HINDGUT SECRETIONS IN *CAMPONOTUS PENNSYLVANICUS* (DE GEER) (HYMENOPTERA: FORMICIDAE): ATTRACTANTS AND NITROGENOUS EXCRETORY MATERIALS

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ABSTRACT

The anatomical source of the trail pheromone in the black carpenter ant, *Camponotus pennsylvanicus*, was investigated by presenting workers with crude or synthetic hindgut extracts to test for attractancy and trail-following behavior. Chemical analysis was used in conjunction with behavioral bioassays to detect and identify volatiles from the rectal sac, poison, and Dufour's glands. The rectal material was also examined to determine levels of total nitrogen and identify metabolites in relation to other solid material present.

Under laboratory conditions, foragers demonstrated a significant level of attraction to a combined Dufour's gland, poison gland, and rectal sac extract. No response was observed to synthetic compounds (formic acid and saturated hydrocarbons) from the poison or Dufour's gland. Two volatiles, n-undecane and n-tridecane, were identified from the Dufour's gland. Fatty acids and esters were found to be ubiquitous in the Dufour's and poison glands. Palmitic acid was identified in the poison gland. A compound described as a component of the trail pheromone in *Camponotus atriceps* (3,4-dihydro-3,5-dihydroxy-6-methyl-pyran-4-one) was identified in the rectum, but was not verified behaviorally as being part of the trail pheromone for *C. pennsylvanicus*. Trail following was not elicited from any of the rectal sac extracts.

Dry weight analysis determined that the rectal material was only 14% solid material and total nitrogen levels were estimated at 19.2 ± 2 ug/mg of ant feces. Most of the components contributing to the total nitrogen excreted were left unidentified, but ammonia (2.7 ± 1.2 ug/mg), two tryptophan intermediates (kynurenic and xanthurenic acid) and one pteridine (biopterin), were identified.

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DEDICATION

This thesis is dedicated to my mother, Sharon, and my father, Paul, for teaching me to never take my eyes off the goal, and to Alice and John, for always having faith in me.

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CHAPTER 1. INTRODUCTION

As invertebrates, ants are by no means equivalent in intelligence to man, but the dominance and ecological success demonstrated by the eusocial superorganism has succeeded in capturing the attention of man. Energy use and biomass of ants overshadow vertebrates in many terrestrial habitats where the two coexist. Ants have dealt with ecological problems normally combated by individuals by use of caste specialization, altruism, and chemical communication. Eusocial behavior is a term used to describe a group where care of the young is cooperative, labor is divided by reproductive status, with sterile members working for the benefit of reproductives, and two overlapping generations with the ability to contribute to colony labor are present (Wilson 1971). Tasks for daily survival are carried out simultaneously rather than sequentially, and chemical communication plays a major role in achieving and maintaining cohesion in the colony.

Ants have been described as walking batteries of exocrine glands, and chemicals (pheromones) released from these glands regulate a range of behaviors from mating to kin recognition to alarm behavior. The odor trail primarily used in foraging is considered the most advanced system of chemical communication. Successful foraging is dependent, in part, on odor trails to excite and orient nestmates to a food source. Such behavior is termed "recruitment" and is believed by some to have evolved from ritualized defecation (Traniello 1977). The chemical nature and source of the odor trail is still largely unknown in many ant species and is the primary subject of this study.

The ant we used for studying the odor trail was the black carpenter ant, *Camponotus pennsylvanicus* De Geer, both for its importance as a pest insect and its ready availability in the Commonwealth of Virginia. *Camponotus pennsylvanicus* has been shown to use both group recruitment and mass communication in its foraging behavior (Traniello 1977). In early studies on the biology of *C. pennsylvanicus* colonies, Pricer (1908) observed that chemical trails could be laid outside the nest, but he was uncertain of the connection with orientation and suggested ants used memory to guide them instead. Later studies in the 1970s showed that workers exhibit trail-laying behavior by contacting the gaster, or tip of the abdomen, to the surface of substrate along which they are traveling (Traniello 1977, Hartwick et al. 1977). The gaster was the first clue researchers had in determining the anatomical source of the trail pheromone. One organ, the rectal sac, and two accessory glands, the poison reservoir and Dufour's gland, have since been the focus of behavioral and chemical studies.

The results of behavioral bioassays and chemical analysis of hindgut material in ants have been complicated by a number of factors. First, the difference between attraction and recruitment has not been addressed in the literature as the two concepts have always been examined separately. A number of aliphatic hydrocarbons have been identified from the Dufour's gland of *C. pennsylvanicus* and shown to serve as attractants for up to 32 minutes (Ayre and Blum 1971). However, their data did not differentiate between recruitment and attraction. If the trail pheromone did indeed evolve from ritualized defecation, then we may deduce that material from the rectal sac may be involved.

The hindgut and rectum have been cited interchangeably in the literature as being the source of the trail pheromone, but specific material used in preparation of hindgut extract samples is unclear (Blum 1974, Attygalle and Morgan 1984, Bestmann et al. 1995). The "hindgut" could refer to the rectum plus the accessory glands, or the rectum alone. Interpretation of results becomes inconclusive under such confusion. Variability in the design of bioassays also causes problems when critically comparing results of previous studies. Artificial trail techniques have generally involved the deposition of pencil line choice tests prepared from gland extracts (Hartwick et al. 1977). However, choice of substrate and applicator can affect the response of workers. Some researchers have used wooden applicator sticks and paper cards; others have used glass plates and microsyringes. The choice of solvent in which the glands are extracted also affects what compounds become a part of the artificial trail. When unknown compounds are being tested, solubility factors must be taken into account.

While only two studies have focused specifically on the trail pheromone of *C. pennsylvanicus* (Traniello 1977, Hartwick et al. 1977), the results of other studies on the Formicinae have confirmed that the hindgut is the anatomical source (Blum and Wilson 1964). The chemistry of the trail pheromone for *C. pennsylvanicus* has not been described to date. Bestmann et al. (1997) identified two δ-lactones in the rectal bladder of *C. pennsylvanicus*, but no behavioral tests were discussed to confirm that either compound actually functioned as a trail pheromone.

The objectives of the studies reported here were to determine the glandular source of the odor trail from behavioral studies and compare the difference between attraction and trail following. Rectal material was collected prior to defecation to determine what percentage was solid material with the goal of further identifying both volatile and

nitrogenous compounds that have yet to be described. Pheromone mixtures and the role of synergists was also investigated. All studies involved workers of *C. pennsylvanicus*, which is the most commonly found carpenter ant species in Virginia (Mallis 1982).

CHAPTER 2. LITERATURE REVIEW

The exocrine secretions of hymenopterans are among the most chemically complex and diverse of the animal kingdom. Despite a rich abundance of chemicals that may offer important keys to social behavior and communication, only the major glands of relatively few social insect species have been described (Blum and Brand 1972). One of the most intricate types of chemical communication resulting from exocrine secretions is the odor trail strategy and is believed to have evolved from tandem running in some ant groups. In tandem running, a scout returns from a food source and waits for her abdomen to be contacted by the antennae of a nestmate. The scout then runs ahead a certain distance while releasing a secretion and waits to be prompted again by the excited nestmate. Intermediate stages exist between the tandem running and the odor trails in ants such as *Camponotus paria* Emery, and *C. compressus* Fabricius, where the scout does not need tactile stimulation to lead workers along a trail. The more advanced stages of odor trails don't require a scout for recruitment to occur as secretions from the scout alone are enough to guide workers to a food source (Wilson 1971).

In 1959, Wilson demonstrated recruitment in *Solenopsis saevissima* Smith by devising artificial trails made from gland extracts. From this study and others, it was learned that ants secrete a trail pheromone that generates a semiellipsoidal active space in which the pheromone concentration exceeds the threshold and triggers a following response. Ants have the ability to contact the trail and orient to the concentration gradient by osmotropotaxis in which they move antennae from side to side to maintain contact with the active space. The amount of trail substance secreted determines how many workers respond. Recruitment trails differ from exploratory trails in that workers only lay them when returning from a food source or a new potential nest site. Trail following is exhibited in the Myrmicinae, Dolichoderinae, and Formicinae (Wilson 1971).

Studies involving pheromones have been complicated by the fact that signals created by exocrine glands involve major and minor compounds that differ in volatility. The highly volatile compounds function in initial excitement and attraction to a trail, while the less volatile compounds maintain trail integrity by keeping the insects oriented for a sustained period of time. Gas-chromatographic analyses have shown that minor compounds are present in trace amounts and are compounds left after the more volatile components have evaporated. Newly laid trails stimulate initial excited trail following and minor components ensure an adequate trail is maintained for food retrieval. Compounds

that function in alarm and defense behavior have been the most successfully investigated pheromones and low concentrations have been observed to increase the recruitment efficiency of an odor trail (Blum 1974).

In order for pheromones to transmit signals through the air, they have to fall in the range of carbon number 5-20 and a molecular weight of 80-300 (Hölldobler and Wilson 1990). Such molecular specifications apply to the majority of described pheromones as large molecules are less volatile, and require more energy to biosynthesize and transport across membranes. The multifunctionality of pheromones lies in the fact that the concentration can dictate different behaviors. A low level of a particular compound may allow recruitment of ants, while a high level may result in alarm (defense) behavior. Some ant species are sensitive to a particular chemical configuration or isomerism, resulting in a lower concentration needed to create a signal. Species-specific trails are created by secreting a mixture of compounds from the same gland or from different glands. Compounds that don't need to be specific, such as alarm substances, have a lower molecular weight than other trial substances, and are therefore less unique (Hölldobler and Wilson 1990).

2.1 Trail Substances in the Formicinae

Scientists have known that chemicals could be detected in ants since 1670 when formic acid was identified in *Formica rufa* Latreille workers (Blum and Brand 1972). Formic acid is a fatty acid that was later found to be characteristic of other fomicine species and is the only volatile compound to have been identified in the poison gland (Blum and Hermann 1978). Alarm pheromones are secreted in greater quantities than other pheromones as ants can make and store large amounts (Blum 1974). Lopez et al. (1993) discovered that the poison apparatus is lined with hexadecanol and its esters to protect the tissue from the venom's corrosive properties. The apparatus is composed of tubular filaments, the convoluted gland, and the reservoir in which the venom is stored (Lopez et al. 1993). Formicine ants have an acidopore instead of a stinger, and the venom secreted can consist of an aqueous formulation of formic acid in concentrations as much as sixty percent (Attygalle and Morgan 1984). Alarm pheromones are not used solely in defense as they have also been found to increase the attractiveness of a recruitment trail in *Formica* spp. Linneaus (Blum 1974). One of the earliest trail systems to be investigated was that of *Lasius fuliginosus* Latreille, and was found to serve as a guide to assist workers in finding the aphid colonies containing the honeydew on which the ants feed (Kern 1997).

Attempts to identify trail pheromones have generally utilized two types of approaches: 1. Dissection of exocrine glands and creation of artificial trails from which behavioral observations could be drawn, and 2. GC-MS analysis for identification of compounds. Some studies have incorporated both these methods. For example, thirty-three compounds have been found in the mandibular gland of the African weaver ant, *Oecophylla longinoda* Latreille, each at a level of at least 5 ng per gland. Gas chromatographic analysis showed the major components of the mandibular glands to be hexanol and 1-hexanol, which are used in alarm. The effects of the individual components of the mandibular glands were determined to be additive rather than synergistic by presenting the ants with the compounds and measuring the distance of attraction within a certain radius (Bradshaw et al. 1975).

In the Formicinae subfamily, the hindgut is the source of trail pheromones for most species studied. Blum and Wilson (1964) examined response to artificial trails prepared from hindgut extracts of two formicine ants, *Myrmelachista ramulorum* Wheeler and *Paratrechina longicornis* Latreille. Behavioral responses were compared to myrmecine and dolichoderine species. Although separated widely by different branches within the subfamily, only the formicine species (*M. ramulorum* and *P. longicornis*) followed the trail, pointing to the hindgut as the source. The authors were unable to determine the mechanism of trail pheromone production. Bestmann (1997) investigated the synthesis of compounds in the hindgut, but endosymbionts living in the hind gut make it difficult to determine outside roles in the creation of signals. To date, it is not known if microorganisms have an effect.

One of the hindgut glands, the Dufour's gland, was first described in 1841 as an accessory to the poison gland apparatus in ants and bees. In ants, the gland synthesizes hydrocarbons, with the major collection of compounds being undecane and tridecane in the formicines (Attygalle and Morgan 1984). Thirty-nine substances have been identified in the gland of *F. rufa*, twelve of which are greater than 1% of the total secretion, indicating the importance of multiple compounds in releasing behavior (Lofqvist 1976). In *L. fuliginosus*, undecane comprises 76.5% of the contents of the Dufour's gland (Ali et al. 1988a). Dufour's gland contents change with the age of workers in colonies of *Formica sanguinea* Latreille. Analysis of newly emerged workers versus older foragers showed that undecane levels decline over time (Ali et al. 1988b).

The Dufour's gland can produce hydrocarbons or oxygenated compounds in ants of the genus *Formica*, making it possible to group species according to which components are secreted. In *Formica selysi* Bondroit, 28 hydrocarbons have been described, with the major compound being undecane as 68% of the total. In contrast, *Formica rufibarbis* Fabricius secrete oxygenated compounds, with decyl acetate comprising 35% of the total (Bagneres et al. 1991). The hydrocarbons, along with acetates, serve as a spreading agent for formic acid produced by the poison gland (Attygalle and Morgan 1984). Ability to secrete compounds separate from the poison gland has always been questioned as the Dufour's gland opens in the short duct of the poison vesicle (Bergstrom and Lofqvist 1973). The Dufour's gland is not only unique in diversity of compounds, but in morphology as well; the bi-lobed structure found in *Camponotus* and *Formica* species is rare in other Hymenoptera (Blum and Hermann 1978).

2.2 Biology of *Camponotus*

Carpenter ants, genus *Camponotus* Mayr, belong to the second largest subfamily of ants, Formicinae, with the largest being the Myrmicinae (Hölldobler and Wilson 1990). One of the most advanced subfamilies, the Formicinae include *Formica* (wood ants), *Camponotus*, and *Lasius* spp. Fabricius. They are identified by the single node petiole and stinger modified to an acidopore (Dumpert and Johnson 1981). Four species of *Camponotus* are found in the northeastern portion of North America: *C. herculeanus* Linnaeus, *C. pennsylvanicus* De Geer, *C. noveboracensis* Fitch and *C. ferrugineus* Emery (Sanders 1964).

Camponotus is a large genus within the ant family, encompassing approximately 1000 species world-wide. Winged reproductives (alates) leave their colony of origin and mate between April and June, depending on species and geography. The mating flight is triggered by a variety of factors, including photoperiod, temperature, humidity, and day length. The sexes are brought together by a pheromone secreted by the male mandibular gland. Males die after mating and queens seek new nest sites. The first set of brood consists of 9 to 16 eggs which adhere to one another via tiny hairs to allow the queen to move the entire set when disturbed (Hansen and Akre 1990). The eggs reach the larval stage in two to three weeks (Hansen and Akre 1990), and complete development in approximately 66 days (Pricer 1908).

The queen is presumed to feed herself and the first brood through metabolism of her fat body and flight muscles, an assumption supported by Cannon's (1990) analysis of nutrient reserves during early stages of nest founding. The larvae spin cocoons upon entering the pupal stage and emerge at the completion of development with help from the workers. As the colony expands, workers take over liberating new adults from cocoons. The queen lays the second brood in July or August and the larvae overwinter as first instars. Initial brood all consist of minor workers that are fairly homogeneous in size (nanitics) and all assist the queen with brood care and nest maintenance or enlargement, in addition to foraging for food. Workers, reproductives, and larvae in various stages of development, enter diapause during the winter and resume normal activity in the spring. No overwintering occurs in the egg or pupal stages (Price 1908, Hansen and Akre 1990).

Worker size is determined by the quality of the food supply with greater resources resulting in greater size variation. Intermediate and major workers don't appear until the third season in *C. ferrugineus, C. pennsylvanicus,* and *C. modoc* Wheeler. Mature colonies that have been in existence over a year often have year-round satellite components. Such populations stay in contact with the parent colony (or "brood colony"), which is located in an area of higher humidity (Sanders 1964, Hansen and Akre 1990) . Mature colony size varies among species. For *C. herculeanus* counts have been estimated to be 12,000 workers while *C. novaboracensis* colonies are somewhat smaller (8900). *C. pennsylvanicus* have been recorded as having 3000 workers while *C. ferrugineus* have approximately 2000. Reproductives are produced when the colony is between three to six years old, depending on whether queen founding was monogynous or polygynous (Pricer 1908, Hansen and Akre 1990).

As forest dwellers, carpenter ants, particularly *C. modoc,* build a tunnel system through the ground litter that can range from a centimeter to a meter deep. The tunnels often parallel tree roots where the ants feed on honeydew from aphid colonies of the genus *Cinara* (Sanders 1964). *C. pennsylvanicus* colonies have been found to be infested with a red mite (*Uropoda* spp.),which may have a parasitic relationship with the ants, but no conclusive studies have been done (Pricer 1908). Orientation of workers is achieved through the release of a hindgut trail pheromone in *C. pennsylvanicus, C. americanus* Mayr, and *C. modoc*. The majority of *C. pennsylvanicus* ants exhibit nocturnal foraging during June and July, while other *Camponotus* species vary activity according to geography, season, and competition with *Formica* species (Fowler and Roberts 1980, Klotz 1984). Nests are constructed by excavating galleries in wood and carpenter ants

infest both sound and rotted portions of living or dead trees, with the exception of trees less than 15.2 cm in diameter or wood in progressed stages of rotting (Sanders 1964, Hansen and Akre 1990). Sometimes the colony will move the brood into the surrounding soil or rocks during the winter to help with thermoregulation (Hansen and Akre 1990).

2.3 Pest Status

There are twenty-three species of carpenter ants in North America that are classified as structural or nuisance pests, but only seven of the pest species can potentially cause significant damage to wood (Akre et al. 1995). *Camponotus modoc* in the Pacific Northwest and *C. pennsylvanicus* in the Northeast and Midwest are the two most common carpenter ant species that damage wood structures. *Camponotus modoc* workers tend to do the most damage as colonies tend to be larger, but carpenter ant pest status has been debated as either a nuisance pest to homeowners or a structural pest because actual damage is usually minor or cosmetic (Klotz and Akre 1991).

Suburbanization in the 1950s and 60s put houses near wooded areas where carpenter ants nest and the species became more of a concern as a structural pest. Control strategies are not very efficient and carpenter ants have even surpassed termite infestation in northeastern areas of the country (Fowler 1983). In New Jersey, *C. pennsylvanicus* nest in urban shade trees and have been attributed to the cause of increased wind throw damage (Fowler and Roberts 1982). Hardwood trees are most commonly infested and colonies have been found in apple (*Malus* spp.), oak (*Quercus* spp.), hickory (*Carya* spp.), boxelder (*Acer negundo* L.), ash (*Fraxinus* spp.), linden (*Tilia* spp.), and cherry (*Prunus* spp.) (Pricer 1908).

2.4 Trail substances in *Camponotus* spp.

Camponotus is one of the largest genera in size and distribution, but few investigations have been done regarding chemical communication, and many questions still remain (table 2.1). Ants have been known to utilize chemical trails as early as 1900. In 1959, E. O. Wilson found that fire ants *Solenopsis* Westwood, display certain behaviors, such as trail following, when exposed to contents of abdominal organs. Wilson later found the source of the chemical trails to be the hindgut in four species of the Formicinae subfamily and the poison, tarsal, Dufour's, and Pavan's glands in other subfamilies tested (Hartwick et al. 1977).

Table 2.1 Summary of Trail Following Studies for *Camponotus* spp. Those compounds that serve as trail pheromones are listed as functioning in recruitment. Some studies examined only behavior and thus anatomical sources or identified compounds are not described.

Gland	Species	Compound	Function	Reference
Rectum	*C. herculeanus*	2,4-dimethyl-5-hexanolide	recruitment	Bestmann et al. 1995
	C. silvicola *C. rufipes*	3,4-dihydroisocoumarins	recruitment	Ubler et al. 1995
	C. atriceps *C. floridanus*	3,5-dimethyl-6-(1'-methylpropyl)-tetrahydropyran-2-one nerolic acid	recruitment	Haak et al. 1996
	C. pennsylvanicus	no data	recruitment	Hartwick et al. 1977
	C. modoc	no data	attraction and recruitment	Hansen and Akre 1985
Dufour's	*C. ligniperda* *C. herculeanus*	tridecane	no data	Bergstrom and Lofqvist 1972
	C. intrepidus	undecane tridecane pentadecane	no data	Brophy et al. 1973
Dufour's Poison	*C. pennsylvanicus*	n-undecane formic acid	attraction	Ayre and Blum 1971
no data	*C. pennsylvanicus*	no data	recruitment	David and Wood 1980

Hartwick et al. (1977) used Wilson's bioassay to investigate the role of odor and light in the orientation of *C. pennsylvanicus.* Artificial trees were used to determine the degree to which *C. pennsylvanicus* used odor trails. Food and lights were used as stimuli and alternated with a control (empty food vial) among several dowel "branches". As soon as the majority of ants were following the trail leading to food, the light source was switched to the opposite trail. Pencil line trails from Dufour's, poison, and rectal extracts resulted in 85% of workers tested following the rectal trail. The results of the bioassay confirmed Wilson's findings that the source of the trail in Formicinae spp. is indeed the hindgut. In contrast to findings from Hölldobler (1971) on orientation in *C. socius* Roger, motor displays in conjunction with chemical trails were not

observed to be as crucial to orientation in *C. pennsylvanicus*. The results were less conclusive concerning the role of light and seemed to indicate that changes in direction or intensity can cause confusion while odor may in fact be the more important cue of the two in recruitment of workers to a food source.

David and Wood (1980) showed that chemicals are used in ant trails through the removal and replacement of leaf litter in areas where colonies of *Camponotus pennsylvanicus* traveled. They studied the effect of visual cues on orientation as well, but discovered that the chemicals alone are enough, and ants will follow a trail even in the absence of visual cues. However, visual cues can be used to re-create a chemical trail that has been disturbed. As part of a comprehensive study on *C. modoc* ant biology, Hansen and Akre (1985) investigated potential attractants by dissecting the hindgut, poison reservoir, and Dufour's glands separately into distilled water. After crushing the glands into methylene chloride, a sample of each extract was presented on filter paper disks to a colony. Trails were also laid on strips of pine wood to determine the degree of orientation and trail following. Only the hindgut extraction resulted in attraction and trail following.

The chemistry of trail pheromones is only known for a few formicine species and a component of the genus *Camponotus* was first identified by Bestmann et al. (1995). Identification of the compounds was achieved by dissection of the rectal bladder (hindgut) of *C. herculeanus*, extraction, and analysis by gas chromatography, coupled with mass spectrophotometry. The main component of the pheromone was determined to be 2,4-dimethyl-5-hexanolide. Species of *C. herculeanus*, *C. ligniperda* Latreille, *C. vagus* Scopoli, *C. socius*, and *C. pennsylvanicus* all followed artificial trails of the pheromone. The pheromone is a lactone that has been found in prior research to serve as a sex pheromone in species of formicine ants such as *Calomyrmex* Emery, *Camponotus thoracicus fellah* Fabricius, and *Lasius niger* Linnaeus. The research was expanded by Ubler et al. (1995) to include *C. silvicola* Forel and *C. rufipes* Fabricius. Using artificial trails and electroantennograms to analyze the volatiles from the two species of *Camponotus*, 3,4-dihydroisocoumarins were found to be prevalent in the rectal bladder.

The Dufour's gland secretions of the ants *Camponotus ligniperda* and *Camponotus herculeanus* were compared, and in conjunction with similarities in morphology between the species, the volatile compounds from workers were similar. The principal components found in the two species were straight chain saturated hydrocarbons with tridecane being a major component (Bergstrom and Lofqvist 1972). Haak et al. (1996) compared the gland

contents (Dufour's gland and rectal bladder) of *Camponotus atriceps* Smith and *Camponotus floridanus* Buckley found in the subtropics. Differences found through GC-MS analysis showed gland contents to be species specific. After behavioral bioassays and GC-MS analysis, the rectal gland was the only source attributed to the secretion of trail pheromones. The major components of the pheromone were identified as 3, 5-dimethyl-6-(1' -methylpropyl)-tetrahydropyran-2-one and nerolic acid.

Ayre and Blum (1971) extracted mandibular, Dufour's, and poison glands from *C. pennsylvanicus*. Mass spectrometry revealed the Dufour's gland contents to be a mixture of n-decane, n-undecane, tridecene, n-tridecane, and n-pentadecane. The mandibular glands were not significant in affecting behavior in contrast to the Dufour's and poison glands. The greatest response occurred to n-undecane and formic acid (poison gland), which, when combined, acted as an attractant that lasted up to 32 minutes. Ayre and Blum (1971) concluded that these chemicals were attractants normally used in alarm responses. In agreement with the findings for formicines as a group, the secretions of the Dufour's gland in *C. intrepidus* Kirby were found to contain n alkanes, such as undecane, tridecane, and pentadecane, as the major components, and are released in conjunction with formic acid from the poison gland (Brophy et al. 1973).

Barlin et al. (1976) found that *C. pennsylvanicus* followed pheromone extracts isolated from *C. americanus* and *C. socius*. Assays were carried out using a pentane extract and the ants only responded to a concentration of 0.010 hindguts per cm. Extracts taken from the walls of the mid or hindgut had no effect. Traniello (1977) demonstrated that the hindgut was the source of pheromone in *C. pennsylvanicus*. Benzene extracts from the poison gland, the Dufour's gland, and the hindgut were tested and the workers responded 71% better to the hindgut, based on degree of trail-following behavior, over the other extracts. Traniello also noted that prior motor stimulation was not necessary for trail following in *C. pennsylvanicus* as opposed to *C. socius*. He emphasized the importance of identifying trail pheromone components in *C. pennsylvanicus* because chemical mass communication is more advanced than in *C. socius* or *Formica fusca* Linnaeus.

2.5 Other potential trail substances

All animals, including insects, ingest more nitrogen than is needed for bodily functions and therefore must excrete the excess material. The majority of insects void excess material in a uricotelic pathway similar to mammals, birds, bacteria, and yeast. The

rectum and Malpighian tubules are responsible for nitrogenous excretion. Uric acid is the major product released because it is high in nitrogen, highly oxidized, and can be voided as a solid, but there are also a number of other products. Allantoin and allantoic acid are two products that are closely related to uric acid, and those insects that produce them have two additional enzymes: uricase to produce allantoin from uric acid, and allantoinase to produce allantoic acid from allantoin (Cochran 1985). Some Hymenoptera that have been investigated, such as *Hemichroa alni* Linnaeus and *Pteronidea salicis* Rohwer or *P. ribesi* Rohwer (black gooseberry sawfly), excrete either uric acid or allantoin and allantoic acid (Bursell 1967).

Purines can also result from modifications in the uric acid metabolic pathway to give hypoxanthine, xanthine, adenosine, guanine, allantoin, allantoic acid, and uric acid riboside (Cochran 1985). Guanine has been found to be 73 to 87% of the nitrogen excreted in spiders. Hypoxanthine and uric acid have been isolated in some species of scorpions (Anderson 1966). Urea is also present in insect feces, but is a minor product in most terrestrial insects because it is normally secreted in liquid form and comes from metabolism of nitrogen rather than direct dietary sources. Tryptophan is an amino acid used to form ommochrome pigments, but the free form is not detected very often in various parts of insects. Intermediates of tryptophan catabolism that are more common to find include kynurenic acid, xanthurenic acid, and 8-hydroxyquinaldic acid (Cochran 1985). All three tryptophan metabolites have been found in the American cockroach and account for 1-3% of the total nitrogen excreted (Mullins and Cochran 1973a). Depending on diet and sex, total nitrogen levels in fecal material of the American cockroach have been estimated between 20 and 40 ug/mg with 2-10 ug/mg of the total in the form of ammonia nitrogen (Mullins and Cochran 1973b). Pteridines that also function in pigmentation, such as xanthopterin, biopterin, and isoxanthopterin, have been found in insect feces (Cochran 1985).

The literature offers very little information on the products that have been found in Hymenoptera, and there are virtually no data with regard to ants. In the entire bodies of *Formica rufa* Linnaeus, *F. polyctena* Foerster, and *F. cordieri* Bondroit, several pterines were isolated, mainly from the muscle, integument, and gut (Schmidt and Viscontini 1964). Thirteen amino acids were located in the poison gland secretion of *C. pennsylvanicus* through two dimensional chromatography analysis and found to be dispersing agents for formic acid sprayed in defense (Hermann and Blum 1968).

2.6 Olfactory responses to trail substances

Chemical analysis followed by bioassay of behavioral response is not the only way to test the significance of a pheromone. Electrophysiological techniques can be used to measure the action potential of whole antennae (or individual sensilla) with tiny electrodes as a summed response. An amplifier allows sensory reception to be magnified enough for the change in voltage potential to be recorded by an oscilloscope. The antennae represent one of the major ways that ants receive signals from chemical communication. These organs are divided into two sections: the scape and funiculus. The funiculus consists of up to 12 segments, the first (pedicel) of which contains Johnston's organ, which is used for orientation. *C. vagus* Scop. has 500 sensory cells in this organ. There are two joints: a ball and socket between the scape and head, and a hinge between the scape and pedicel. Movement is controlled by muscles located in the joints and changes in hemolymph pressure (Dumpert and Johnson 1981).

Electrophysiological studies on *Formica rufa* have shown that formic acid and hydrocarbons previously discussed in this review belong to different reaction groups and are received by different receptor cells on the antennae. Electrophysiological studies have demonstrated an additive effect on alarm response when formic acid and hydrocarbons are combined. The intensity of response also increases when two or more hydrocarbons are combined, indicating that formicine ants use a mixture of substances rather than one to elicit chain reactions involved in alarm or recruitment (Lofqvist 1976).

Payne et al. (1975) measured the olfactory response of *C. pennsylvanicus* queens, males, and workers to mandibular gland secretion (sex pheromone). EAGs were recorded from antennae on head capsules removed from colony members. Clean air was used as a control, and 2-heptanone, an alarm pheromone not found in *C. pennsylvanicus*, but in most other ant species, was used as a standard. All three castes responded to the mandibular gland secretion, but the males had a reduced EAG response. Haak et al. (1996) obtained a positive dose response curve after exposing an antennae from *Camponotus atriceps* to hindgut extracts, which unlike the hydrocarbons found in most formicine species, represent new compounds (lactones) yet to be isolated in this subfamily. Kern and Bestmann (1994) identified a new group of trail pheromones from the rectal bladder (3,4-dihydroisocoumarins) in *Lasius niger* and *Formica* species. The compounds and their analogs were tested for comparison of olfactory and trail following behavior of workers. The natural compounds showed greater electrophysiological and behavioral responses than

the analogs and the number and position of various methyl groups were observed to play a role in chemoreception as well.

CHAPTER 3. RESPONSE OF *C. PENNSYLVANICUS* WORKERS TO CRUDE HINDGUT EXTRACTS AND SYNTHETIC COMPOUNDS

3.1 Introduction

Trail pheromones in ants serve to recruit nestmates to a site where work is required. The deposition of trails by contacting the gaster with the substrate led researchers to investigate and conclude that the hindgut is the source of trail pheromone in formicine ants. Hindgut secretions involved in the trail pheromone may involve the rectal sac, poison gland, and Dufour's gland. Attractive signals are created by a blend of multifunctional chemicals. High concentrations of formic acid from the poison gland, for example, result in alarm behavior, whereas lower concentrations increase the stimulatory effect of a recruitment trail (Blum 1974).

Certain blends secreted by the hindgut cause workers to form clusters for sustained periods of time. Several hydrocarbons identified in the Dufour's gland (n-undecane, n-tridecane, n-pentadecane, 1-tridecene, and n-decane), in combination with formic acid, have been found to produce such behavior without recruitment (Ayre and Blum 1971). The rectal sac in contrast, has been credited with releasing material that is both attractive and stimulatory in terms of oriented trail following (Hartwick et al.1977, Traniello 1977).

The objectives of this study were to first test attractive response to crude gland extracts from the hindgut. Synthetic gland extracts from the poison and Dufour's glands were included in the behavioral bioassays to test the effect of mixtures and concentrations. Trail following response to contents of the rectal sac and gland chemistry analysis are investigated in the next chapter.

3.2 Materials and Methods

3.2.1 Carpenter ant colony maintenance

Colonies of *Camponotus pennsylvanicus* were collected from hardwoods (oak, pine, and locust) in southwestern Virginia during the summers of 1997 and 1998. Once the nests were located, dead portions of trees containing partial colonies were cut, contained within plastic garbage bags, and transported to Price's Fork Research Center where a modified car vacuum cleaner was used to extract the ants. Colonies were housed

in 57 liter glass aquariums in an insulated room where temperature could be kept close to ambient (10 to 27± 5°C from May to early September). Petroleum jelly was used to coat the inner perimeter of the aquariums to prevent escape. Moisture was provided with water-filled polystyrene bottles plugged with cotton. Colonies were fed on an artificial diet modified from Bhatkar and Whitcomb (1970) (Fell unpublished). Six grams of agar were combined with 250 ml of distilled water and brought to a boil while stirring. Cane sugar (150 g), cottonseed flour (5 g), salt (3.5 g) and one egg were combined with another 250 ml of distilled water and placed on a cold stirring block until the agar mixture had boiled 5-10 minutes. The agar was then added to the cold mixture and diet was delivered to 5 ml test tubes and stored in the freezer (- 10 °C) until use.

3.2.2 Crude gland bioassays

C. pennsylvanicus workers were frozen and the hindgut, Dufour's, and poison glands of each were dissected and extracted in methylene chloride. A square foraging arena constructed of plexiglass and measuring 0.6 m long and 6.4 cm deep was connected to the nesting chamber (glass aquarium) by a bridge of white plastic pipe (Figure 3.1). Samples (poison, Dufour's, and hindgut assembly of both glands plus the rectum) equivalent to two glands (200 ul) were presented simultaneously to ants on 5.5 cm filter paper disks. Disks were placed on 10 cm square glass chromotography plates to prevent the foraging arena from becoming contaminated between trials. A filter paper disk with methylene chloride served as the control. The number of workers aggregating on or antennating the samples were recorded every 5 minutes for 30 minutes. Since *C. pennsylvanicus* workers exhibit nocturnal foraging patterns during the summer months (Fowler and Roberts 1980), all bioassays were conducted between 8 and 11 pm at night under red light. Trials were replicated four times and each consisted of a control and the three samples mentioned above.

A second bioassay was designed to test whether workers would follow trails constructed from the gland extracts showing greatest attraction. In the same foraging arena as described above, a Y-shaped choice trail was elevated above the arena floor using four inverted screw-cap glass jars (6.5 cm tall). The first jar was placed flush with the plastic pipe bridge connected to the nest (Figure 3.1). The other jars were greased with petroleum jelly (sides only) and used to complete the Y formation. Three glass rods (0.5 cm in diameter) were placed on top of the glass jars to connect the points of the "Y" and held in

place with modeling clay. The first rod (base of the "Y"), measured 19.5 cm and the two branch rods measured 21.4 cm each. A combined sample of rectum, poison, and Dufour's glands was collected as described for the crude gland samples (chapter 3). Using disposable 1 ml syringes, samples equivalent to two glands (200 ul) were deposited on the first rod and one of the "branches". The other branch was treated with methylene chloride (200 ul) to serve as the control. Food (artificial diet described in chapter 3) was placed at the branch terminal treated with the hindgut extract. Each trial consisted of 10 workers which were allowed to enter the arena via the plastic pipe bridge and observations were made as to the direction each ant traveled down the "Y". Once a worker reached a branch terminal, she was removed to ensure only naive ants were encountering the extracts and not responding based solely on location of food. The branch chosen for treatment with the hindgut extract was randomized and trials were replicated eight times between 9 and 11 p.m. at night under red light.

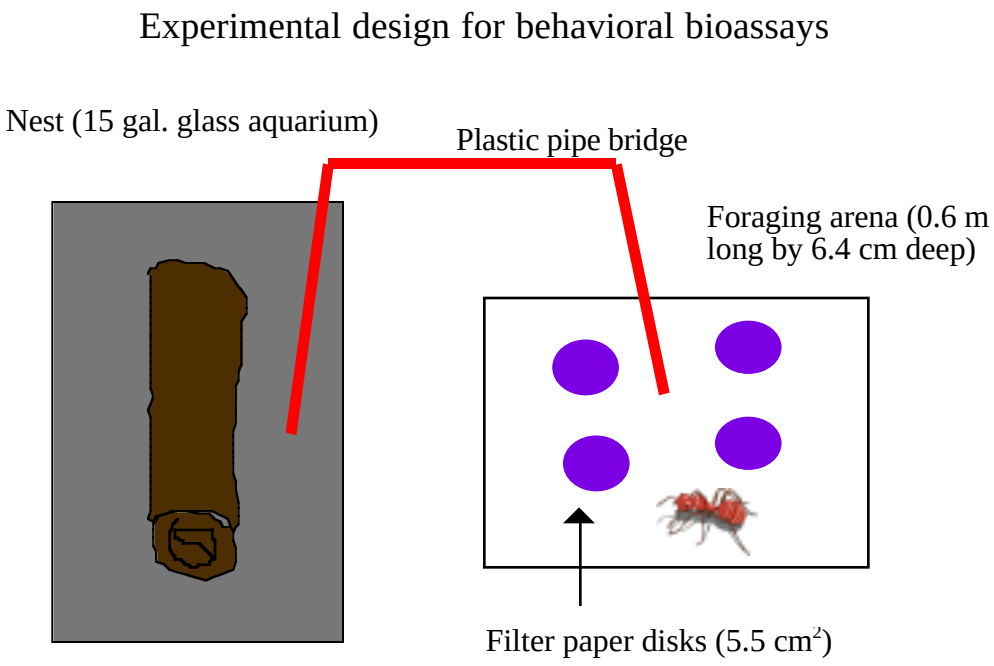

Figure 3.1 Experimental design showing nesting site for lab colonies of carpenter ants connected to an artificial foraging arena where gland and trail following behavioral bioassays were conducted. Samples were presented simultaneously with a control on filter paper disks and workers were allowed to enter the foraging arena via a plastice pipe bridge.

3.2.3 Analysis of response to synthetic compounds

Compounds documented in the literature as being components of the Dufour's gland (n-undecane, n-decane, 1-tridecane, 1-tridecene, and n-pentadecane) (Ayre and Blum 1971) were tested for attractancy. The main component to be identified in the poison gland (formic acid) was included in the comparisons (Blum and Hermann 1978). Five bioassays were designed to determine if any one compound was more attractive than the others. The effect of combinations and concentrations on worker response was investigated as well. In the first test, n-undecane, n-decane, 1-tridecane, 1-tridecene, n-pentadecane, and formic acid were all compared simultaneously against a control. Concentration was held at 2 ug (20 ul of a 100 ug/ml sample in ethanol) and all samples were presented to foragers on 5.5 cm filter paper disks laid over 10 cm^2 glass chromotography plates. Encounters (as described for the crude gland bioassays) were recorded every two minutes for 10 minute intervals under red light conditions between 9 and 11 pm. Each trial, consisting of a control (20 ul of ethanol), the five hydrocarbons described above, and formic acid, was replicated 5 times.

The remaining tests followed the same experimental design as the first test. The second test involved combined samples of tridecene/formic acid (1.7 ug: 0.3 ug), tridecene/undecane (1 ug: 1 ug), undecane/formic acid (1.7 ug: 0.3 ug), and all three substances combined (0.6 ug each) versus a control. The third test contained samples of undecane in log dose concentrations of 2 ug to 0.002 ug versus a control. The remaining tests examined responses to samples of formic acid and decane in the same concentration range as the third test.

3.2.4 Statistical analysis

The average number of encounters to the crude gland extracts was considered to be a continuous response with a normal distribution and same variance but possibly different means across gland samples. One way analysis of variance was chosen to fit the means for each group (gland samples) and test that the means are not significantly different. Further individual pairwise comparisons of the means were computed using Fisher's LSD (α = 0.05). The same techniques were applied to data from synthetic gland tests. In addition to student's t test, a normal linear regression was performed on the response of workers to crude gland samples over the first 10 minutes (maximum response) of the 30 minute test

interval. All statistical operations were performed using JMP IN software, although some graphical information was organized using Microsoft Excel (Sall and Lehman, SAS Institute 1996).

3.3 Results

3.3.1 Crude Gland Extracts

Significant differences were observed between the number of workers responding to the gland samples. The entire hindgut apparatus showed the greatest level of attractancy ($\alpha = 0.05$) compared to the other extracts or control (Figure 3.2). A graph of cumulative response shows that the maximum response during the 30 minute interval occurred after 10 minutes for the poison and Dufour's glands, and after 15 minutes for the hindgut samples (Figure 3.3). Behavior analyzed over the first 10 minutes resulted in slopes of 1.6 ($R^2 = 0.92$) for the hindgut, 0.75 ($R^2 = 0.63$) for the Dufour's gland, 0.50 ($R^2 = 0.49$) for the poison gland, and 0.12 ($R^2 = 0.09$) for the control. Sixty-two percent of the workers tested in the Y-shaped bioassay followed the hindgut extract, but the response was not significantly different ($\alpha = 0.05$) from the control (Figure 3.4).

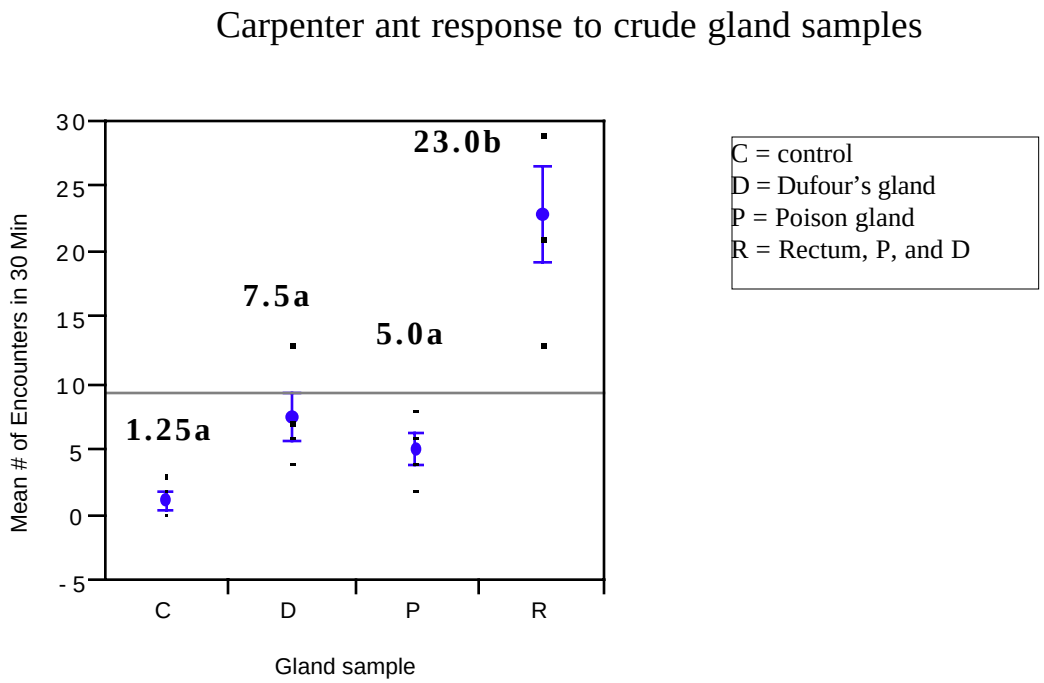

Figure 3.2 Response of carpenter ants to crude gland samples recorded as average number of encounters in 30 minutes. The total response sample mean is indicated by the horizontal line. The large marker dots indicate the mean of each sample and the error bars depict one standard error above and below each sample mean. Means followed by the same letter are not significantly different, $\alpha = 0.05$, using Fisher's LSD (Student t test) (Sokal and Rohlf 1995).

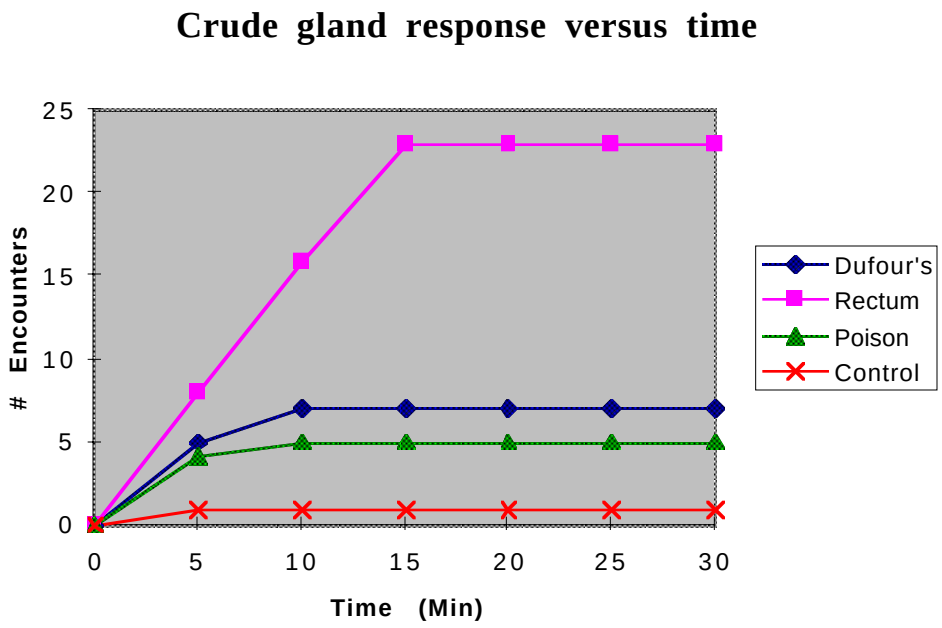

Figure 3.3 Response of carpenter ant workers to gland extracts (Dufour's, poison, and a combination of the two glands with the rectum) over a 30 minute interval. All four gland samples were presented simultaneously to the workers. Data for each 5 minute count were averaged from four replications.

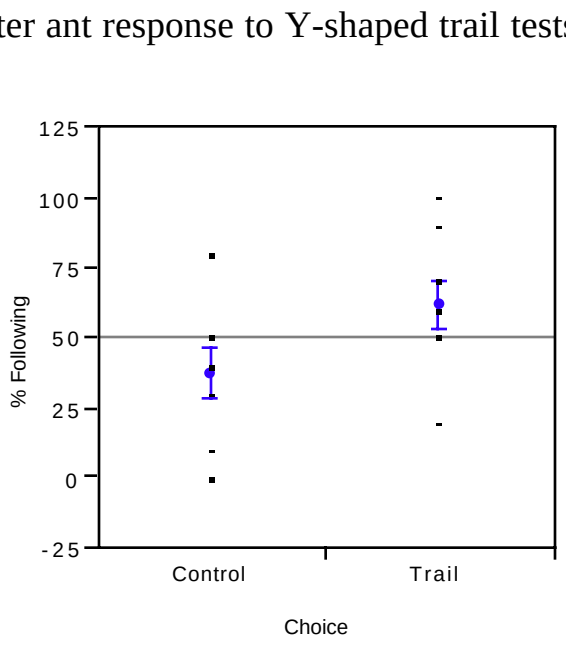

Figure 3.4 Response of carpenter ant workers to a Y-shaped choice test with one branch (trail) treated with extract from the hindgut (rectum, poison, and Dufour's gland) and the other branch serving as the control (methylene chloride). Response was recorded as percent of ants out of 10 following either the control or trail. Each trial was replicated 8 times. Group means (control and trail) are indicated by the large marker dots and the error bars show one standard error above and below the mean. The total response sample mean is indicated by the horizontal line.

3.3.2 Response to synthetic compounds

For the ten minute synthetic gland bioassays, the number of encounters did not differ significantly among the treatments tested (Table 3.1). When combined, tridecane and undecane elicited a significantly greater response ($\alpha = 0.05$) than did the control (Table 3.2). In a dose response evaluation of decane only, concentrations of 0.002 ug differed from the control (Table 3.3). Concentration was not a factor in the tests with undecane or formic acid (Tables 3.4 and 3.5).

Table 3.1 Response of carpenter ant workers to individual comparisons of synthetic gland samples versus a control. Each trial consisted of all seven samples and total number of workers aggregating at or antennating a sample over a 10 minute interval was recorded.

Sample[1]	Sample Size	No. Encounters[2]	Fisher's LSD[3]
Control	7	1.11 ± 0.13	abc
Decane	7	2.8 ± 0.97	ab
Formic acid	7	2.2 ± 1.1	abc
Pentadecane	7	0.4 ± 0.25	c
Tridecene	7	2.6 ± 1.1	abc
Tridecane	7	0.8 ± 0.58	bc
Undecane	7	3.4 ± 0.87	a

1. Each sample consisted of 2 ug, with the exception of the control, which consisted of a 5.5 cm^2 filter paper disk with 20 ul of ethanol
2. All samples were presented to workers simultaneously and number of encounters were recorded as average of five replications ± standard error.
3. Means followed by the same letter are not significantly different, $\alpha = 0.05$, using Fisher's LSD (Student t test) (Sokal and Rohlf 1995)

Table 3.2 Response of carpenter ant workers to hydrocarbon and formic acid combinations versus a control. Each trail consisted of all samples and total number of workers aggregating at or antennating a sample over a 10 minute interval was recorded.

Sample[1]	Concentration (ug)	Sample Size	No. Encounters[2]	Fisher's LSD[3]
Control	0	5	0.05 ± 0.05	b
TC + FA	1.7 : 0.3	5	1.8 ± 0.66	ab
TC + UD	1 : 1	5	2.8 ± 1.5	a
UD + FA	1.7 : 0.3	5	0.6 ± 0.40	ab
all	0.6 : 0.6 : 0.6	5	1.0 ± 0.32	ab

1. TD= tridecane, FA = formic acid, TC = tridecene, and UD = undecane
2. Average of five replications ± standard error
3. Means followed by the same letter are not significantly different, $\alpha = 0.05$, using Fisher's LSD (Student t test) (Sokal and Rohlf 1995)

Table 3.3: Carpenter ant response to samples of decane at different concentrations versus a control. Each trail consisted of all samples and total number of workers aggregating at or antennating a sample over a 10 minute interval was recorded.

Sample	Sample Size	No. Encounters[1]	Fisher's LSD[2]
Control	5	0.00 ± 0.00	b
2 ug	5	0.00 ± 0.00	ab
0.2 ug	5	0.00 ± 0.00	b
0.02 ug	5	0.20 ± 0.20	abc
0.002 ug	5	0.60 ± 0.40	c

1. Average of five replications ± standard error
2. Means followed by the same letter are not significantly different, $\alpha = 0.05$, using Fisher's LSD (Student t test) (Sokal and Rohlf 1995)

Table 3.4 Carpenter ant response to samples of undecane at different concentrations versus a control. Each trail consisted of all samples and total number of workers aggregating at or antennating a sample over a 10 minute interval was recorded.

Sample	Sample Size	No. Encounters[1]	Fisher's LSD[2]
Control	5	0.60 ± 0.24	ab
2 ug	5	0.60 ± 0.40	ab
0.2 ug	5	0.20 ± 0.20	ab
0.02 ug	5	0.20 ± 0.20	ab
0.002 ug	5	0.06 ± 0.40	ab

1. Average of five replications ± standard error
2. Means followed by the same letter are not significantly different, $\alpha = 0.05$, using Fisher's LSD (Student t test) (Sokal and Rohlf 1995)

Table 3.5 Carpenter ant response to samples of formic acid at different concentrations versus a control. Each trail consisted of all samples and total number of workers aggregating at or antennating a sample over a 10 minute interval was recorded.

Sample	Sample Size	No. Encounters[1]	Fisher's LSD[2]
Control	5	0.20 ± 0.20	ac
2 ug	5	0.40 ± 0.24	ac
0.2 ug	5	0.00 ± 0.00	a
0.02 ug	5	1.00 ± 0.55	c
0.002 ug	5	0.60 ± 0.40	ac

1. Average of five replications ± standard error
2. Means followed by the same letter are not significantly different, $\alpha = 0.05$, using Fisher's LSD (Student t test) (Sokal and Rohlf 1995)

3.4 Discussion

The crude gland results agree with observations made on *Camponotus modoc* and *C. pennsylvanicus* in response to hindgut extracts (Traniello 1977, Hansen and Akre 1985). It is safe to conclude, therefore, that a blend of compounds from the hindgut rather than just single compounds is responsible for creating an attractive signal. It is also reasonable to find an extract with high levels of attraction (rectum) to involve a longer time interval to reach maximum response levels as this sustains aggregation behavior for a

longer period. It is interesting to note that the crude gland extracts ellicited greater levels of response than the synthetic gland samples: the highest response in the crude gland tests (16.2 workers) was five times greater than the highest response in the synthetic bioassays (3 workers) during a ten minute interval. The difference suggests involvement not only of multiple compounds, but also potential material in gland walls not included in the synthetic studies.

This study could not duplicate the results of Ayre and Blum (1971), who reported combination of n-undecane and formic acid to act as a strong attractant. A combination of tridecene and undecane was found to be more attractive than the control, but not more attractive than the other compounds tested. Response to the 0.002 ug sample of decane was significantly different from all samples tested except 0.02 ug ($\alpha = 0.05$). In studies by Ayre and Blum (1971), the greatest level of attraction was observed to a mixture of n-undecane and formic acid in a 3.5 ug to 6.1 ug ratio. In the present study, the ratio was higher for the hydrocarbons (1.7 versus 0.3 for formic acid) to account for the alarm behavior normally ellicted by formic acid. Ayre and Blum (1971) may have obtained such positive results because they used formic acid as a control when comparing the synergism of n-undecane and formic acid. As a major component comprising 90% of the known volatiles identified in the Dufour's gland, n-undecane produces a slower reaction from ants in comparison to the strong excitation resulting from formic acid (Ayre and Blum 1971). By using formic acid as a control, as opposed solvent (ethanol) used in the creation of synthetic extracts in this study, the authors may have driven workers to aggregate on the n-undecane/formic acid mixture.

The rectal extract used in the crude gland bioassays was composed of the rectal sac, Dufour's gland and poison gland. Chemical identity of compounds from the hindgut and accessory glands is the subject of the following chapter.

CHAPTER 4. TRAIL FOLLOWING AND GLAND CHEMISTRY ANALYSIS

4.1 Introduction

In the previous chapter, crude gland tests showed that a combination from the rectal sac, Dufour's gland, and poison gland creates an attractive signal to carpenter ants. Aggregations were not observed in response to synthetic extracts from the Dufour's and poison gland. The role and identity of rectal sac extracts still needs to be addressed

Trail pheromones have been described as exocrine secretions deposited during foraging and used in recruitment by the formicine ants. As with the aggregation pheromones of the Dufour's gland, the signals created are a result of mixtures of compounds, and the less volatile a substance, the longer the orientation is sustained. Hindgut exocrine glands can contain compounds in nanogram amounts, and yet, workers are able to detect concentrations in the picogram range (Blum 1974). Trail pheromone mixtures can also contain compounds, that while not attractive themselves, prolong the volatile components to sustain a trail (Blum and Brand 1972).

While the behavioral function of trail pheromones has been established, there is still much to be done in the area of chemical analysis. Five compounds have been detected and identified from the rectal bladder of *Camponotus* spp. in recent years, but *C. pennsylvanicus* has not been included in this group even though the species has been shown to follow compounds from other carpenter ants (Bestmann et al. 1995, Ubler et al. 1995, and Haak et al. 1996). Pheromone research takes chemical communication down to a molecular level and can provide information gained that can be used to control pests (Silverstein 1982). Since *C. pennsylvanicus* has been defined as a structural pest, further investigation into the behavioral and chemical aspects of the hindgut would be a valuable step towards development of more effective methods of control (Akre et al. 1995).

The goals of this study were to: (a) design behavioral bioassays to test rectal extracts for trail following, (b) use GC-MS analysis to identify the chemical components in hindgut extracts used in this study and in the previous chapter, and (c) examine the remaining solid material that could be involved in the creation of a trail through the analysis of nitrogen levels, nitrogen metabolites, and dry weight studies.

4.2 Materials and Methods

4.2.1 Colony Maintenance

Partial colonies of *C. pennsylvanicus* were collected in the same fashion described in chapter 3. Ants were fed an artificial diet (chapter 3) or one that approximated more natural food sources. The following ingredients were prepared in a similar fashion to the maintenance diet but were fed only to colonies being analyzed for nitrogen levels and metabolites: 30 g of cane sugar, 1 g silkworm pupae (protein source), 0.7 g salt, 1.2 g agar, and 100 ml of distilled water.

4.2.2 Collection of Samples

Fecal contents were collected by dissecting out and separating the rectal sac from the Dufour's gland and poison gland. Both workers from lab maintained colonies and natural foragers were used to compensate for effect of diet on behavioral and chemical analyses. The sac was punctured and the contents were drawn into 1 ul microcapillary tubes. Tubes were stored within 1.5 ml microcentrifuge tubes in a freezer (- 18 °C) with no defrost cycle until use.

4.2.3 Trail following studies

Samples of ant rectal contents and whole rectums were collected and extracted into methanol (~ 10 glands per 20 ul of methanol). Trails 14 cm long were laid using disposable microcapillary syringes on glass chromotography plates and presented to lab foragers in an artificial arena as described for the attraction tests (chapter 3). The number of foragers following half or all of the trail were recorded during a 5 minute interval. A trail of methanol served as the control and trials were replicated 10 times. All tests were done between 9 and 11 pm under red light.

4.2.4 Gland analysis (GC-MS)

Rectal samples from *C. pennsylvanicus* workers were collected on two different occasions. The first sample set was taken from workers maintained on artificial diet (chapter 3) and included 4-5 glands per sample of the Dufour's gland, poison gland, and

rectal sac as well as a combination of all three extracted into a few drops of methanol. Remaining samples were collected only from the rectal sacs using the dissection technique described earlier (section 4.2.2). Samples from both natural foragers and those maintained on the artificial diet (chapter 3) were analyzed. Gas chromatographic-mass spectrometric analysis of 2.5 ul of each sample was carried out on a Shimadzu QP-5000 equipped with a 30 meter by 0.25 millimeter i.d. RTX-5 column. The oven was programmed from 60 °C to 250 °C for 25 minutes. Compounds were identified by comparison with authentic spectra from the NIH/NIST spectra data base.

4.2.5 Dry Weight Analysis of ant fecal material

C. pennsylvannicus workers were dissected and fecal contents collected with 1 ul microcapillary tubes. A pre-cleaned glass slide was weighed and 5-6 mg of fecal material was squeezed from the capillary tubes on to the slide. A wet weight was recorded and the slide was placed in a drying oven for 24 hours at 70 °C. The slide was re-weighed and a dry weight for the fecal material was determined.

4.2.6 Nitrogen metabolite analysis

The hindguts of live *C. pennsylvanicus* workers were dissected and the contents collected via 1 ul microcapillary tubes. The material was then extracted into a few drops (10 ul) of methanol or spotted directly. The presence of nitrogen metabolites was examined using high performance thin layer chromotography analysis (modified from Mullins 1971). Samples (1 ul) were spotted on 10 cm square cellulose plates (without indicator) along with analytical standards (2 ug/ul) of tryptophan, tryptophan intermediates, pteridines and purines (see Table 4.1 for sources). Each sample was replicated 2 to 4 times. Silica gel plates were also used in some cases for tryptophan isolation. Plates were prewashed in methanol and developed once in one of five solvent systems: (1) n-butanol: methanol: water: NH_4OH 60: 20: 20: 1, (2) isobutyric acid: water 4:1, (3) isopropanol: 2% NH_4OH 2: 1, (4) isobutyric acid: NH_4OH: water: 66: 1: 33, (5) acetonitrile: water 85: 15 (silica gel only). Solvent systems were allowed to travel 8.5 cm. Detection methods involved the use of short wave UV light (254 nm) for fluorescing or absorbing materials (mainly tryptophan and tryptophan intermediates) and several spray reagents : (1) Ammonical 10% $AgNO_3$ spray reagent (1 g $AgNO_3$ in 10 ml H_2O, 10 ml NH_4OH, and 80 ml methanol) for detection of purines as well as xanthurenic acid, (2) Ehrlich's spray reagent (10 ml of 10%

Dimethylaminobezaldehyde in ethanol, and 10 ml of Hydrochloric acid for a total volume of 100 ml ethanol) for detection of allantoin, allantoic acid, and urea, (3) Diphenyl Carbazone Spray reagent or DPC (0.0625 g mercuric acetate in 25 ml of 95% ethanol with several drops of glacial acetic acid to make 0.25% mercuric acetate, and 0.0125 g diphenylcarbazone in 25 ml of 95% ethanol to make 0.05% DPC) for detection of purines and pyrimidines, and (4) Ninhydrin Spray Reagent (500 mg Ninhydrin, 95 ml of 95% ethanol, and 5 ml of collidine) for the detection of amino acids.

Table 4.1 HPTLC Analytical Standards used in the detection of nitrogen metabolites in carpenter ant fecal material

Compound	Concentration	Solvent System	Source of standards
adenine	2 ug/ul	methanol	Pabst Laboratories, Milwaukee, WS
guanine	2 ug/ul	methanol	P-L Laboratories, Milwaukee, WS
hypoxanthine	2 ug/ul	methanol	Pabst LaboratoriesMilwaukee, WS
xanthine	2 ug/ul	methanol	Pabst Laboratories,Milwaukee, WS
xanthosine	2 ug/ul	methanol	Pabst Laboratories, Milwaukee, WS
uric acid	2 ug/ul	ethanol: 0.6% Li_2CO_3 1:1	Sigma Chemical Co., St. Louis. MO
allantoin	2 ug/ul	methanol	Sigma Chemical Co., St. Louis. MO
allantoic acid	2 ug/ul	methanol	Sigma Chemical Co., St. Louis. MO
urea	2 ug/ul	methanol	Sigma Chemical Co. St. Louis. MO
biopterin	2 ug/ul	methanol	Regis Chemical Co, Chicago, Ill
8-hydroxquinaldic acid	2 ug/ul	methanol	Dr. Donald Mullins, Department of Entomology,Virginia Tech
isoxanthopterin	2 ug/ul	methanol	Sigma Chemical Co. St. Louis. MO
kynurenic acid	2 ug/ul	methanol	Sigma Chemical Co. St. Louis. MO
leucopterin	2 ug/ul	methanol	Sigma Chemical Co. St. Louis. MO
tryptophan	2 ug/ul	distilled water	Sigma Chemical Co. St. Louis. MO
xanthurenic acid	2 ug/ul	methanol	Sigma Chemical Co. St. Louis. MO
amino acids (casein, acid hydrolysate)	5 ug/ul	distilled water	Sigma Chemical Co. St. Louis. MO

4.2.7 HPTLC Plate Photography

A photographic record of HPTLC plates was made during the course of this study. Guidelines were taken from Mullins (1971, personal communication) for photography of fluorescing or absorbing materials visualized under UV light. A Canon FTP camera was fitted with a 35 mm single lens and a 52 mm normal lens was used in conjunction with Kodak Ektachrome 160T film and Wratten filters #85c, CC10R, and 2E (Eastman Kodak Company). Exposure at 5 seconds at f 5.6 was found to be the optimal settings. The camera was mounted on a Desega UVIS thin layer copy stand and a 254 nm light source was used. For plates visualized with spray reagents, pictures were taken without Wratten filters, 120 volt 4 x 75 Tungsten lights, and a exposure of 1/60 seconds with an 18% Gray Kodak neutral test card used to determine f-stop.

4.2.8 Quantification of total nitrogen

A micro-Kjeldahl procedure (Mullins 1971) was used to assay samples to determine total levels of organic nitrogen. Fecal material was collected from *C. pennsylvanicus* workers as for the nitrogen metabolite studies except that the samples were not extracted into methanol, but instead kept in 1 ul pre-weighed microcapillary tubes. The microcaps with fecal material were re-weighed and samples were created by delivering 0.5 to 2 mg of fecal material into 18 mm Pyrex test tubes with 250 ul of distilled water. Standard samples of $(NH_4)_2SO_4$ (250 ul) ranging in concentration from 50 ug/ml to 5 ug/ml were also measured into test tubes. Each sample then received 250 ul of an oxidizing-digestion mixture (34.8 ml of 17.996 M (96%) H_2SO_4 (2.5M H_2SO_4), 122.41 mg of $CuSO_4$ and 32.25 mg of H_2SeO_3) and was placed in a 41.3 by 42.5 by 20.3 cm stainless steel heating unit lined with asbestos board and 100 holes for test tubes resting above a wire screen that allowed only 8 cm of tubes to contact the heat while the rest was exposed to the air for condensation. The tubes were heated (120 - 150°C) until all water had been driven off as indicated by the absence of water beads at the top of the tubes. The temperature was increased to 300°C and the samples were refluxed for 8 to 12 hours by placing a glass marble at the top of each tube.

After removal from the digestion oven, each tube received 750 ul of distilled water and was rotated to collect all the residual material. In immediate sequence, 750 ul of 3.3M NaOH and 500 ul of Nessler's reagent (1.75 g/L gum ghatti and 7 g of K_2HgI_4) was added

to each tube, followed by vortexing. The tubes were allowed to sit for 10 to 15 minutes and read in a Perkin-Elmer dual beam spectrophotometer at 490 nm against a blank of distilled water treated in the same fashion as the other samples minus the $(NH_4)_2SO_4$.

Procedural recovery rates were determined in two ways. First, standards were prepared as described except that the digestion oven step was skipped. The absorbances recorded from the spectrophometric analysis at the end were averaged with the absorbances measured from standards that were digested. The equation of the line from the standard curve of concentration versus the averaged absorbances was used to determine the ug of nitrogen present in the standards and the result was divided by the expected amount (ug) of nitrogen (i.e. 50, 40, 30, 20, 10, or 5) to obtain a percent recovery. The second method of determining recovery rates was based on a spike-over analysis. Two subsamples (250 ul each) of ant fecal material were taken from one large sample, the amount of which had been determined by weight. One of the two subsamples received an equal volume of the 5 ug/ml of $(NH_4)_2SO_4$ standard in distilled water and both samples were subjected to the rest of the micro-Kjeldahl as described above. Two to three spike-overs were tested periodically with certain runs of micro-Kjeldahl analysis. Standard recovery analysis resulted in $99.8 \pm 0.49\%$ for 50 ug/ml, $96.5 \pm 0.71\%$ for 40 ug/ml, $104.4 \pm 1.6\%$ for 30 ug/ml, $105.2 \pm 1.2\%$ for 20 ug/ml, $98.4 \pm 3.2\%$ for 10 ug/ml, and $83.5 \pm 4.4\%$ for 5 ug/ml. Spike-over analysis yielded $103 \pm 4.1\%$ recovery for fecal samples.

4.2.9 Quantification of ammonia nitrogen

A method of microdiffusion was used to determine the amount of nitrogen from ammonia in the carpenter ant samples (Mullins 1971). Fecal samples were collected from carpenter ant workers and delivered to 18 mm Pyrex test tubes as with the Micro-Kjeldahl analysis. The same $(NH_4)_2SO_4$ standards were used from the total nitrogen analysis. Samples (250 ul each) were transfered to the outer ring of a Thomas Microdiffusion dish. Standards were treated in the same way. An equal volume of the oxidizing-digestion mixture used in the Micro-Kjeldahl analysis (250 ul) was measured into the center ring of the dish. The samples and standards were made basic by adding 750 ul of saturated Na_2CO_3 to the outer ring and all dishes were immediately sealed with 12 cm^2 glass tops greased with petroleum jelly. Dishes were kept flat on a lab bench and swirled periodically for a total diffusion time of 2 hours. The contents of each center well was drawn up with a

Pasteur pipet and transfered to test tubes with 750 ul of distilled water. Each well was rinsed three to four times. The remainder of the procedure follows the steps in section 4.2.8 starting with addition of NaOH and Nessler's reagent to each sample. A Perkin-Elmer dual beam spectrophotometer (490) nm was again used to read samples against a blank of distilled water treated in the same fashion as the samples minus the $(NH_4)_2SO_4$. Standard recovery analysis resulted in 99 ± 0.71% for 50 ug/ml, 101 ± 1.3% for 40 ug/ml, 102 ± 0.3% for 30 ug/ml, 97 ± 2.1% for 20 ug/ml, 90 ± 1.8% for 10 ug/ml, and 93 ± 0.78% for 5 ug/ml. Procedural difficulties with spike-over analysis in this assay prevented determination of a recovery rate from unknowns.

4.2.10 Statistical analysis

One way analysis of variance was chosen to fit the means for each group (rectal trails versus control) and test that the means are not significantly different. Further comparisons of the means were computed using Fisher's LSD ($\alpha = 0.05$). All operations were performed using JMP IN software (Sall and Lehman, SAS Institute 1996).

4.3 Results

4.3.1 Trial following studies and gland analysis

No trail following was observed in response to extracts from whole rectal sacs or just rectal contents. Behavioral comparisons between the rectal contents and whole rectums were unable to be made as a result of the negative data. The two hydrocarbons, n-undecane and n-tridecane, were identified in material from the Dufour's gland of lab colony workers (Figure 4.2). A number of carbon-18 fatty acids and esters were also detected in the Poison and Dufour's gland. The poison gland has been described in the literature as the source of formic acid, composing 50% of the contents (Hermann and Blum 1968). In this study, an additional compound, palmitic acid, was identified (Figure 4.3). Only one compound (3,4-dihydro-3,5-dihydroxy-6-methyl-pyran-4-one), previously identified in *C. atriceps*, was found in the rectal material of both lab and natural foragers of *C. pennsylvanicus* (Figures 4.4 and 4.5).

Gas chromatogram of compounds detected in the Dufour's gland

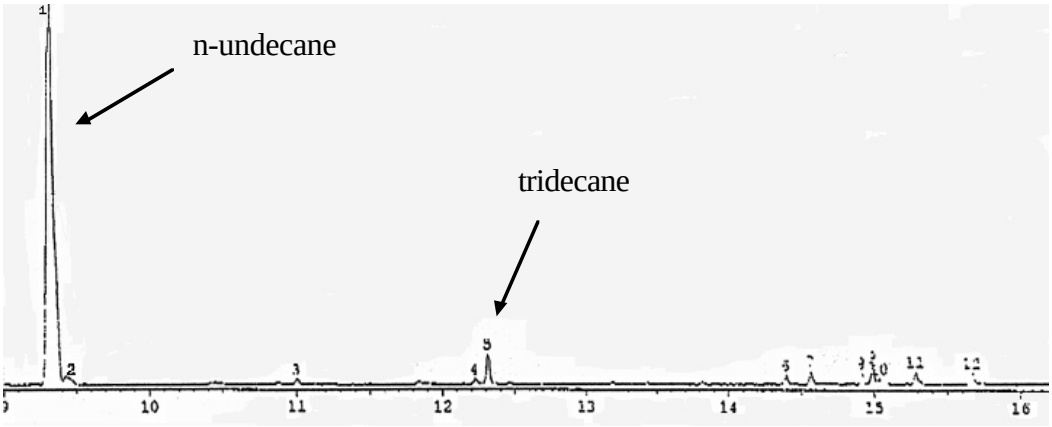

Figure 4.1 Gas chromatogram of n-undecane (peak #1) and tridecane (peak #5) detected in the Dufour's gland of carpenter ant workers.

Gas chromatogram of compounds detected in the poison gland

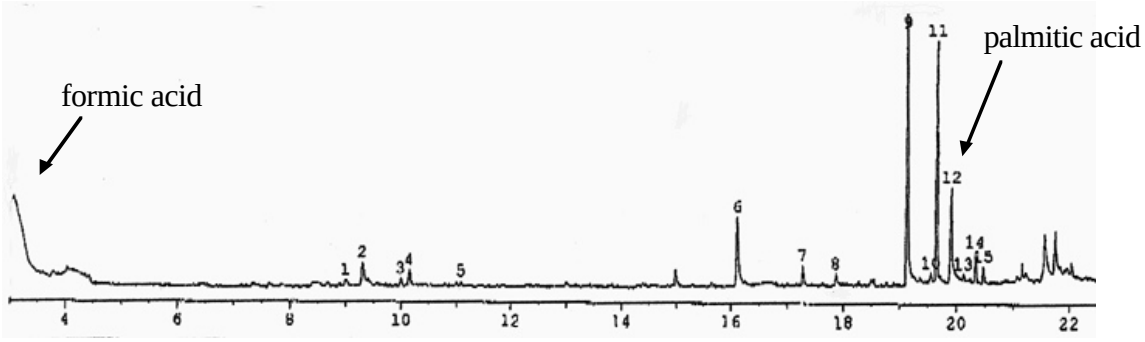

Figure 4.2 Gas chromatogram of formic acid and palmitic acid (peak # 12) detected in the poison gland of carpenter ant workers.

Gas chromatogram of compounds detected in the rectum

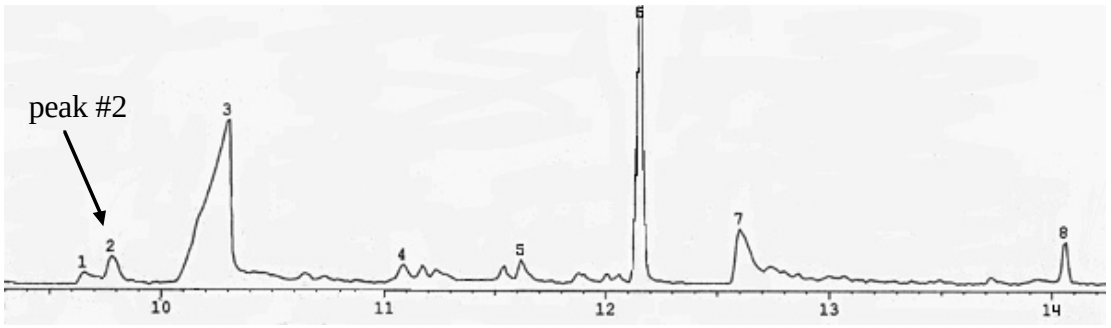

Figure 4.3 Gas chromatogram of 3,4-dihydro-3,5-dihydroxy-6-methyl-pyran-4-one (peak # 2) detected in the rectal material of carpenter ant workers.

Mass spectrogram from carpenter ant fecal material

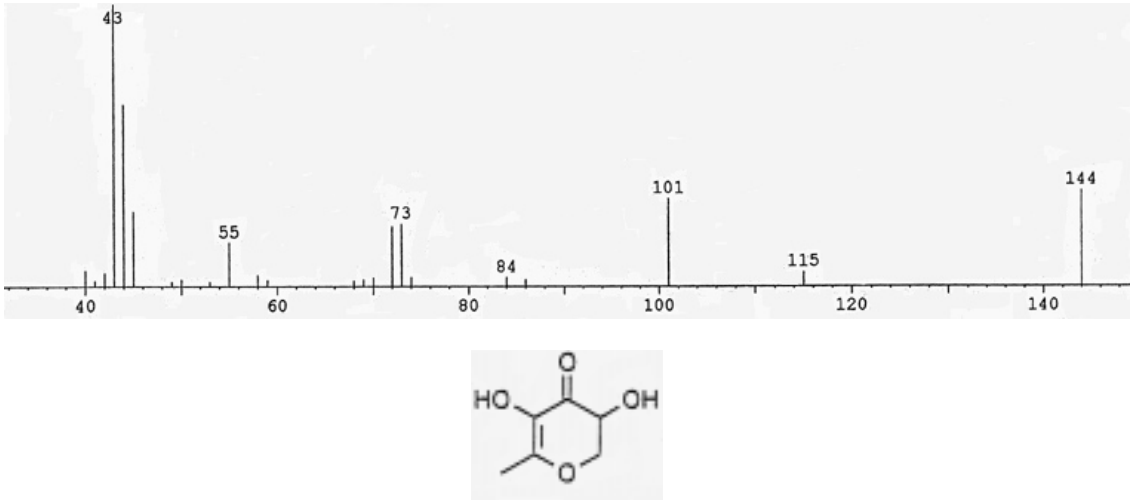

Figure 4.4 Mass spectrogram and chemical structure of 3,4-dihydro-3,5-dihydroxy-6-methyl-pyran-4-one detected in the rectal material of *Camponotus pennsylvanicus* workers

4.3.2 Nitrogen levels, metabolite isolation and dry weight analysis

Total nitrogen levels averaged 19.2 ± 2 ug per mg of ant feces. Ammonia nitrogen levels were measured to be 2.7 ± 1.2 ug per mg of ant feces. Dry weight analysis showed the rectal material to be 14% solid material. Nitrogen composes 14% of the solid material and 14% of the nitrogen is in the form of ammonia. Two tryptophan intermediates, kynurenic and xanthurenic acid, and one pteridine, biopterin, were detected in rectal samples from both natural foragers of *C. pennsylvanicus* and those maintained on artificial diet (Figures 4.6 and 4.7). The presence of amino acids was detected when several samples of casein acid hydolysate were spotted on silica gel plates along with fecal samples and standards of tryptophan. The amino acids were not identified but visualization with Ninhydrin Spray detected trace amounts of tryptophan as well in the fecal material, but the results were unable to be verified in subsequent analysis (Figure 4.8). The presence of metabolites was verified using multiple solvent systems in addition to visualization with spray reagents or UV light (Table 4.2).

Cellulose plate from HPTLC analysis of carpenter ant fecal material

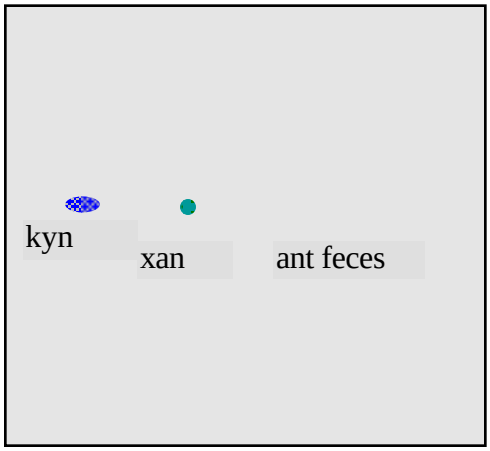

Figure 4.5 Reconstructed cellulose plate (visualized with UV light, 254 nm) indicating the presence of kynurenic (kyn) and xanthurenic (xan) acid in carpenter ant fecal material (three replications). Subsequent plates developed in various solvent systems were used to verify the presence of both metabolites (see Table 4.2).

Cellulose plate from HPTLC analysis of carpenter ant fecal material

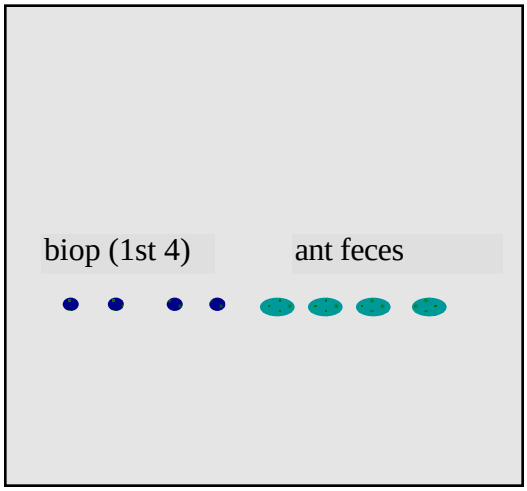

Figure 4.6 Reconstructed cellulose plate (visualized with UV light, 254 nm) indicating the presence of biopterin (biop) in carpenter ant fecal material (four replications).

Silica gel plate from HPTLC analysis of carpenter ant fecal material

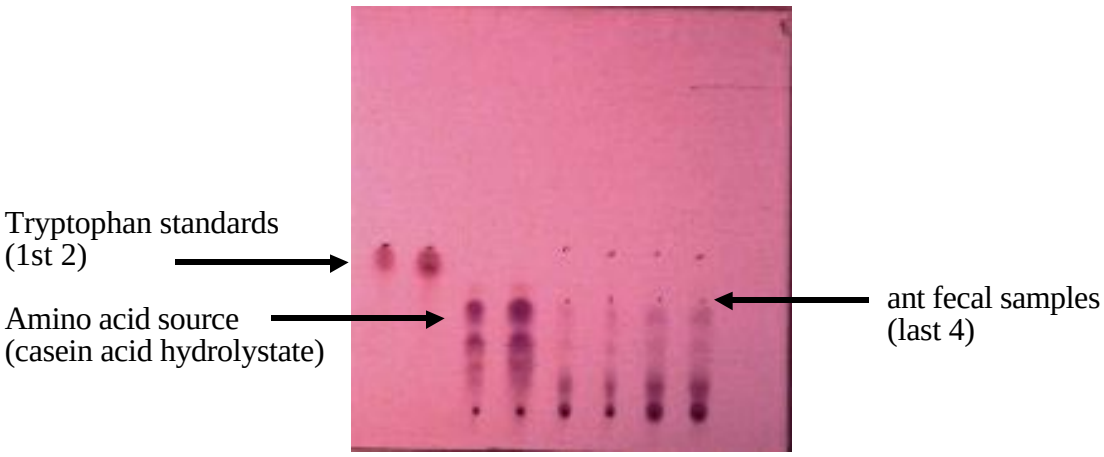

Figure 4.7 Silica gel plate (visualized with Ninhydrin spray reagent) indicating the presence of several unknown amino acids from carpenter ant fecal material.

Table 4.2 Standard and Unknown Rf values for nitrogen metabolites detected in fecal material of *C. pennsylvanicus* workers

Plate type[1]	Solvent[2]	Compounds detected	Standard Rf	Unknown Rf	Detection[3]	Reps[4]
cellulose	1	kynurenic acid	0.47	0.45	UV	3
cellulose	3	kynurenic acid	0.67	0.69	UV	4
cellulose	1	kynurenic acid	0.52	0.49	UV	3
cellulose	1	kynurenic acid	0.40	0.37	UV	2
cellulose	1	biopterin	0.18	0.16	UV	4
cellulose	1	kynurenic acid	0.43	0.42	UV	4
cellulose	3	kynurenic acid	0.59	0.55	UV	3
cellulose	3	xanthurenic acid	0.49	0.48	UV	4
cellulose	2	biopterin	0.34	0.34	UV	4
cellulose	3	xanthurenic acid	0.49	0.52	$AgNO_3$	4
cellulose	2	biopterin	0.34	0.34	UV	4
cellulose	3	xanthurenic acid	0.49	0.52	$AgNO_3$	4
*cellulose	1	xanthurenic acid	0.47	0.44	UV	4
*cellulose	1	kynurenic acid	0.43	0.44	UV	4
*cellulose	1	xanthurenic acid	0.23	0.26	$AgNO_3$	4
*cellulose	2	biopterin	0.38	0.38	UV	4
*cellulose	2	kynurenic acid	0.39	0.38	UV	4
*cellulose	2	xanthurenic acid	0.39	0.37	UV	2
*cellulose	2	xanthurenic acid	0.25	0.28	$AgNO_3$	2

1. "*" indicates rectal samples that were taken from natural foragers not maintained on artificial diet
2. Solvent used were as follows: (1). n-butanol: methanol: water: NH_4OH 60: 20: 20: 1, (2). isobutyric acid: water 4:1, (3). isopropanol: 2% NH_4OH 2: 1, (4). isobutyric acid: NH_4OH: water: 66: 1: 33
3. UV = ultraviolet light (254 nm) used to visualize fluoreseing or absorbing materials and $AgNO_3$ is an Ammonical (10%) spray reagent
4. Number of ant fecal samples replicated on that plate

4.4 Discussion

A number of factors could explain why trail following was not observed as a response to rectal extracts. The behavioral responses in a laboratory situation are more subjective than responses found under natural conditions as visual and geographical cues are not the same. During the course of this study, workers were often observed wandering the artificial foraging arena in a highly random fashion despite the presence of food. Such few studies on trail following behavior have been conducted on *C. pennsylvanicus* that doubt is still cast on the degree to which these ants even rely on a trail pheromone to recruit foragers to a food source. Workers were never observed laying their own trail on the Y-shaped design even though they were permitted to feed and return to the nest on several occasions. The experimental design (Figure 3.1) did not allow for much distance between the lab nest and the foraging arena, and the workers could have easily used the plastic bridge as a guide to food rather than relying on chemical signals.

GC-MS analysis in this study showed that diet can affect compounds produced in the hindgut. Initial detection of large quantities of benzoic acid from rectal samples of workers maintained on artificial diet led to the mistaken assumption that a new compound had been discovered. However, the source of the compound was traced to use of sodium benzoate and potassium sorbate, which were being tested as a diet preservative at the time of the chemical analysis. Such a result led to collection of fecal samples from workers foraging naturally and not maintained in lab colonies.

The compound identified from the fecal material of both natural and lab maintained colonies (3,4-dihydro-3,5-dihydroxy-6-methyl-pyran-4-one) is only a minor component of the trial pheromone of *C. atriceps*. The major component of this species, 3,5-dimethyl-6-(1'-methylpropyl)-tetrahydropyran-2-one) was not found in *C. pennsylvanicus*. Compounds identified as the trail pheromone in the *C. herculeanus* (2,4-dimethyl-5-hexanolide), *C. silvicola* (3,4-dihydroisocoumarins), *C. rufipes* (3,4-dihydroisocoumarins), and *C. floridanus* (nerolic acid) were also not found in *C. pennsylvanicus* (Bestmann et al. 1995, Ubler at al. 1995, Haak et al. 1996). No attempt to synthesize 3,4-dihydro-3,5-dihydroxy-6-methyl-pyran-4-one in the lab was made, otherwise behavioral tests could have examined whether this compound was indeed a component of the trial pheromone in *C. pennsylvanicus*. In agreement with previous findings from Ayre and Blum (1971), n-undecane was found to be a major peak in the GC traces whereas tridecane was present in trace amounts (Figure 4.1). Ayre and Blum (1971)

found n-undecane to be greater than 90% of the volatiles isolated while n-tridecane was only a minor component. It would be interesting to include palmitic acid and fomic acid in artificial trail extracts to determine if both compounds are part of the trail pheromone.

The nitrogen levels measured in the fecal material were slightly lower than levels cited in the literature for the American cockroach (Mullins and Cochran 1973b). The *C. pennsylvanicus* workers had 19.2 ± 2 ug/mg of total nitrogen, and 2.7± 1.2 ug/mg ammonia nitrogen compared to 20 to 40 ug/mg total nitrogen and 2 to 10 ug/mg ammonia nitrogen in the cockroach, respectively. Two of the nitrogen metabolites isolated in *C. pennsylvanicus* fecal material, kynurenic and xanthurenic acid, were also found in the American cockroach (Mullins and Cochran 1973b). It is interesting to note that this study represents the first investigation of nitrogen levels and isolation of metabolites from carpenter ant feces. Tryptophan is an amino acid not normally detected in free form in the blood of insects, so detection of intermediates instead (kynurenic and xanthurenic acid) is not unusual (Cochran 1985). Pteridines, like biopterin found in this study, function in pigmentation, and presence in the feces may again be the result of excess amounts being voided from the blood.

CHAPTER 5. SUMMARY

Carpenter ants belong to a widespread genus and one of the most advanced subfamilies in the Formicidae (Holldobler and Wilson 1977, Dumpert and Johnson 1981). *Camponotus pennsylvanicus*, the most commonly found species in Virginia, is considered a structural pest based on nesting habits contributing to increased wind throw damage in shade trees and nuisance foraging in houses and other buildings (Mallis 1982, Fowler 1983). Workers utilize both group recruitment and mass communication in their foraging strategies, yet the anatomical source and chemical identity of the odor trial has not been thoroughly examined. Ritualized defecation has been proposed as a theory of trail pheromone evolution (Traniello 1977). The focus on trail laying behavior led researchers to narrow the search for the source of the trail pheromone to the hindgut. This study investigated behavioral and chemical aspects of the odor trail by examining the response to extracts from the hindgut accessory glands (Dufour's and poison gland), and rectum, as well as analyzing contents through identification of glandular volatiles and solid material in the feces.

Crude gland tests showed that compounds from a combination of the rectal sac, Dufour's gland and poison gland, create an attractive signal to carpenter ants. The signal for such a mixture sustained aggregation longer than did individual extracts from the Dufour's or Poison gland, in agreement with previous behavioral studies (Traniello 1977, Hansen and Akre 1985). Synthetic hydrocarbons tested from the Dufour's gland contradicted previous findings in that none elicited attraction. Formic acid, as a more volatile compound, did not produce initial excitement as expected, nor did the fatty acid serve as a synergist with hydrocarbons from the Dufour's gland as seen in other studies (Blum 1974). Crude gland extracts elicited stronger responses from workers compared to synthetic tests, suggesting involvement of material in gland walls in creating an attractive signal. The difference in response could also be a factor of analyzing a combination of compounds in an appropriate quanitative amount. Undecane, as a major component, and tridecane, in trace amounts, were identified in the Dufour's gland, as in previous studies. The other hydrocarbons mentioned in the literature (n-decane, n-pentadecane, and 1-tridecene) were not identified (Ayre and Blum 1971). A number of fatty acids and esters were identified in the poison and Dufour's glands and may serve as stabilizers for the secretion that is normally released simultaneously from the two glands (Bergstrom and Lofqvist 1973). Palmitic acid was identified in the poison gland, but behavioral studies were not carried out to confirm involvement in the trail pheromone. Prior to this study,

formic acid was the only compound to have been described in the poison gland (Blum and Hermann 1978).

No trail following was observed in response to rectal extracts, unlike results reported by Traniello (1977), and Barlin et al. (1976). The only compound identified in the rectal material, 3,4-dihydro-3,5-dihydroxy-6-methyl-pyran-4-one, was shown to be a minor component of the trail pheromone in *C. atriceps* (Haak et al. 1996), but a synthesized version would need to be tested to determine whether it serves as a pheromone for *C. pennsylvanicus*. Since positive response (attraction) was obtained from the a combined extract of Dufour's and poison gland in addition to rectal material, it is likely the Dufour's gland, if not the sole source, has a role in secretion of the trail pheromone.

The fecal material was determined to be 14% solid material, 14% of which is nitrogen, and 1.9% is ammonia nitrogen. Among the nitrogenous metabolites, biopterin, xanthurenic acid and kynurenic acid were isolated. Nitrogenous materials determined for American cockroaches (Mullins and Cochran 1973b) are similar to what was found in carpenter ants, but more than 50% of the remaining solid material was left unidentified. Further analysis of carpenter ant feces would be useful in describing the other components. The metabolites isolated may be excess voiding of materials from the blood that were not absorbed or digested.

Artificial conditions of this study may explain some of the negative results. Diet was found to affect compounds produced in the hindgut as some preservatives added to the maintenance diet resulted in identification of large quantities of benzoic acid, which was not found in foragers from the field. The distance between the nesting site and foraging arena was not an accurate representation of nature, making the use of chemicals in recruitment potentially unnecessary or sporadic in use. Few studies have been conducted on *C. pennsylvanicus* to confirm level of trial pheromone use in foraging. Some research has shown that chemical cues are more important for trail following in *C. pennsylvanicus* than visual cues, such as light, but results were rather inconclusive (Hartwick et al. 1977, David and Wood 1980). Other species of *Camponotus* demonstrate very noticeable trail following, such as *C. modoc*, in which both attraction and trail following to hindgut extracts have been observed (Hansen and Akre 1985). Bacterial symbionts have been found in the hindgut, and as trail extracts were collected prior to secretion, the actual pheromone may contain different compounds from those assayed (Bestmann 1997). The response of workers to compounds from the hindgut depends on age of workers as

determined by reduced oocyte development (Traniello 1977). Worker development was not evaluated in this study as all (minors, intermediates, and majors) were allowed to enter the foraging arena. If the majority of workers randomly entering the foraging arena were not “old enough” then attraction may have been observed, but trail following would not.

CHAPTER 6. LITERATURE CITED

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VITAE

Anne Elizabeth Hillery was born in Washington, DC on December 9, 1973, and spent her formative years living 30 minutes north in Bethesda, MD. From 1978 to 1988 she attended Our Lady of Lourdes in Bethesda. The next four years were spent at Connelly School of the Holy Child High School in Potomac where her interest in biology was pursued with greater fervor and early interest in entomology was demonstrated in a science fair competition where she won third place for her project titled: "Reaction of Crickets to different environments". Upon graduation in 1992, she attended Western Maryland College where she conducted research on the effect of nutrient content on ant foraging patterns. She graduated *cum laude* in 1996 with a BA in Biology and began a program of study at Virginia Polytechnic Institute and State University. Her Master's work has involved investigations of the hindgut secretions of the black carpenter ant and she successfully defended her thesis on April 20, 1999.

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