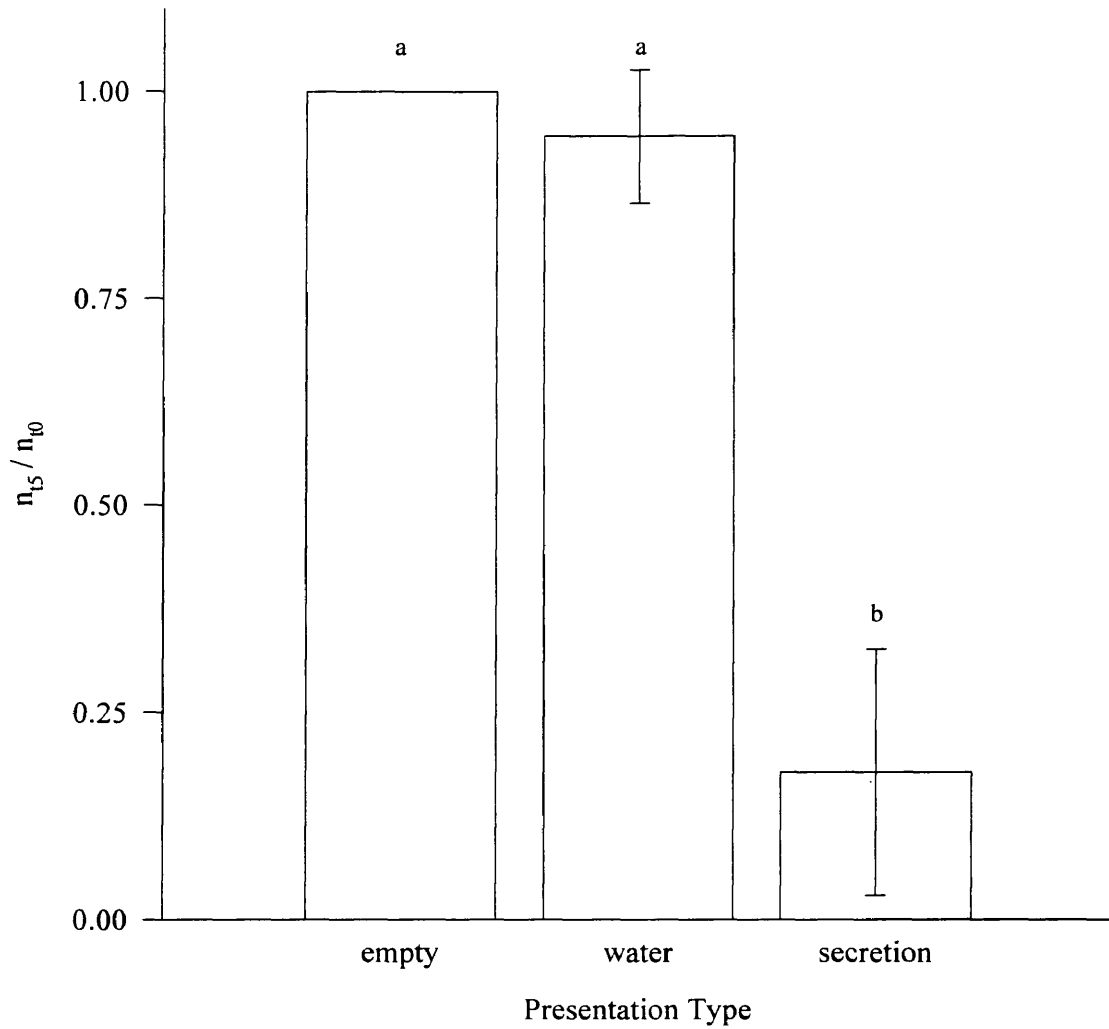
Several studies have shown that the presence of an odorous or topically irritating compound at a potential food source can function to repel ants (Jefson et al. 1983, Peschke and Eisner 1987, Eisner et al. 1976). Analysis of the proportion of ants remaining at a potential food source following presentation of various compounds reveals that the secretion of *E. floridana* has this effect (**Tables 11, 12; Fig. 16**). Whereas the proportion of *M. pharaonis* remaining at the honey droplet was the same following

<b>Presentation</b>	<b>N</b>	<b>Mean</b>	<b>Std. Dev.</b>	<b>Std. Error</b>	<b>Mean Rank</b>
<b>empty</b>	8	1.00	0.00	0.00	18.00
<b>water</b>	8	0.9454	0.0817	4.1595	15.00
<b>secretion</b>	8	0.1770	0.1476	4.5696	4.50
	df	2			
	Chi-Square	19.103			
	Sig.	0.000			

**Table 11. Descriptive statistics of the proportion of ants remaining at honey at t = 5s.**

Comparison	$R_A - R_B$	SE	Q
empty – water	$18.00 - 15.00 = 3.00$	3.24	0.9259
empty – secretion	$18.00 - 4.50 = 13.50$	3.24	4.167*
water – secretion	$15.00 - 4.50 = 10.50$	3.24	3.241*

**Table 12. Results of Nemenyi's post-hoc test for multiple comparisons of ant repellency data ( $m = 1$ ,  $\Sigma t = 2184$ ) ( $Q_{0.05,3} = 2.394$ ; \* - data significantly different at  $\alpha = 0.05$ ).**



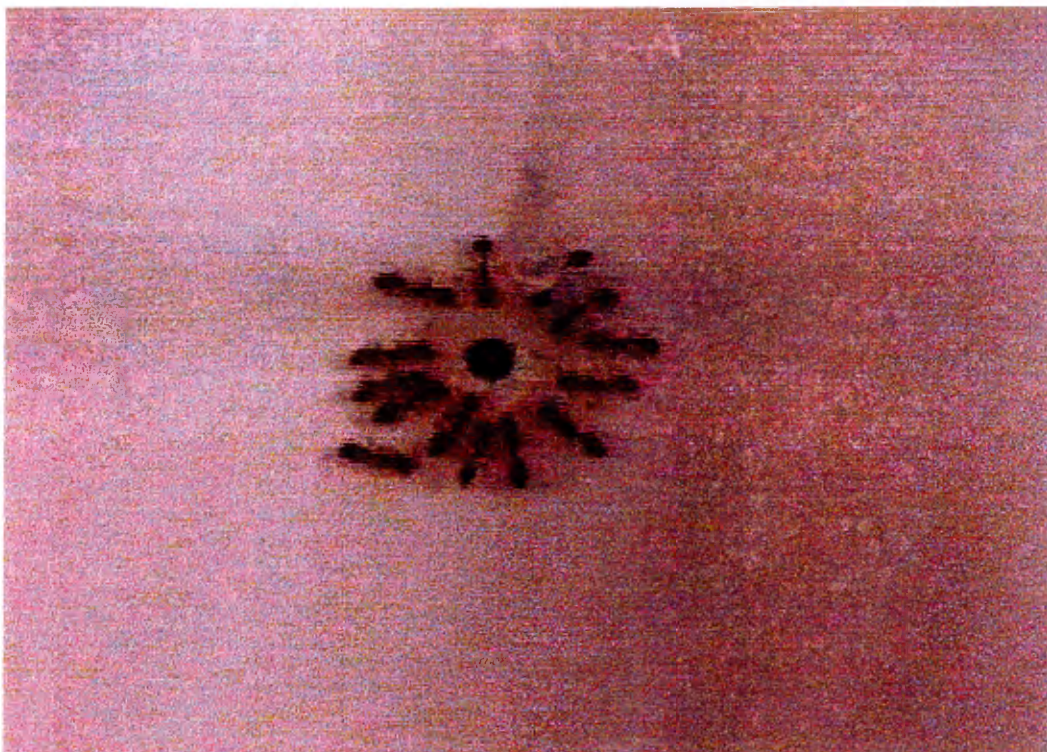
**Figure 16. Mean proportion ( $n_i / n_o$ ) of *M. pharaonis* remaining at honey at  $t = 5s$  (columns with same letter are not significantly different at  $\alpha = 0.05$ ; bars represent standard deviation).**

presentation of an empty capillary tube and one with distilled water ( $p = 0.25$ ), the difference was highly significant between the secretion and water presentation ( $p < 0.01$ ) (**Figs. 17, 18**).

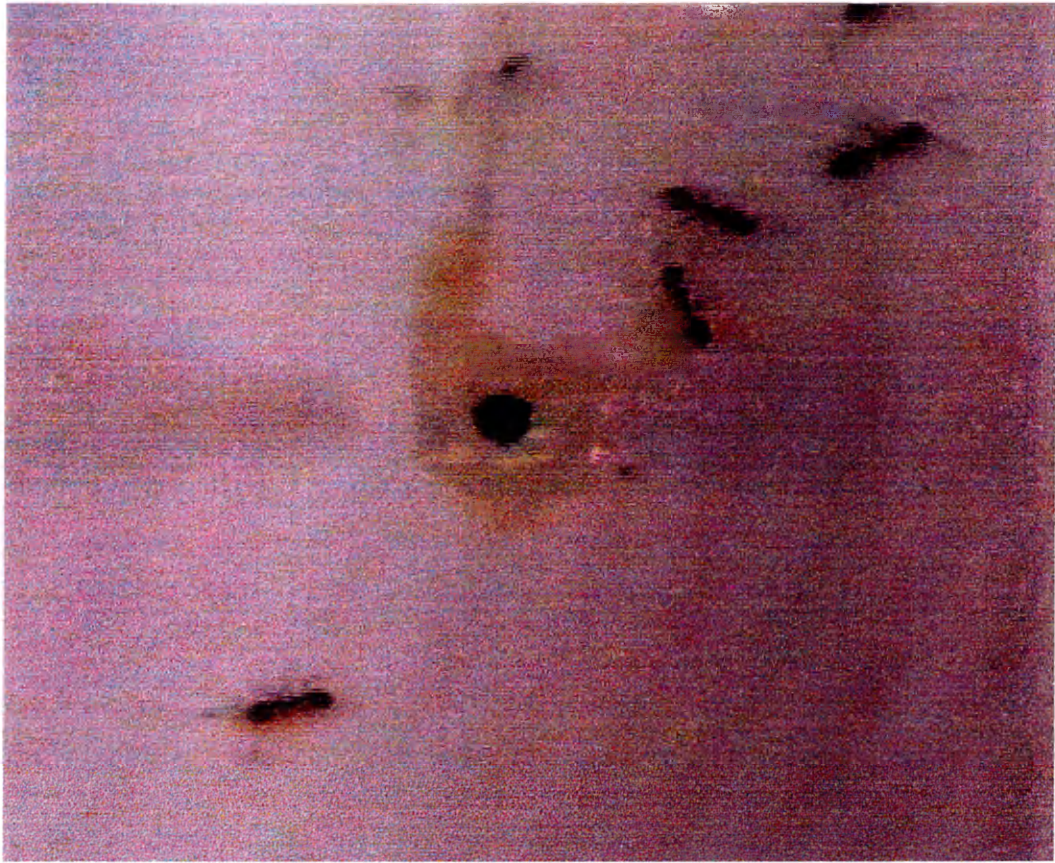
Only 3 times ( $n = 16$ ) did the proportion of ants feeding drop during the  $t_0$  to  $t_5$  interval in the blank and water presentation. The tube presenting the secretion produced dramatically different results, however. Not only did the proportion of ants feeding decrease during the first 5 second interval in every trial ( $n = 8$ ), it was usually a very large decline. The smallest percentage decrease was 60% ( $nt_0 = 5$ ); 6 of 8 trials had a decrease of 75% or greater within the initial 5 second interval. Additionally, the proportion feeding at the honey remained very low through the course of the 30 second presentation in all of the secretion trials (**Fig. 19**).

The 3 types of cockroaches assayed by contact were highly irritated by the application of *E. floridana* secretion to the mesothoracic legs (**Figs. 20, 21**). In all 3 types the response difference between application of distilled water and secretion was found to be highly significant following analysis by a test of independence (**Fig. 22**).

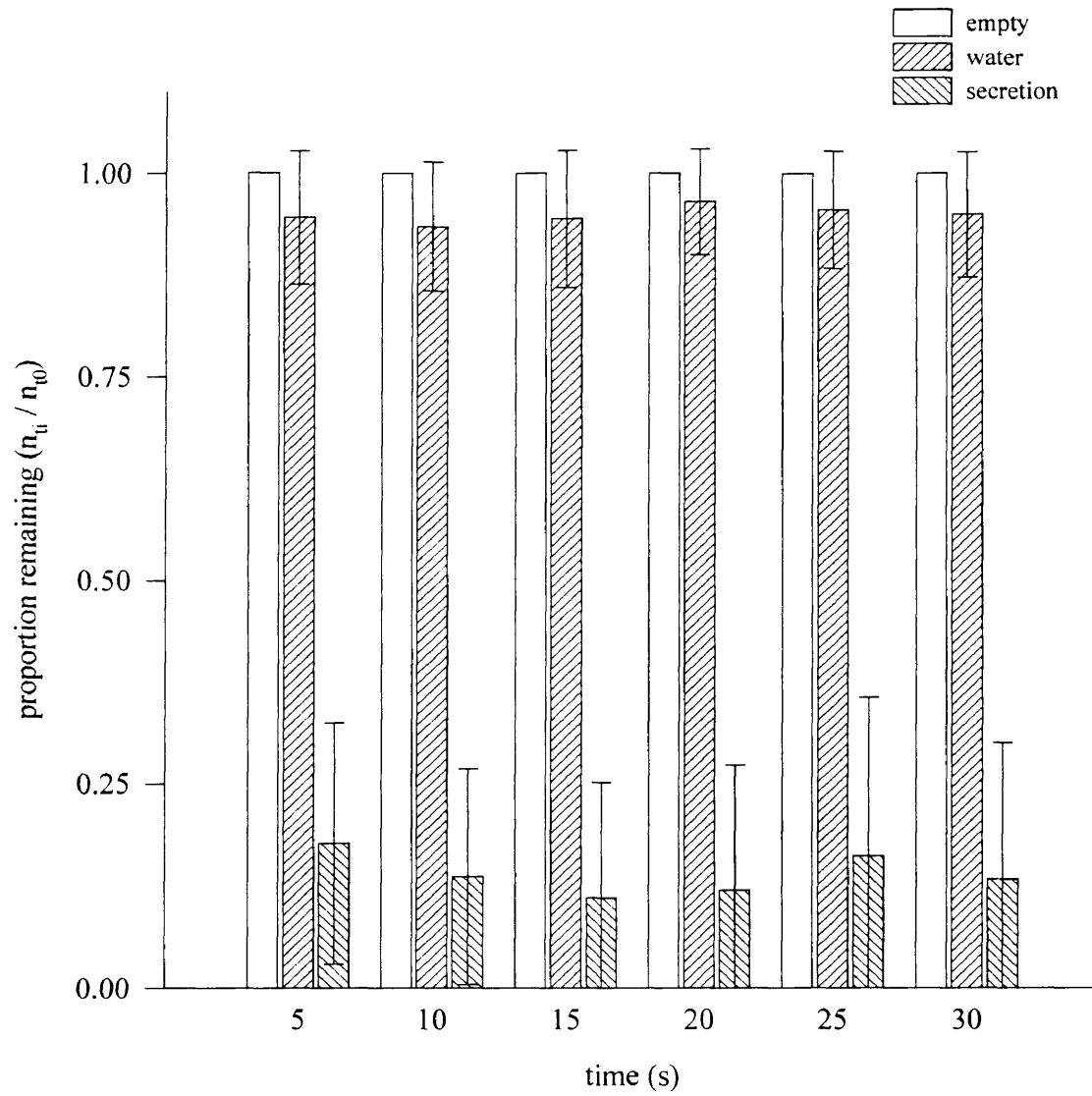
The irritancy data do not show several items of interest. Firstly, the *E. floridana*, both adults and juveniles, were much more mobile following the decapitation than were the American roaches. Secondly, the protocol called for the use of both mesothoracic legs. Each trial was scored as positive if either leg was brought forward for grooming. Each application of secretion ( $n = 45$ ) resulted in both legs being brought forward. The few positive results elicited by water application involved only 1 leg in each case ( $n = 3$ ). Thirdly, application of the secretion always elicited a response within 2 seconds; the 3 reactions to water occurred after more than 5 seconds. Finally, 5 American roaches



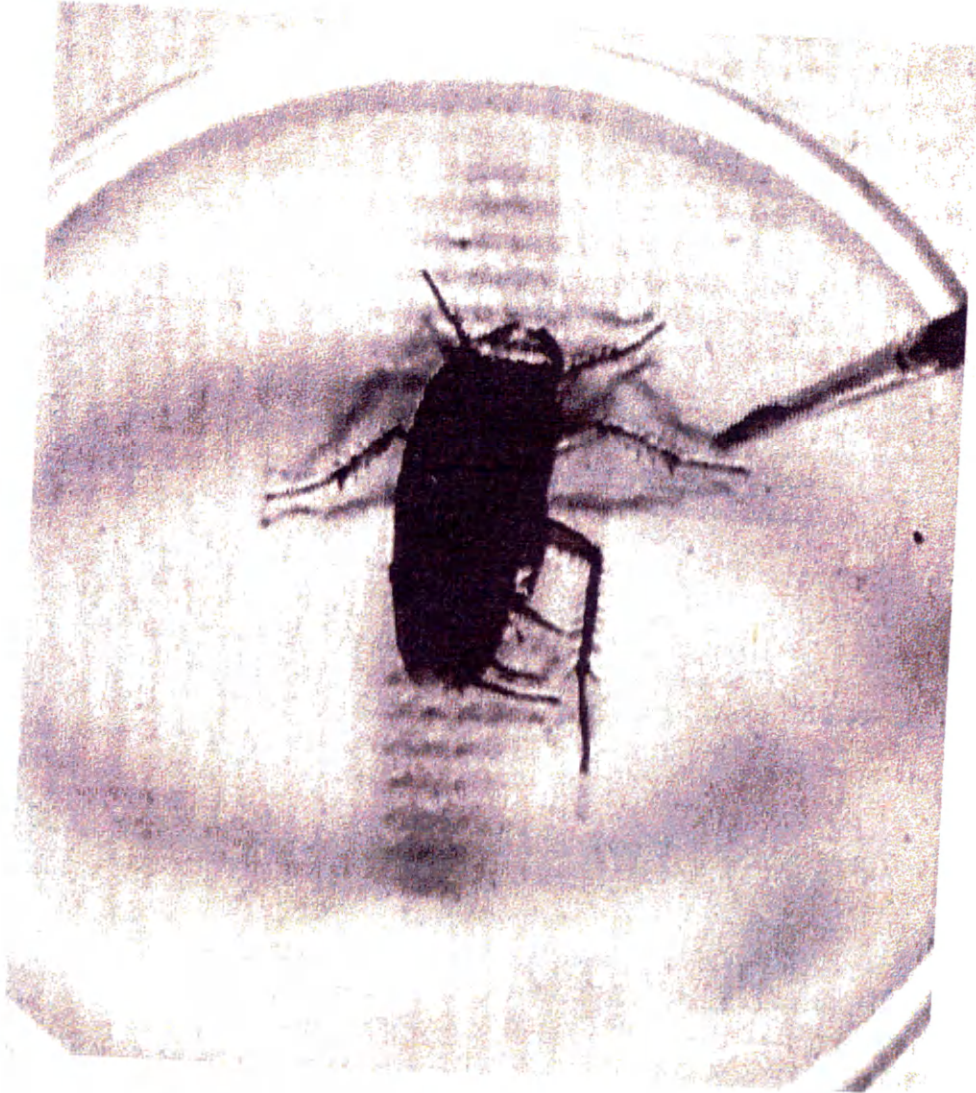
**Figure 17.** *Monomorium pharaonis* surrounding honey during water presentation,  $t = 5s$ .



**Figure 18.** *Monomorium pharaonis* surrounding honey during secretion presentation,  $t = 5s$ .



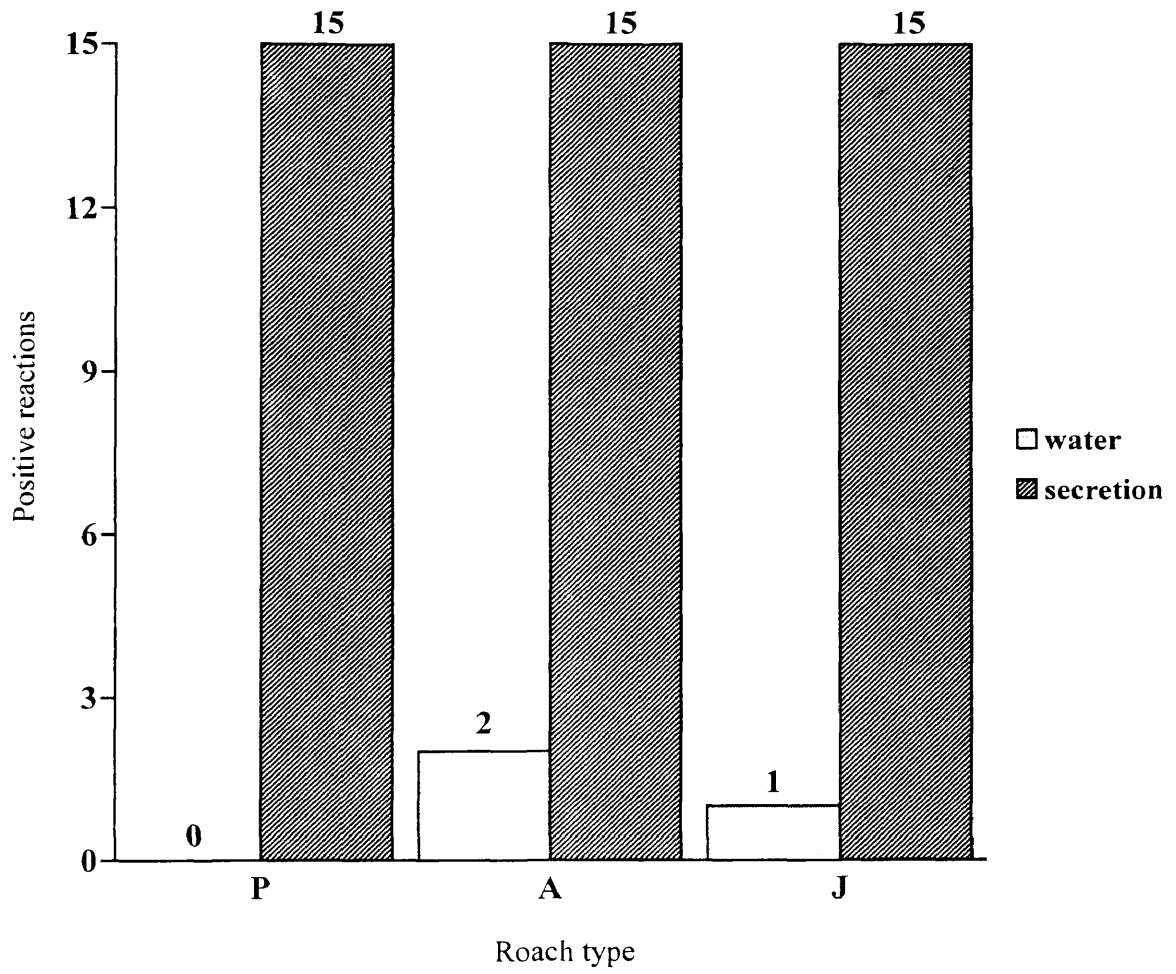
**Figure 19. Mean proportion ( $n_{ti} / n_{t0}$ ) of *M. pharaonis* remaining at honey at 5 second intervals to  $t = 30s$  (bars represent standard deviations).**



**Figure 20.** *Periplaneta americana* prior to application of secretion.



**Figure 21. *Periplaneta americana* following application of secretion. Note position of mesothoracic leg.**



**Figure 22.** Number of roaches responding positively to application of water and secretion (P – *P. americana*; A – Adult *E. floridana*; J – Juvenile *E. floridana*)

were treated with glacial acetic acid in a similar fashion as the secretion application to test their response to a known severe irritant. There was no reaction to the distilled water application, but there was a strong, positive reaction to the acid in all 5 cases (data not shown). The response times and behaviors were very similar to that induced by the application of the secretion.

Although both data sets demonstrate the effectiveness of the secretion, additional data (not shown) were obtained with a congener of a possible natural predator of *E. floridana*. In 2 trials where an adult *E. floridana* was presented to 10 carpenter ants (*Camponotus sp.*), the ants behaved very antagonistically toward the roach, resulting in several secretion events. The ants responded to contact with the secretion as is typical of contact with uncomfortable chemical stimuli: each ant ran in chaotic patterns, dragging its abdomen along and swiping its head frequently against the substrate. Typically, several minutes passed before other ants would approach the roach, although the roach was killed within 10 minutes in both presentations. Blum (1965) made similar observations concerning the spray of the roach *Pelmatosilpha coriacea*. *Iridiomyrmex melleus* worker contacted by the secretion did not attack for up to 3 minutes, giving the roach time to escape.

Adult and juvenile *E. floridana* were used in the irritancy assay due to the observation that the insects are very sensitive to the odor of their own secretion, and in fact use it as an alarm agent (Farine et al. 1997). Additionally, it was hypothesized that the chemicals which comprise the secretion, particularly (E)-2-hexenal, act as general antagonists, thus affecting *E. floridana* as well. This would seem to be a problem, as the ergonomics of the adult are such that it is unlikely that it could spray the secretion

without contacting itself. However, this could be beneficial, as the presence of the secretion on the cockroach's own cuticle may aid in prolonging the repellent effects of the secretion on nearby predators (Eisner 1970).

The possibility that the secretion therefore acts not as an alarm pheromone, but rather as a general deterrent which affects even conspecifics, is raised. Farine et al. (1997) noted that both juveniles and adults are sensitive to the presence of the secretion, and explained their fleeing from the odor as a response to an alarm pheromone. However, *E. floridana* males are territorial and agonistic (Diboine in Farine 1997), and are thought to use the secretion against one another in aggressive interactions (David-Henriet 1995). Rather than being an alarm pheromone, the spray simply may affect *E. floridana* in a similar fashion to its action on other insects. If this is the case, the behavior noted by Farine et al. (1997) could be an attempt to flee from an aversive stimulus, rather than a response to an altruistic alarm pheromone. The general symptoms observed in *E. floridana* are consistent with an hypothesis of general repellency.

It is likely that (E)-2-hexenal causes the majority of symptoms seen in response to contact with the secretion. Aldehydes, particularly those that are  $\alpha,\beta$ -unsaturated, are very reactive and thus cytotoxic (Feron et al. 1991). It appears that a majority of seizure-like symptoms observed in insects may be due in part to an increase in cytosolic  $\text{Ca}^{2+}$  levels, an effect of (E)-2-hexenal on mammalian cell cultures (Griffin and Segall 1989). As insect muscles rely upon actin and myosin, alteration of the  $\text{Ca}^{2+}$ /calmodulin equilibrium could cause tetanic contractions, resulting in seizure-like symptoms. Increased  $\text{Ca}^{2+}$ /calmodulin activation of CaM kinase II family members also could act to release neurotransmitters by phosphorylation in neurons (Cooper 1997), further

contributing to the seizure-like symptoms. (E)-2-hexenal also has been observed to interfere with gap junction intercellular communication (Seliverstov 1984), presumably by altering receptor proteins (de Haan et al. 1994) and thus interfering with ion exchange. In the insect antenna, this could occur via the reaction of (E)-2-hexenal with nucleophiles such as  $\text{NH}_2$  and  $\text{SH}_2$  on chemoreceptor proteins, effectively blinding olfactory-dependent insect predators (Blum 1978).

The compounds present in lower concentrations likely act to fulfill accessory molecule functions (e.g., penetrating the insect cuticle, reducing evaporation rate, and increasing spreading rate). Several are likely intermediates involved in synthesis of the secretion (Wallbank and Waterhouse 1970) and would be relatively unimportant in providing a defensive capability to the secretion. Gunawardena and Herath (1991) found that the medium chain alkanes and alkenes accompanying the bioactive components of insect defensive secretions optimize the functionality of the secretion by providing the characteristics noted above. Unfortunately, the wide variety of constituents in defensive compounds makes the characterization of the specific effects of one constituent more difficult. The array of potential predators also complicates matters. Certain generalizations are possible, though. For example, unsaturated aldehydes tend to be more toxic than alkanes (Feron et al. 1991), and medium length hydrocarbons function more efficiently than longer hydrocarbons as accompanying compounds (for review see Gunawardena and Herath 1991). Presumably the former effect is due to the more reactive nature of unsaturated hydrocarbons, particularly  $\alpha,\beta$ -unsaturated aldehydes, as compared to saturated hydrocarbons; the latter probably is due to the greater volatility of smaller compounds.

The biphasic nature of the secretion is important in aiding in its function. The hydrophobic organic phase allows the secretion to spread on (Lewis 1980) as well as penetrate the wax layer of arthropods (Treherne 1957). An active insect, reacting to the secretion, may further aid in its spread through movement induced deformations of the cuticle (Lewis 1980). Whereas hydrophobic molecules penetrate and move through the cuticular matrix (Lewis 1980), polar molecules likely take another route to avoid the cuticle. It has been demonstrated that hydrophilic molecules move through the pore-canal and epicuticular filaments of the epicuticle, thereby reaching the basement layer below the epidermis (Brück and Komnick 1971, Scheie and Smythe 1967). The ability of these epidermal cells to retard penetration would depend on several factors, including metabolic rate and potential detoxifying enzymes as well as cytotoxicity of the epidermal cells. Regardless, the next stage would be influx into the hemolymph and nervous systems, followed by presentation of the secretion's toxins to various cells.

A further contributing point governing the entrance of the secretion into the insect system is the vulnerability of thinner areas of cuticle. Wigglesworth (1942) demonstrated that setal articulating joints are favorable areas of oil penetration, and the susceptibility of the thin cuticle of the tracheal system is unknown. Effects on contact chemoreceptors, and whether there are generalized or specific receptors for constituents of the secretion, also are unknown. Antennal chemoreceptors do react to compounds in the secretion (Dickens et al. 1993), including (E)-2-hexenal (Visser and Fu-Shun 1995).

The complexity of the secretion of *E. floridana* may increase its efficacy as a biochemical defense. In insects contacted by the secretion, this complexity appears to allow penetration of a hydrophobic cuticle and temporary disruption of neuromuscular

junctions through a variety of cytotoxic mechanisms. Juvenile *E. floridana* are unable to discriminate between very high levels and low levels (Farine et al. 1997), leading to the belief that the compounds present also may act to overwhelm a predator's sensory system, allowing the secreting roach to escape. The majority of the secretion's effects appear to be temporary, although certain effects of the secretion could have longer lasting effects. The green leaf volatiles (e.g., (E)-2-hexenal, hexenol, and hexanal) emitted by plants under attack by insect herbivores are known attractants of the herbivore's predators and parasitoids (Karban and Baldwin 1995); a similar situation could result from an attack on *E. floridana* (an interesting study would be to see if the sternal secretion acts as a kairomone for any parasitoids of *E. floridana*). Another potential mechanism for increased hyper-predation is the disorientation caused by the secretion, which may temporarily lower anti-predator defenses. Temporal costs involved in foraging would also have to be considered.

## Chapter III.

### Observations on Orientation Behaviors Associated With Discharge of Secretion in *Eurycotis floridana*

#### Introduction

In pilot studies *Eurycotis floridana* were observed to orient their abdomen in response to antagonistic behaviors by *Peromyscus leucopus*. This was investigated independently of the mice to determine whether the orientation of the abdomen is an actual controlled behavior rather than an experimental artifact. The pattern of secretion and various other behaviors were noted in order to better describe the efficacy of the secretion as a deterrent.

#### Materials and Methods

Adult *E. floridana* were placed in a freezer (approximately -20°C) for 10 minutes to anesthetize them (CO<sub>2</sub> anesthesia was unusable as it stimulated secretion in most individuals). This time period was sufficient to reduce ambulatory movements in the roach without appearing to effect long term changes in behavior. Following removal from the freezer a 15 cm dowel-rod (r ~ 2 mm) was attached to the thorax with heated parafilm. Once the wax hardened, the roach could be immobilized and manipulated without stimulation of the abdomen and legs. Following attachment of the dowel, the roach was allowed a 12 hour recovery period under conditions described earlier (Chapter I).

Each roach then was subjected to gentle irritation with forceps in an attempt to induce discharge. These activities included pinching, tugging, and rubbing various parts of the abdomen, thorax, and legs. It was thought that these body parts were appropriate

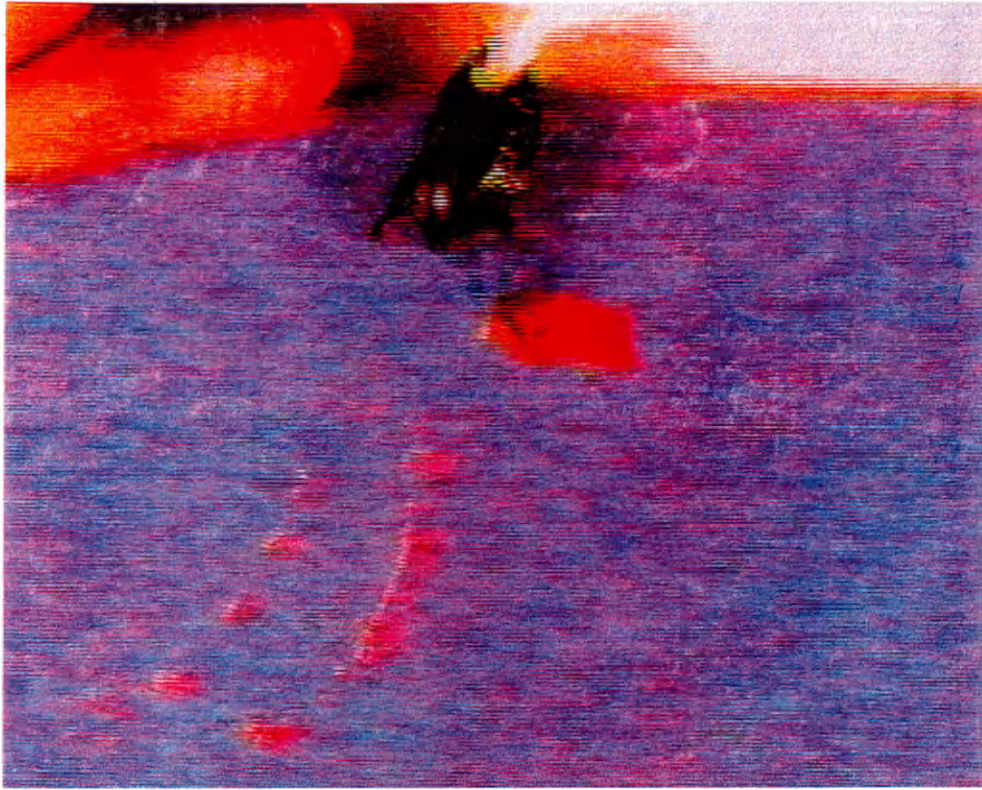
due to the location of the duct for the gland: abdominal, facing to the posterior of the roach. Also, observational pilot studies corroborate this, as roaches only discharged their secretion ( $n = 67$ ) in response to abdominal contact (data not shown). The process was recorded on videotape for description of behaviors associated with discharge, such as rotation of the abdomen, and analysis of accuracy and distance.

## Results and Discussion

*Eurycotis floridana* was found to react in differing degrees to stimuli. Thoracic and head trauma invariably brought about an attempt to move away; abdominal rotation often was not apparent. Abdominal stimulation usually, but not always, brought about rotation of the abdomen. This also was accompanied by an attempt to move away from the stimulus. Antagonism to the metathoracic legs, although not the other two pairs, elicited the same behaviors.

Analysis of other data (Chapter 1) showed that the roach is highly accurate in spraying a potential attacker, even one that is moving and reacting quickly. Not surprisingly, steady pinching with tweezers resulted in accurate spraying. Typically a V-pattern was observed (**Fig. 23**), with the vertex of the angle at the roach's posterior. The mean angle of discharge was near the angle of stimulus incidence in every case (see Chapter I for definitions).

*Eurycotis floridana* appears able to direct the spray unilaterally to some extent, regardless of abdominal orientation. This is an interesting observation, as the roach possesses only one centrally located ostiole. The normally seen pattern was the V-shape shown in **Fig. 23**, but it does appear that the roach is able to selectively fire its secretion to one side or another. As *E. floridana* lacks sphincter muscles associated with the gland

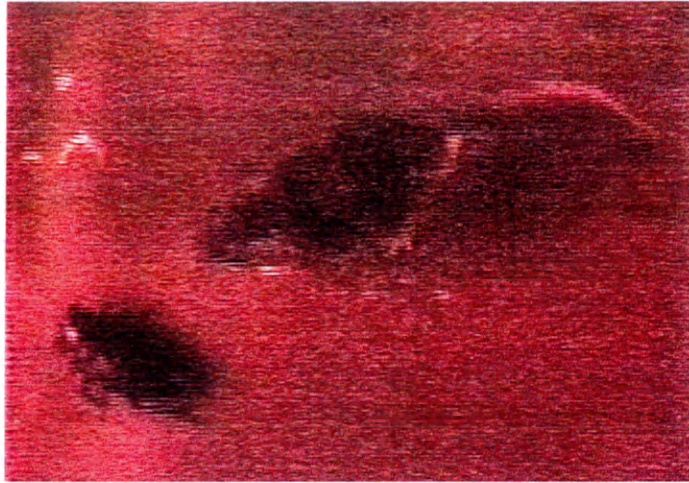


**Figure 23. Typical pattern of discharge in *E. floridana* following abdominal stimulation.**

(Stay 1957), the mechanism controlling this phenomenon is probably behavioral, related to abdominal orientation and differential pressure.

In addition to orientation of its abdomen, *E. floridana* exhibits other behaviors associated with control of the spray. The secretion is sprayed by a combination of two mechanisms. The roach is capable of shortening the abdomen by contracting one pair of longitudinal muscles of the abdomen, thereby reducing the volume of the reservoir (Stay 1957). Secondly, a generalized contraction of the abdomen would provide the pressure necessary to discharge gland contents. Regardless of the mechanism, the changes in the roach's body are obvious, as the roach frequently appears to shorten in increasing abdominal pressure.

*Eurycotis floridana* does not appear to exhibit any type of abdominal yaw or stiling, as is seen in *Platyzosteria castanea* and *P. ruficeps* (Waterhouse and Wallbank 1967). However, these relatives possess aposematic coloration that is exposed when the roach stilt; *E. floridana* lacks aposematic coloration. The result of the lack of stiling is a low angle of discharge in relation to the ground, which decreases the likelihood of long spraying distances. This helps to better understand the reasons for the movement behaviors observed in the roach, as it must alter its position to better aim its spray. The roach frequently orients its body in a crescent fashion following a discharge by shortening the length of one side of its body (**Fig. 24**). This was done in such a fashion as to keep the abdomen directed at the disturbance as the roach moved laterally relative to the device, and not directly away. This would increase the likelihood of hitting a target while decreasing the response time to an attack.



**Figure 24. Body curvature orientation exhibited by *E. floridana* following an attack.  
Note lengthening of opposite side from mouse.**

One surprising discovery was the variation in response to stimulus observed between roaches. Several roaches required a great amount of force or rapid, repeated stimuli to incite spraying; others needed only a slight touch to incite release of its secretion. As roaches were not aged for this study, it is unknown whether age had an effect. Furthermore, little is known about control of the gland: the gland is poorly innervated (Stay 1957), and it is unknown how, or even whether, the roach can assess how much secretion remains in the gland. The behaviors associated with discharge were often observed in absence of actual discharge when the roach was properly stimulated, even in instances when it was apparent that the roach had discharged its full reservoir. Two hypotheses may be postulated from this: either the roach is unaware that it has used its full complement of secretion, or the behaviors function as part of the roach's aposematic suite (Eisner and Grant 1981) and function to warn an experienced predator even without the accompanying release of secretion.

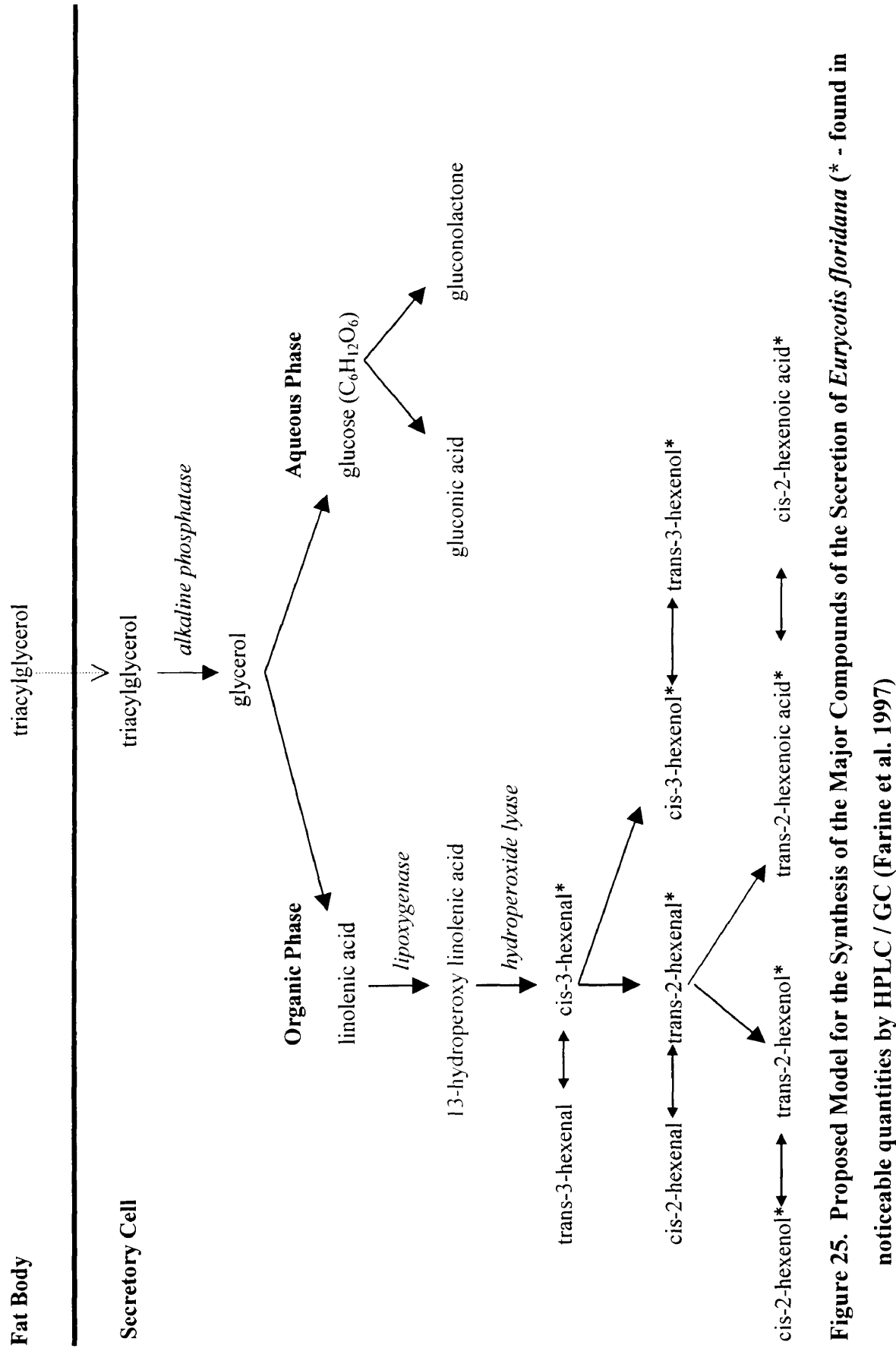
## Chapter IV.

### A Model for the Biosynthesis of the Major Components of the Secretion

#### Introduction

The biosynthesis of insect defense chemicals is a fairly new investigative field. Since the advent of radio-labeling, studies have focused on the incorporation of radioisotopes into compounds *in vivo*. This has allowed analysis of whether compounds are obtained from food or synthesized *de novo*. However, research has focused on those species that are economically important, while neglecting species that are behaviorally but not economically interesting. Also, for purposes of pest control, research has focused on pathways involved in the incorporation and/or synthesis of amino acids and fatty acids. Due to this, the chemical precursors and important enzymatic links of biochemical pathways leading to the formation of many defensive compounds are unknown, even for a chemical as prevalent as (E)-2-hexenal (see Blomquist et al. 1991).

The model below (**Fig. 25**) is proposed as a pathway for the synthesis of both aqueous phase components and hexenal-related components of the organic phase of the secretion of *E. floridana*. The pathway is based on the presence of several precursor compounds in the secretory cells and fat body of the cockroach, along with models proposed for the synthesis of “green flavors” (e.g., hexenal, hexenol, and hexenoic acid) in plants (Karban and Baldwin 1995, Kasu et al. 1995). Potential mechanisms of action, and links from the secretion’s properties to them, are not suggested here, as these have been discussed in some depth elsewhere (Chapters I and II).



**Figure 25. Proposed Model for the Synthesis of the Major Compounds of the Secretion of *Eurycotis floridana* (\* - found in noticeable quantities by HPLC / GC (Farine et al. 1997)**

## Synthesis Model

Triacylglycerol, used by insects as an energetic storage and transport molecule (Schneider and Dorn 1994), is synthesized in gland-associated fat body and transported to the secretory cells of the gland. The presence of alkaline phosphatase in the secretory cells during times of secretion synthesis (Stay 1957) indicates the lipolysis of triacylglycerol to glycerol (Stryer 1995). For the creation of the organic phase, some of the glycerol is converted to linoleic acid ( $18:3:\Delta^{9,12}$ ) or linolenic acid ( $18:3:\Delta^{9,12,15}$ ). The polyunsaturated fatty acid is converted to 13-hydroperoxy linolenic acid via a lipoxygenase. The 13-hydroperoxy linolenic acid is then broken down into (Z)-3-hexenal. The (Z)-3-hexenal is converted to (Z)-3-hexenol and (E)-2-hexenal; both should convert to their stereoisomers, although evidence of this is not seen for (E)-2-hexenal in the final product (Farine et al. 1997). Some of the alkenal is converted to (E)-2-hexenol (Karban and Baldwin 1997) and (E)-2-hexenoic acid (Feron et al. 1991).

The synthesis of the aqueous phase is much simpler: some of the glycerol in the secretory cells is converted to glucose (Stryer 1991); the product is then converted to gluconic acid by hydrolysis, and on to glucolactone. Aldrich et al. (1978) have found that the aqueous phase of the biphasic secretion of *Leptoglossus phyllopus* (Hemiptera: Coreidae) contains enzymes involved in the synthesis of the organic phase. This is certainly a possibility here, though the aqueous phase has not been studied as well as the organic phase in *E. floridana* (Aldrich et al. 1978, Dateo and Roth 1967).

Although data concerning the actual pathway are not available for insects, the necessary enzymes and the intermediate components are found in insects closely related

to *E. floridana*. Lipoxygenases responsible for the conversion of fatty acids are known to occur in insect cells, primarily in the cytosol (Gadelhak et al. 1995), and are important in the derivation of several cellular defense compounds (Miller et al. 1996). (Z)-3-hexenal, the immediate precursor of (E)-2-hexenal, is found in low concentration in the secretion (Farine et al. 1997). (E)-2-hexenol and (E)-2-hexenoic acid, immediate derivatives of (E)-2-hexenal, are also found in the upper phase of the secretion in low quantities (Farine et al. 1997); both compounds are known to be active biologically, causing oxidative damage to cells as lipid peroxidation products (Berlett and Stadtman 1997). (E)-2-hexenol also is known to have toxic effects (Hamilton et al. 1985), which might explain the continuance of the pathway to its development. Lack of (Z)-2-hexenal in the final product (Farine et al. 1997) is interesting. This may be related to the observation that many insects are able to synthesize specific stereoisomers (e.g., cis- or trans-) of unsaturated compounds (Blum 1978), and may have implications for functionality of the secretion (e.g., the trans- isomer may stimulate a receiver, whereas the cis- isomer may depress it).

Stay (1957) found a correlation between the initial signs of secretion production in final instar nymphs, as well as the production of secretion in the glands of depleted adults, and the presence of both glycogen and alkaline phosphatase in the secretory cells. Neither molecule's presence was found to correlate exactly with secretion production, although both were found to occur in higher quantities in those cells that either were initializing production of, or were currently producing, secretion. Regardless of the presence of glycogen and alkaline phosphatase in the secretory cells, the possibility exists that certain compounds of the secretion are synthesized in the cuticular cells. This has

been noted of cytotoxic aldehydes, including (E)-2-hexenal, in true bugs (Aldrich et al. 1978); likely this is due to the impermeability of the cuticular cells, which would protect the insect from the cytotoxic compounds (for review see Blum 1981).

The production of linoleic, or linolenic, acid is problematic. Perhaps because neither fatty acid can be synthesized in humans (David and Sittman 1995), the enzymes managing this pathway are not known. However, de novo synthesis of both fatty acids has been described in insects (Blomquist et al. 1991, Borgeson et al. 1991, Rule and Roelofs 1989).

Analysis of the synthesis of the hexenal-laden secretion of *E. floridana* should prove interesting for several reasons. Although there is a large number of insects that produce (E)-2-hexenal for defense (Blum 1981), the biochemical pathway has yet to be described. As the chemistry of *E. floridana*'s secretion is already well known (Farine et al. 1997), this organism should provide an excellent tool for this study. Knowledge gleaned from this work would contribute both basic knowledge about *E. floridana* and a better understanding of de novo synthesis of non-essential compounds in insects, which would be beneficial.

## Discussion

The abdominal secretion of *Eurycotis floridana* is defensive in nature. Data suggest that the secretion is an effective deterrent against both vertebrates and invertebrates. As known predators of *E. floridana* have not been recorded, the assays performed attempted to test for effects across a broad taxonomic range of organisms.

It is apparent from the study that the secretion is effective through contact on both vertebrates and invertebrates. To characterize the efficacy of the secretion further, a second assay was performed with both vertebrates and invertebrates. In both studies, it became apparent that the secretion was capable of causing discomfort without contacting what are thought of as primary targets, namely the sensitive membranes around the eyes and in the mouths of vertebrates and contact receptors in insects.

That the roach is antagonized by the secretion begs the question of what the functions of the chemical are in intraspecific interactions. Although Farine et al. (1997) demonstrated that the compound functions as an alarm chemical in high quantities, the use of the compound in territorial behaviors is unknown. Observations have been made that the sternal secretion is discharged in male – male agonistic interactions (David-Henriet et al. 1995, Farine et al. 1997), but what governs spraying under these circumstances is not known. Additionally, Farine et al. (1997) found that males are less sensitive to low concentrations of secretion than females (though more sensitive than juveniles); presumably this is due to use of the secretion in these dominance contests. In *E. floridana* the male instigates sexual relations (David-Henriet 1995, Farine et al. 1996) by exposure and release of tergal gland compounds. It also has been established that *E. floridana* males are territorial (Diboine, in Farine et al. 1997). Thus, there is the

possibility, in agreement with Blum's observations on the parsimony of chemical compounds in insects (Blum 1996), that the defensive, sternal gland secretion of *E. floridana* has been co-opted to signal proper spacing among territorial males. This would seem to occur due to the general toxicity of the secretion, rather than an actual pheromonal function (i.e., alarm pheromone) being established. That *E. floridana* is as irritated by application of the secretion as other invertebrates supports this conclusion.

It has been hypothesized that the main biological agent in the secretion is the alkene (E)-2-hexenal, a lipid peroxidation product (Berlett and Stadtman 1997) commonly known as leaf aldehyde. Many key behavioral symptoms of lipid peroxidation product-mediated toxicity are apparent in the effects of the secretion (e.g., the seizures observed in both vertebrates and invertebrates). Although cytological toxicity data were not collected, it is thought that these also would support the hypothesis, as would studies investigating ion balance at neuromuscular junctions. Future studies examining the effects of various components of the secretion in situ should provide evidence of this. Regardless, such studies are necessary, as there is a dearth of such observations in the field of chemical ecology.

It is also interesting to note other roles of (E)-2-hexenal in biotic secretions. A parallel situation is the use of green compounds (e.g., hexenal, hexenol, and hexanal) by plants as an herbivore deterrent. Several groups of plants are known to synthesize the compound, along an arachidonic/linolenic acid enzymatic pathway (for review see Hatanaka et al. 1995), following tissue damage to leaves by herbivores such as aphids (Hildebrand et al. 1986, Kasu et al. 1995). Under these circumstances, the release of (E)-2-hexenal has been documented to aid the plant in multiple ways. The green volatiles are

known to act as attractants for various insectivores and parasitoids that kill insect herbivores (for review see Tumlinson et al. 1993). It has also been shown that the green volatiles, (E)-2-hexenal in particular, are potent microbicides (Deng et al. 1993) and fungicides (Brown et al. 1995), thus reducing incidence of pathogenesis in the plant (and in insects, which would provide more evidence of parsimony of chemicals in insects).

The presence of compounds of the sternal secretion on the cuticle surrounding the duct (Farine et al. 1994) again becomes an issue, as an evolutionary relationship between lack of sphincter muscles and these protective functions may be postulated. The many benefits of the defensive secretion may be such that the cost of a steady loss of secretion through the duct may be negligible when compared to the advantage conferred by the multiple other functions. It would appear, though, that the secretion evolved primarily as a defensive chemical, and that the general toxicity of its constituents have allowed the secretion to be co-opted into other functions, such as conspecific spacing and protection versus various pathogens.

### Literature Cited

1. **Abed, D., J. Farine, J. Francois, and R. Brossut. 1994.** Protein and amino acid composition of the tergal gland secretions of *Blatta orientalis* and *Eurycotis floridana* (Dictyoptera: Blattidae). *Chemoecology* 5/6, 1: 19-25.
2. **Aldrich, J.R., M.S. Blum, A. Hefetz, H.M. Fales, H.A. Lloyd, and P. Roller. 1978.** Proteins in a Nonvenomous Defensive Secretion. *Science* 201: 452-453.
3. **Alsop, D.W. 1970 (in Roth and Alsop 1978).** Defensive glands of arthropods: Comparative morphology of selected types. Ph.D. Dissertation, Cornell University, NY.
4. **Barcay, S.J. and G.W. Bennett. 1991.** Influence of Starvation and Lighting on the Movement Behavior of the German Cockroach (Blattodea: Blattellidae). *J Econ Entomol* 84(5): 1520-1524.
5. **Bell, W.J., R.E. Gordon, M.K. Toutellot, and M.D. Bred. 1979.** Comparison of male agonistic behavior in five species of cockroach. *Insectes Soc* 26: 252-253.
6. **Benn, M.H., R.F.N. Hutchins, and R. Folwell. 1977.** Defensive Scent of the Black Stink-Roach *Platyzosteria novaeseelandiae*. *J Insect Physiol* 23: 1281-1284.
7. **Berlett, B.S. and E.R. Stadtman. 1997.** Protein Oxidation in Aging, Disease, and Oxidative Stress. *J Biol Chem* 272(33): 20313-20316.
8. **Blomquist, G.J., C.E. Borgeson, and M. Vundla. 1991.** Polyunsaturated fatty acids and eicosanoids in insects. *Insect Biochem* 21(1): 99-106.
9. **Blum, M.S. 1965.** Insect defensive secretions: Hex-2-enal-1 in *Pelmatosilpha coriacea* (Blattaria) and its Repellent Value Under Natural Conditions. *Ann Entomol Soc Am* 57: 600-602.
10. **Blum, M.S. 1974.** Deciphering the Communicative Rosetta Stone. *Bull Entomol Soc Am* 20: 30-35.
11. **Blum, M.S. 1978.** Biochemical Defenses of Insects, pp. 466-513. *In* Rockstein, M. [ed.], *Biochemistry of Insects*. Academic Press, NY.
12. **Blum, M.S. 1981.** *Chemical Defenses of Arthropods*. Academic Press, NY.
13. **Blum, M.S. 1996.** Semiochemical Parsimony in the Arthropoda. *Annu Rev Entomol* 41: 353-374.
14. **Blum, M.S. and J.M. Brand. 1972.** Social Insect Pheromones: their chemistry and functions. *Am Zool* 12: 553-576.

15. **Blum, M.S., R.M. Crewe, J.H. Sudd, and A.W. Garrison. 1969.** 2-Hexenal: Isolation and Function in a *Crematogaster (Atopogyne) sp.* J GA Entomol Soc 4(4): 145-148.
16. **Borgeson, C.E., T.J. Kurtti, U.G. Munderloh, and G.J. Blomquist. 1991.** Insect tissues, not microorganisms, produce linoleic acid in the house cricket and the American cockroach. *Experientia* 47(3): 238-241.
17. **Bowers, M.D. and N.E. Stamp. 1997.** Fate of host-plant iridoid glycosides in lepidopteran larvae of Nymphalidae and Arctiidae. *J Chem Ecol* 23(12): 2955-2965.
18. **Brenner, R.J. 1988.** Focality and mobility of some peridomestic cockroaches in Florida. *Ann Entomol Soc Am* 81(4): 581-592.
19. **Brenner, R.J. and R.R. Pierce. 1991.** Seasonality of Peridomestic Cockroaches (Blattoidea: Blattidae): Mobility, Winter Reduction, and Effect of Traps and Baits. *J Econ Entomol* 84(6): 1735-1745.
20. **Brossut, R. 1983.** Allomonal Secretions in Cockroaches. *J Chem Ecol* 9(1): 143-158.
21. **Brossut, R. and L. Sreng. 1980.** Ultrastructure Comparee des Glandes Exocrines Abdominales des Blattaria (Dictyoptera). *Int J Insect Morphol Embryol* 9: 199-213.
22. **Brown, G.C., G.L. Prochaska, D.F. Hildebrand, G.L. Nordin, and D.M. Jackson. 1995.** Green Leaf Volatiles Inhibit Conidial Germination of the Entomopathogen *Pandora neophidis* (Entomophthorales: Entomophthoraceae). *Env Entomol* 24(6): 1637-1643.
23. **Brück, E. and H. Komnick. 1971.** Histochemischer Nachweis der Penetrationswege von Salzlösungen durch die isolierte Insektencuticula. *J Insect Physiol* 17: 2027-2034.
24. **Cooper, G.M. 1997.** *The Cell: A Molecular Approach.* Sinauer Associates Inc., Sunderland, MA.
25. **Crewe, R.M., M.S. Blum, and C.A. Collingwood. 1972.** Comparative Analysis of Alarm Pheromones in the Ant Genus *Crematogaster*. *Comp Biochem Physiol B Comp Biochem* 43: 703-716.
26. **Dateo, G.P. and L.M. Roth. 1967.** Occurrence of Gluconic acid and 2-Hexenal in the Defensive Secretions of Three Species of *Eurycotis* (Blattaria: Blattidae: Polyzosteriinae). *Ann Entomol Soc Am* 60(5): 1025-1030.
27. **David, V.L. and D.B. Sittman. 1995.** *Biochemistry.* Harwal Publishing, Baltimore, MD

28. **David-Henriet, A., J. Farine, and R. Brossut. 1995.** Les signaux chimiques intervenant dans le comportement sexuel d'*Eurycotis floridana* (Dictyoptera, Polyzosteriinae). Bull Soc Entomol Fr 100(2): 109-118.
29. **Dell'Omo, G. and E. Alleva. 1994.** Snake Odor Alters Behavior, but Not Pain Sensitivity in Mice. Physiol Behav 55: 125-128.
30. **Deng, W., T.R. Hamilton-Kemp, M.T. Nielsen, R.A. Andersen, G.B. Collins, and D.F. Hildebrand. 1993.** Effects of Six-Carbon Aldehydes and Alcohols on Bacterial Proliferation. J Agric Food Chem 41: 506-510.
31. **Dickens, J.C., G.D. Prestwich, C. Ng, and J.H. Visser. 1993.** Selectively fluorinated analogs reveal differential olfactory reception and inactivation of green leaf volatiles in insects. J Chem Ecol 19(9): 1981-1991.
32. **Diboine, O. 1993 (in Farine 1997).** Le comportement antagoniste chez *Eurycotis floridana* (Dictyoptera, Blattidae). D.E.A., Université de Bourgogne, Dijon, France.
33. **Eisner, H.A., D.W. Alsop, and T. Eisner. 1967.** Defense Mechanisms of Arthropods. XX. Quantitative Assessment of Hydrogen Cyanide Production in Two Species of Millipedes. Psyche 74: 107-117.
34. **Eisner, T. 1961.** Demonstration of Simple Reflex Behavior in Decapitated Cockroaches. Turtox News 39(8): 196-197.
35. **Eisner, T. 1970.** Chemical Defense Against Predation in Arthropods, pp. 157-217. In E. Sondheimer and J.B. Simeone [eds.], Chemical Ecology. Academic Press, NY.
36. **Eisner, T. and R.P. Grant. 1981.** Toxicity, odor aversion, and "olfactory aposematism". Science 213(4506): 476.
37. **Eisner, T. and J. Meinwald. 1966.** Defensive Secretions of Arthropods. Science 153 (3742): 1341-1350.
38. **Eisner, T., F. McKittrick, and R. Payne. 1959.** Defense Sprays of Roaches. Pest Control (June): 9-12,44-45
39. **Eisner, T., I. Kriston, and D.J. Aneshansley. 1976.** Defensive Behavior of a Termite (*Nasutitermes exitiosus*). Behav Ecol Sociobiol 1: 83-125.
40. **Farine, J., J. Le Quere, J. Duffy, C. Everaerts, and R. Brossut. 1994.** Male Sex Pheromone of Cockroach *Eurycotis floridana* (Walker) (Blattidae, Polyzosteriinae): Role and Composition of Tergites 2 and 8 Secretions. J Chem Ecol 20(9): 2291-2306.

41. **Farine, J., C. Everaerts, D. Abed, M. Ntari, and R. Brossut. 1996.** Pheromonal Emission During the Mating Behavior of *Eurycotis floridana* (Walker) (Dictyoptera: Blattidae). *J Insect Behav* 9(2): 197-213.
42. **Farine, J., C. Everaerts, J. Le Quere, E. Semon, R. Henry, and R. Brossut. 1997.** The Defensive Secretion of *Eurycotis floridana* (Dictyoptera, Blattidae, Polyzosteriinae): Chemical Identification and Evidence of an Alarm Function. *Insect Biochem Molec Biol* 27(6): 577-586.
43. **Feron, V.J., H.P. Til, F. de Vrijer, R.A. Woutersen, F.R. Cassee, and P.J. van Bladeren. 1991.** Aldehydes: occurrence, carcinogenic potential, mechanism of action and risk assessment. *Mutation Research* 259: 363-385.
44. **Gadelhak, G.G., V.K. Pedibhotla, and D.W. Stanley-Samuelson. 1995.** Eicosanoid biosynthesis by hemocytes from the tobacco hornworm, *Manduca sexta*. *Insect Biochem Molec Biol* 25(6): 743-749.
45. **Goelzer, P., C. Janzowski, B.L. Pool-Zobel, and G. Eisenbrand. 1996.** (E)-2-hexenal-induced DNA damage and formation of cyclic 1,N<sup>2</sup>-(1,3-propano)-2'-deoxyguanosine adducts in mammalian cells. *Chem Res Toxicol* 9(7): 1207-1213.
46. **Griffin, D.S. and H.J. Segall. 1989.** Effects of the pyrrolizidine alkaloid senecionine and the alkenals trans-4-OH-hexenal and trans-2-hexenal on intracellular calcium compartmentation in isolated hepatocytes. *Biochem Pharmacol* 38(3): 391-397.
47. **Gunawardena, N.E. and H.M.W.K.B. Herath. 1991.** Significance of Medium Chain n-Alkanes as Accompanying Compounds in Hemipteran Defensive Secretions: an investigation based on the defensive secretion of *Coridius janus*. *J Chem Ecol* 17(12): 2449-2458.
48. **de Haan, L.H.J., I. Bosselaers, W.M.F. Jongen, R.M.L. Zwijsen, and J.H. Koeman. 1994.** Effect of lipids and aldehydes on gap-junctional intercellular communication between human smooth muscle cells. *Carcinogenesis* 15(2): 253-256.
49. **Hamilton, J.G.C., A.J.E., Gough, B.W. Staddon, and D.E. Games. 1985.** Multichemical defense of plant bug *Hotea gambiae* (Westwood) (Heteroptera: Scutelleridae): (E)-2-hexenol from abdominal gland in adults. *J Chem Ecol* 11(10): 1399-1410.
50. **Hatanaka, A., T. Kajiwara, and K. Matsui. 1995.** The Biogenesis of Green Odour by Green Leaves and Its Physiological Functions – Past, Present and Future. *Z Naturforsch* 50C: 467-472.
51. **Hebard, M. 1917.** Blattidae of North America, North of the Mexican Border. *Memoirs of the Ann Entomol Soc Am*, No. 2. American Entomological Society at the Academy of Natural Sciences.

52. **Hildebrand, F.F., J.G. Rodriguez, G.C. Brown, K.T. Luu, and C.S. Volden. 1986.** Peroxidative responses of leaves in two soybean genotypes injured by twospotted spider mites (Acari: Tetranychidae). *J Econ Entomol* 79: 1459-1465.
53. **Jacobson, M. 1966.** Chemical Insect Attractants and Repellents. *Annu Rev Entomol* 11: 403-422.
54. **Jefson, M., J. Meinwald, S. Nowicki, K. Hicks, and T. Eisner. 1983.** Chemical Defense of a Rove Beetle (*Creophilus maxillosus*). *J Chem Ecol* 9(1): 159-180.
55. **Karban, R. and I.T. Baldwin. 1995.** Induced Responses to Herbivory. The University of Chicago Press, Chicago, IL.
56. **Kasu, T., G.C. Brown, and D.F. Hildebrand. 1995.** Formation of lipoxygenase products in *Phaseolus vulgaris* L. Leaves as a response to twospotted spider mite (Acari: Tetranychidae) feeding and their effect on spider mite populations. *J Kans Entomol Soc* 68(1): 27-34.
57. **Konček, S.K. 1924 (in Stay 1957).** Zur Histologie der Rückendrüse unserer einheimischen Blattiden. *Z Wissen Zool* 122: 311-322.
58. **Lewis, C.T. 1980.** The Penetration of Cuticle by Insecticides, pp. 367-400. In T.A. Miller [ed.], *Cuticle Techniques in Arthropods*. Springer-Verlag, NY.
59. **Miller, J.S., R.W. Howard, T. Nguyen, A. Nguyen, R.M.T. Rosario, and D.W. Stanley-Samuelson. 1996.** Eicosanoids mediate nodulation responses to bacterial infections in larvae of the tenebrionid beetle, *Zophobas atratus*. *J Insect Physiol* 42(1): 3-12.
60. **Minitab Inc. 1998.** Minitab 12.0 User's Guide. Minitab Inc., State College, Pa.
61. **Nicotera, P., D. McConkey, S.A. Svensson, G. Bellomo, and S. Orrenius. 1988.** Correlation between cytosolic Ca<sup>2+</sup> concentration and cytotoxicity in hepatocytes exposed to oxidative stress. *Toxicology* 52(1-2): 55-63.
62. **Peschke, K. and T. Eisner. 1987.** Defensive secretion of the tenebrionid beetle, *Blaps mucronata*: Physical and chemical determinants of effectiveness. *J Comp Physiol A Sens Neural Behav Physiol* 161: 377-388.
63. **Petruzzi, S., M. Fiore, G. Dell'Omo, and E. Alleva. 1995.** Exposure to Ozone Inhibits Isolation-Induced Aggressive Behavior of Adult CD-1 Male Mice. *Aggressive Behavior* 21: 387-396.
64. **Prestwich, G.D. and G.N. Blomquist. 1987.** Pheromone Biochemistry. Academic Press, NY.

65. **Quicke, D.L.J. 1997.** Parasitic Wasps. Chapman and Hall, NY.
66. **Rohlf, F.J. 1995.** Statistical tables, 3<sup>rd</sup> ed. W.H. Freeman and Co., NY.
67. **Roth, L.M. and D.W. Alsop. 1978.** Toxins of Blattaria, pp. 465-487. *In* S. Bettoni [ed.], Arthropod Venoms. Springer-Verlag, NY.
68. **Roth, L.M. and T. Eisner. 1962.** Chemical Defenses of Arthropods. *Annu Rev Entomol* 7: 107-136.
69. **Roth, L.M. and E.R. Willis. 1960.** The Biotic Associations of Cockroaches. Smithsonian Miscellaneous Collections 141. Smithsonian Institution, D.C.
70. **Roth, L.M., W.D. Niegisch, and W.H. Stahl. 1956.** Occurrence of 2-Hexenal in the Cockroach *Eurycotis floridana*. *Science* 123: 670-671.
71. **Rule, G.S. and W.L. Roelofs. 1989.** Biosynthesis of sex pheromone components from linolenic acid in arctiid moths. *Arch Insect Biochem Physiol* 12(2): 89-97.
72. **Schei, P.O. and T. Smythe, Jr. 1967.** Electrical measurements on cuticles excised from adult male *Periplaneta americana* (L.). *Comp Biochem Physiol* 21: 547-571.
73. **Schneider, M. and A. Dorn. 1994.** Lipid storage and mobilization by flight in relation to phase and age of *Schistocerca gregaria* females. *Insect Biochem Molec Biol* 24(9): 883-889.
74. **Seliverstov, G.A. 1984.** The action of ketamine and hexenal on excitable membranes of the frog neuromuscular preparation. *Farmakol Toksikol (Mosc)* 47(4): 45-51.
75. **Sokal, R.R. and F.J. Rohlf. 1995.** Biometry: the principles and practice of statistics in biological research, 3<sup>rd</sup> ed. W.H. Freeman and Co., NY.
76. **SPSS Inc. 1998.** SPSS Base 8.0 Applications Guide. SPSS Inc., Chicago, IL.
77. **Stay, B. 1957.** The Sternal Scent Gland of *Eurycotis floridana* (Blattaria: Blattidae). *Ann Entomol Soc Am* 50: 514-519.
78. **Stryer, L. 1995.** Biochemistry. W.H. Freeman, N.Y.
79. **Sullivan, S. 1997.** William and Mary Senior Honors Thesis, College of William and Mary, VA.
80. **Thor, H., P. Hartzell, and S. Orrenius. 1984.** Potentiation of oxidative cell injury in hepatocytes which have accumulated Ca<sup>2+</sup>. *J Biol Chem* 259(10): 6612-6615.

81. **Treherne, J.E. 1957.** The diffusion of non-electrolytes through the isolated cuticle of *Schistocerca gregaria*. *J Insect Physiol* 1: 178-186.
82. **Tumlinson, J.H., W.J. Lewis, and L.E.M. Vet. 1993.** How parasitic wasps find their hosts. *Sci Amer* 268(3): 100-106.
83. **Visser, J.H. and Y. Fu-Shun. 1995.** Electroantennogram responses of the grain aphids *Sitobion avenae* (F.) and *Metopolophium dirhodum* (Walk.) (Hom., Aphididae) to plant odour components. *J Appl Entomol* 119: 539-542.
84. **von Nickisch-Roseneck, E. and M. Wink. 1993.** Sequestration of pyrrolizidine alkaloids in several arctiid moths (Lepidoptera: Arctiidae). *J Chem Ecol* 19(9): 1889-1903.
85. **Wallbank, B.E. and D.F. Waterhouse. 1970.** The Defensive Secretions of *Polyzosteria* and Related Cockroaches. *J Insect Physiol* 16: 2081-2096.
86. **Waterhouse, D.F. and B.E. Wallbank. 1967.** 2-Methylene butanal and related compounds in the defensive scent of *Platyzosteria* cockroaches (Blattidae: Polyzosteriinae). *J Insect Physiol* 13: 1657-1669.
87. **Wigglesworth, V.B. 1942.** Some notes on the integument of insects in relation to the entry of contact insecticides. *Bull Entomol Res* 33: 205-218.
88. **Zar, J.H. 1996.** *Biostatistical Analysis*. Princeton Hall, Upper Saddle River, NJ.

## VITA

### Matthew William Turnbull

Born in Richmond, Virginia, July 4, 1973. Graduated from Lee-Davis High School in Mechanicsville, Virginia (just outside Studley), June 1991. Graduated from The College of William and Mary, Virginia, December 1994, B.S. in Biology. Candidate for M.A. in Biology, The College of William and Mary, Virginia. Thesis work, in progress, entitled: Characterization of the Defensive Efficacy of the Sternal Secretion of *Eurycotis floridana* (Walker) (Dictyoptera: Blattidae).

Employed by The College of William and Mary as a Teaching Assistant from August 1996 to May 1998. Also, employed as a research assistant searching for invertebrate orthologues of vertebrate B-cell specific activating protein.