INVESTIGATING THE INTEGRATION OF SMALL HIVE BEETLES (*AETHINA TUMIDA* MURRAY, COLEOPTERA: NITIDULIDAE) INTO WESTERN HONEY BEE (*APIS MELLIFERA* L., HYMENOPTERA: APIDAE) COLONIES

By

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To my wife and kids

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The small hive beetle (*Aethina tumida* Murray; Coleoptera: Nitidulidae) is native to sub-Saharan Africa, where it is considered an occasional nuisance in honey bee (*Apis mellifera* L.; Hymenoptera: Apidae) colonies. However, the species is considered a significant pest of honey bees in its introduced range of North America and Australia, where the beetle has been established since 1996 and 2002, respectively. The small hive beetle damages colonies through feeding and reproductive behaviors, and can cause absconding or complete colony collapse. Small hive beetles integrate into honey bee colonies via several adaptations, including: retraction of appendages beneath the body when encountering defensive honey bees, finding hiding areas (confinement sites) within the bee nest that are inaccessible to honey bees, and coercing host honey bees to feed them while confined. Other nitidulids have been found in honey bee colonies and they appear to have lower degrees of integration into honey bee nests than do small hive beetles.

A series of experiments was conducted to investigate potential morphological (Chapter 3), behavioral (Chapters 2, 4-7), and chemical (Chapter 5) adaptations that enable small hive beetles to integrate successfully into honey bee nests. The results of the research suggest that small hive

beetles are attracted to odors present in honey bee colonies (Chapter 2). Also, they possess leg modifications that allow them to retract their appendages beneath their bodies more fully (Chapter 3), thus resisting attack from honey bee hosts who treat them more defensively than they treat other beetles at the nest entrance (Chapter 4). Furthermore, small hive beetles have an altered chemical profile that is dependent upon their post-eclosion diet (Chapter 5), though the significance of the altered profile is unclear. Finally, small hive beetles are unique among other beetle species in their ability to find hiding places within the colony where they are confined by honey bee hosts (Chapter 6) until the ambient temperature decreases, whereafter the beetles enter the thermoregulatory cluster of honey bees (Chapter 7). The research presented herein contributes to a greater understanding of attributes of small hive beetles that enable them to integrate successfully into honey bee nests.

CHAPTER 1
INTRODUCTION

Social insects typically host many colony co-inhabitants, or symbionts, from a broad range of arthropod taxa. Using Wilson's definition (1971), a social insect symbiont is a species that has a close, dependent relationship with a social insect species. Social insect symbionts can be subdivided into three categories: parasites, where one species benefits while the host suffers; commensals, in which one species benefits while the host is neither helped nor harmed; and mutualists where both species benefit. The relationships between symbionts and their social insect hosts range from invaders merely using a host's nest as temporary shelter to the invaders forming relationships with their hosts that may incur both immediate and evolutionary significance (Kistner 1979, 1982, Rosenheim 1990).

Arthropod symbionts of termites and ants (termed termitophiles and myrmecophiles, respectively) are common whereas symbionts of bees (termed melittophiles) presumably are not (Wilson 1971, Kistner 1979). Several hypotheses have attempted to explain the relative scarcity of social bee symbionts, two of which are discussed here. The first hypothesis relates to the quality of the host's diet (Wilson 1971, Kistner 1979). Many bees eat pollen and nectar-derived foodstuffs that are concentrated, nutrient rich, and produce little debris. However, ants and termites eat a variety of foodstuffs, such as insects and cellulose-rich plant material, which produce more refuse (Wilson 1971). Therefore, bee colonies have less debris available in the colony, which minimizes potential food sources for symbionts, while the opposite is true for colonies of termites and ants.

Another explanation for the apparent lack of social bee symbionts is that social bee species tend to nest in trees, whereas most ants and termites nest in the ground. The increased abundance of ground-dwelling arthropods compared to arboreal arthropods (see André et al. 1994, Osler and

Beattie 2001) proportionally favors symbiont development in ground-nesting social insects. Consequently, arthropods presumably must be pre-adapted to arboreal life to locate and thrive in social insect nests in trees. Thus, statistically one would expect fewer symbionts in social bee nests than ant or termite nests, which has been observed in nature (Wilson 1971).

Despite these barriers, some symbionts have managed to establish themselves in social bee colonies. Specific adaptations in morphology, behavior, and/or chemical use have been adopted by most, or perhaps all, social insect symbionts (Wilson 1971, Kistner 1979). The purpose of this dissertation is to investigate the integration of the small hive beetle (SHB), *Aethina tumida* Murray (Coleoptera: Nitidulidae), into nests of the western honey bee, *Apis mellifera* L. (Hymenoptera: Apidae). The following sections review arthropod integration into non-*A. mellifera* social bee nests (Table 1-1) and honey bee nests (Table 1-2), thereby providing the requisite background for understanding the relationship between SHBs and their honey bee hosts. This review is limited to arthropods which enter the nest of eusocial insect hosts. It is not an exhaustive list of all melittophiles but, rather, is a representation of the various means of integration into social bee nests.

Methods Used by Symbionts to Integrate into Non-*Apis mellifera* Social Bee Nests

Morphological

Symbiont morphology contributes significantly to successful integration into social bee nests. Morphological adaptations exhibit two main forms among social bee symbionts, which typically are linked to symbiont behavior. One form involves passive defense and includes adaptations that protect the symbiont from host attacks. The second form includes phoretic adaptations for using the host as a dispersal mechanism.

Passive defense

Several invader species utilize passive defense morphological adaptations to integrate into host bee nests. The springtail species *Pseudocyphoderus melittophilous* Mari Mutt (Collembola: Cyphoderidae) occurs in nests of the stingless bee *Trigona testacea* Klug (Hymenoptera: Apidae) (Mutt 1977). This species possesses a well-sclerotized dorsal cuticle having heavy scales that are used for passive defense. Furthermore, they have an enlarged mesothorax with an anterior notch that protects the back of the head from attack. Also, the meso- and metathorax are expanded lateroventrally to protect the pleural body regions and legs, which can be pulled under the body due to their reduced size (Mutt 1977).

Scotocryptus species (Coleoptera: Leiodidae) are found frequently in nests of *Melipona* species (Salt 1929, Roubik and Wheeler 1982, Wheeler 1985, Davis and Gonzalez 2008). These beetles possess a strongly convex dorsum, which presumably makes them difficult to grasp. Also, they are capable of fitting their head, antennae, and legs under their body (Salt 1929, Roubik and Wheeler 1982).

The beetle *Cleidostethus meliponae* Arrow (Coleoptera: Corylophidae) is found with *Melipona alinderi* Alfken and has several morphological adaptations for passive defense (Salt 1929). The pronotum and fused elytra, in dorsal view, are flattened laterally thereby concealing the head and antennae. Also, the tibiae can be folded into the femora, which themselves can be situated into grooves on the venter. These adaptations may allow the beetle to flatten to the substrate and avoid injury from its bee hosts (Salt 1929, Bowestead et al. 2001). The presence of these morphological adaptations among these melittophilic beetles led me to believe they are relevant in the SHB-honey bee relationship.

Phoresy

Phoresy, i.e. wherein one species is carried by another species (Torre-Bueno 1985), is a ubiquitous behavior used by many arthropods to gain entrance into bee nests. Mites (Acari) are among the most common phoretic invaders found in bee nests (Kistner 1982). In most mite species, the modified deuteronymph stage is adapted for phoresy. Adaptations include anal and ventral suckers for attachment, as in *Kuzinia laevis* (Dujardin) (Acari: Acaridae) on *Bombus* species (Hymenoptera: Apidae) queens (Alford 1975, Schwarz and Huck 1997) and caudal suckers, as in *Histiostoma halictonida* Woodring (Acari: Anoetidae) on *Halictus rubicundus* (Christ) (Hymenoptera: Halictidae) pupae (Woodring 1973).

Alternatively, the leiodid genera *Scotocryptus*, *Scotocryptodes*, *Parabystus*, and *Synaristers* have notched mandibles to grasp the corbicular setae of their *Melipona* hosts, while retracting their appendages beneath the body, leaving only the antennae exposed (Roubik and Wheeler 1982, Bezerra et al. 2000). The triungulin first instar larvae of some ripiphorid beetles (Coleoptera: Ripiphoridae) are phoretic on sweat bees (Hymenoptera: Halictidae) visiting flowers (Falin 2002). These larvae attach to host wings via a terminal abdominal segment and tarsal pulvilli modified into suckers (Tomlin and Miller 1989, Cline and Huether 2011).

Other

Several other integrative morphological adaptations exist in melittophiles. For example, the pseudoscorpion genera *Dasychernes* and *Corosoma* (Pseudoscorpiones: Chernetidae), which occur in *Melipona* nests, presumably disguise themselves with an abundance of vestitural setae (Salt 1929, Roubik 2006, Gonzalez et al. 2007). Similarly, larvae of *Ecthrodape africana* Burks (Hymenoptera: Perilampidae), which are parasites of *Braunsapis* pupae (Hymenoptera: Anthophoridae), closely resemble host larvae and are treated as host larvae as evidenced by

adults frequently moving them about the nest with bee larvae. These larvae are covered with setae which give them structural support, but also may provide tactile mimicry (Michener 1969).

Behavioral

The most prevalent adaptations for integrating into social bee nests are behavioral (Kistner 1982). Several behaviors enable symbionts to persist with social bee hosts, including adaptive feeding and reproduction. The most common behavior, though, is phoresy, and this is largely performed by phoretic mites (Kistner 1982).

Phoresy

Successful phoresy involves morphological and behavioral traits working in tandem to enable the symbiont to use the host as a dispersal mechanism. Phoresy encompasses those behaviors the symbiont uses to "rig" the host for dispersal purposes, behaviors which are made possible by morphological adaptations.

Scutacarus acarorum (Goeze) (Acari: Scutacaridae), which is phoretic on *Bombus*, has been found attached to species of *Parasistus* (Acari: Parasitidae) that are subsequently attached to *Bombus* species (Richards and Richards 1976, Schwarz and Huck 1997). Similarly, *Fuscuropoda marginata* (Koch) (Acari: Uropodidae), which are phoretic on *Bombus*, are also phoretic on *Volucella bombylans* (L.) (Diptera: Syrphidae), which inhabit *Bombus* colonies (Alford 1975).

Cryptostigma (Hemiptera: Coccidae) are phoretic on stingless bees and secrete honeydew and wax within nests of *Schwarzula* in exchange for protection from natural enemies (Camargo and Pedro 2002, Roubik 2006). *Antherophagus* species (Coleoptera: Cryptophagidae) gain entrance to *Bombus* colonies by attaching to the legs and proboscis of foragers at flowers (Frison 1921, 1926, Wheeler 1928, Free and Butler 1959, Chavarria 1994, Gonzalez et al. 2004). Analogously, *Ripiphous smithi* Linsey & McSwain (Coleoptera: Ripiphoridae) lay eggs in closed

flowers. As the flowers open, the eggs hatch, and triungulin 1st instar larvae attach to a visiting *Lasioglossum* and are taken to the nest where they parasitize the host larvae (Tomlin and Miller 1989, Majka et al. 2006).

Feeding/reproduction

Many species integrate into social bee colonies via adaptations that allow them to feed and reproduce within nests. Some species are predatory/parasitic toward their host while others enjoy a commensalist or mutualist type relationship with a host.

Predatory and parasitic taxa on host adults or brood are common. For example, *Locustacarus buchneri* (Stammer) (Acari: Podapolipidae) reside within the tracheae of *Bombus* workers or queens and overwinter in the tracheae of queens. This mite lays eggs about a week after emergence from hibernation. The resulting offspring mate within the tracheae, and gravid females shift to other workers or larvae as the season progresses (Husband and Sinha 1970, Shykoff and Schmid-Hempel 1991, Otterstatter and Whidden 2004, Yoneda et al. 2008). Also, *Varroa jacobsoni* Oudemans, *V. underwoodi* Delfinado-Baker & Aggarwal (Acari: Varroidae), and *Tropilaelaps clarae* Delfinado-Baker (Acari: Laelapidae) are parasitic on drone brood of *Apis cerana* F. (Eickwort 1997).

Many mutillid wasp species are parasitic on halictid and apid bees (Alford 1975). These wasps either fight their way past guard bees at the entrance or dig a new nest entrance and lay eggs on the brood (Alford 1975, Roubik 1990, Brothers et al. 2000, Polidori et al. 2009). *Cryptocerus elongata* Mayr (Hymenoptera: Formicidae) enter nests of *Trigona mosquito* Lutz (Hymenoptera: Apidae), kill the bees, consume the honey, and make a nest (Salt 1929). *Brachycoma sarcophagina* (Townsend), *B. devia* Fallén (Diptera: Metopiidae), and *Melittobia* species (Hymenoptera: Eulophidae) quickly move into *Bombus* nests, oviposit (larviposit in the case of the *Brachycoma* species) on or in larval cells, and the parasite subsequently consumes the

developing larva (Frison 1926, Free and Butler 1959). *Volucella* species enter nests to oviposit in a similar fashion (Wheeler 1928); however, they are adapted to oviposit immediately if killed, and the resulting egg mass possesses a protective viscous cover that solidifies upon exposure to air (Free and Butler 1959).

Achroia grisella (F.) and *Galleria colonella* Hübner (Lepidoptera: Pyralidae) feed on nest debris, but not brood, in *Melipona* spp. colonies (Hymenoptera: Apidae), where they can destroy weak nests (Salt 1929, Cepeda-Aponte et al. 2002). Likewise, *Ephestia kühniella* Zeller and *Vitula edmandsii* (Packard) (Lepidoptera: Pyralidae) deplete food stores of *Bombus* colonies (Frison 1926, Wheeler 1928, Alford 1975, Schmid-Hempel 2001). *Aphomia sociella* (L.) (Lepidoptera: Pyralidae) consume food stores in addition to brood in *Bombus* spp. colonies (Frison 1926, Wheeler 1928).

Many arthropod species that invade social bee nests are innocuous commensals, being scavengers among the nest debris of their host species or using the nest as shelter. *Tyrophagus* mites (Acari: Acaridae) feed on fungi in nests of stingless bees (Hymenoptera: Apidae) (Roubik 2006). Secondary invaders, which parasitize or predate upon primary invaders, include parasitic *Apanteles* species (Hymenoptera: Braconidae) and *Stilpnus gagates* (Gravenhorst) (Hymenoptera: Ichneumonidae), and the predacious mite genus *Parasistus* (Acari: Parasitidae) (Alford 1975). Springtails, such as *Pseudosinella* species (Collembola: Entomobryidae), feed on pollen and fecal material in nests of *Lasioglossum zephyrum* (Smith) and *Augochlora* species (Hymenoptera: Halictidae) (Batra 1965, Eickwort and Eickwort 1972). Leafcutter ants, *Acromyrmex octospinosus* (Reich) (Hymenoptera: Formicidae), build fungus gardens within abandoned cells of active *Bombus pullatus* Franklin nests, doing so without harming the hosts (Chavarria 1996).

In some instances, inquilines activity benefits the host, creating a mutualistic relationship between the host and the invader. For example, the feeding activity of mites (Acari) associated with *Megalopta genalis* Meade-Waldo and *M. ecuadoria* Friese (Hymenoptera: Halictidae) reduce harmful fungal loads on host brood (Biani et al. 2009). Also, the wasps *Habrobacon juglandis* (Ashmead) and *Apanteles nephoptericis* (Packard) (Hymenoptera: Braconidae) parasitize the potentially destructive moth *Vitula edmandsii* (Frison 1926).

Chemical

Chemical use is the most prevalent means of communication in insects, and often is exploited by inquilines that integrate into social bee nests (Wilson 1971). Nest or host semiochemicals may be produced by the inquiline *de novo* or are acquired from the colony itself. However, while chemical means of integration are common among myrmecophiles and termitophiles (e.g. Howard et al. 1980, Vauchot et al. 1998, Lenoir et al. 2001), there are relatively few reported among melittophiles.

Methods Used by Symbionts to Integrate into *Apis mellifera* Nests

Apis mellifera is one of the most socially-complex bee species, as well as one of the most important pollinators of agricultural crops, accounting for 80-90% of all pollination, and having an estimated value of ~$15 billion USD as of 2000 (Wilson 1971, Morse and Calderone 2000). Consequently, the study and control of its pests has been the focus of considerable research and attention (e.g. Morse and Flottum 1997).

Honey bees exhibit most of the traits hypothesized to be important for limiting symbiont invasion. Along with concentrated food sources and arboreal nests, honey bees exhibit a temporal division of labor. At any given time, there are bees in the colony that perform different tasks for the colony, including defense (Breed et al. 2004). Thus, honey bee colonies are well-defended at all times. Honey bee defense acts as an impediment to would-be invaders, thus

minimizing invasion by opportunistic arthropods. Despite these adaptations, some arthropods succeed in infiltrating the nest. These invaders provide an opportunity to investigate how honey bees limit nest invasion, and the traits symbionts such as the SHB must possess to overcome honey bee defenses. Morphological, behavioral, and chemical adaptations all contribute to the success of arthropods at integrating into honey bee colonies (Table 1-2).

Morphological

Passive defense

Passive defense mechanisms of nest invaders are achieved mainly through possession of a relatively thick cuticle. For example, the cuticle of the death's head hawkmoth, *Acherontia atropos*, enables them to deflect and subsequently survive potentially lethal stings of honey bees (Moritz et al. 1991). Similarly, because of their thick cuicle, *Euphoria sepulcralis* (F.) (Coleoptera: Scarabaeidae) can enter honey bee hives unimpeded by their defensive stings (Woodruff 2006).

Phoresy

Afrocypholaelaps africana (Evans) (Acari: Ameroseidae) are phoretic mites that reside on flowers generally, but occasionally may be found in bee hives. These mites possess sucker-like ambulacral pads that lack claws and enable them to attach to honey bees at flowers (Seeman and Walter 1995). Another mite, *Tropilaelaps clarae*, is devastating to honey bee colonies in southern and southeast Asia (Sammataro et al. 2000, Brown et al. 2002). This mite species has an elongate body that allows for movement between hairs of host bees, as well as large legs with claws for attaching to bees (Rath et al. 1991).

Acarapis woodi (Rennie) (Acari: Tarsonemidae) is a mite that feeds and reproduces within the tracheae of honey bees. The females are phoretic for a brief time, and have tarsal claws and specialized setae on the legs that enable attachment to bee hairs (Ochoa et al. 2005). Similarly,

the destructive varroa mite, *Varroa destructor* Anderson & Trueman (Acari: Varroidae), has specialized structures on their legs called apoteles that adhere to hosts (Rosenkranz et al. 2010).

The fly *Braula coeca* Nitzsch (Diptera: Braulidae) is phoretic on adult honey bees, but rarely is problematic for host bees. The adult is wingless and attaches to adult bees by means modified comb-like pretarsal claws (Sammataro 1997).

Other

Acarapis woodi larvae possess enlarged pulvillar pads, rendering their claws useless, but enabling them to move freely around the tracheae. Likewise, both the body and leg setation are oriented distally, which allows the mite to measure the radius of and navigate the tracheae (Ochoa et al. 2005). Furthermore, *Acarapis* mites collectively possess modified chelicerae for piercing the host integument and imbibing their fluids (Eickwort 1997).

Behavioral

Phoresy

Many mite species are phoretic on honey bees including *Varroa destructor*, *Tropilaelaps clarae*, and *Acarapis woodi* (Eickwort 1997). The fly species *Braula coeca* also is phoretic on honey bee adults.

Feeding/reproduction

The phoretic mites *Acarapis externus* Morgenthaler, *A. dorsalis* Morgenthaler, and *Varroa destructor* all feed on the adult bees to which they are attached. *Varroa destructor* also feeds on brood once inside the colony (Eickwort 1997, Rosenkranz et al. 2010). Although *T. clarae* are phoretic on adult bees, they only feed on brood. The non-phoretic *Pyemotes* species (Acari: Pyemotidae) feed on brood within the colony also (Eickwort 1997, Sammataro et al. 2000, Brown et al. 2002).

Oplostoma fuligineus Olivier and *Pachnoda sinuata flaviventris* Gory and Percheron (Coleoptera: Scarabaeidae) enter colonies and feed preferentially on open brood cells, but also will feed on capped brood, honey, and pollen stores secondarily (Donaldson 1989). Another scarab beetle, *Euphoria sepulcralis*, is found occasionally within nests feeding on food stores (Caron 1997b, Woodruff 2006).

Other predators of honey bee adults and brood include species of *Meloe* (Coleoptera: Meloidae), *Paratemnus minor* (Balzan) (Pseudoscorpiones: Atemnidae), and *Salticus* species (Aranae: Salticidae). The latter build silken cases on the inner cover of colonies and feed on passing bees (Wheeler 1928, Caron 1997a, b, Sammataro 1997, Majka 2007). Highly effective predator ants in the genera *Dorylus*, *Eciton*, *Iridomyrmex*, *Formica*, *Crematogaster*, and *Solenopsis* (Hymenoptera: Formicidae) are swift in destroying honey bee colonies, and can be major honey bee pests in some regions of the world. *Camponotus* species can raid a colony of resources as well as destroy hive materials (Fell 1997). Similarly, *Heterotermes tenuis* (Hagen) (Blattodea: Rhinotermitidae) and *Dermestes lardarius* L. (Coleoptera: Dermestidae) can feed on and destroy hive components, and subsequently produce sites that can harbor other pests (Caron 1997b).

Mutilla europaea L. (Hymenoptera: Mutillidae) is a parasite that lays eggs in pupal cells. The developing larvae then eat the bee larvae, and, after pupating within a host cocoon, the emerging adult feeds on the honey stores (Alford 1975).

Several species of Blattodea, Coleoptera, and Lepidoptera feed on wax, honey, and pollen within colonies (Nielsen and Brister 1979, Caron 1997b, Williams 1997). *Acherontia atropos* (L.) (Lepidoptera: Sphingidae) and *Vespula* and *Dolichovespula* species (Hymenoptera: Vespidae) enter colonies occasionally to rob nectar (Moritz et al. 1991, Fell 1997). Eggs of

Drosophila busckii Coquillett (Diptera: Drosophilidae) are laid on wax cappings, and developing larvae feed on honey stores, subsequently fermenting them (Sammataro 1997). Similarly, *Hermetia illucens* L. (Diptera: Stratiomyidae) and *Tenthredomyia australis* Shannon (Diptera: Syrphidae) lay eggs in the colonies and the developing larvae feed on food stores (Sammataro 1997). Adults of the phoretic fly *Braula coeca* take food directly from honey bee mouthparts. Occasionally, the fly will induce the host bee to regurgitate food by scratching the bee's labrum (Sammataro 1997).

Several scavengers can be found among the debris in honey bee nests, including mites, silverfish, earwigs, barklice, moths, and beetles (Lea 1910, 1912, Alford 1975, Caron 1997b, Williams 1997, Neumann and Ritter 2004, Ellis et al. 2008). *Epuraea corticina* Erichson (Coleoptera: Nitidulidae) has been found in supplemental protein patties placed within hives, and *Carpophilus dimidiatus* (F.) (Coleoptera: Nitidulidae) has been found in pollen cells within active hives (Ellis et al. 2008).

Species of *Ellingsensis* and *Chelifer cancroides* (L.) (Pseudoscorpiones: Cheliferidae) serve a beneficial purpose by feeding on destructive *Varroa destructor* and *Galleria mellonella* (L.) (Lepidoptera: Pyralidae) within colonies (Caron 1997a, Donovan and Paul 2005). Similarly, the parasitic wasps *Bracon hebetor* Say and *Apanteles galleriae* Wilkinson (Hymenoptera: Braconidae) parasitize pestiferous *G. mellonella* and *A. grisella* within colonies (Williams 1997, Dweck et al. 2010).

Other

Upon entering a colony, *Galleria mellonella* remains motionless if encountered by a honey bee, thereby evading detection. The moth can sustain this for up to five minutes, and is detected only if it is touched or approached closely by a bee (Nielsen and Brister 1977, Eischen et al. 1986). *Acherontia atropos* disorients guard bees by producing a squeaking noise, which

purportedly appeases the bees and helps the moth enter the colony to steal nectar and honey (Williams 1997).

Chemical

The most thoroughly studied chemical integration systems exist among melittophiles of honey bees. For example, mass attacks on honey bee colonies by the predatory *Vespa mandarinia japonica* Smith (Hymenoptera: Vespidae) are likely assisted by pheromonal markings at the hive site (Fell 1997). *Linepithema humilis* Mayr (Hymenoptera: Formicidae) is a pest ant species that releases few volatiles, and Spangler and Taber (1970) suggest that a lack of identifying odors is one means of integrating into bee colonies. Therefore, not only is a specific chemical profile helpful for integrating into a hive or marking its presence, but also so is a lack of any chemical profile at all.

Acherontia atropos enters colonies by chemical camouflage, wherein the moth produces nest chemicals endogenously, resulting in virtual indifference to the moth by the bee hosts (Moritz et al. 1991). The devastating pest mite *V. destructor* has a chemical profile nearly identical to that of honey bees (Nation et al. 1992). Interestingly, as the chemical profile of the developing immature bee changes, that of the mite changes correspondingly (Martin et al. 2001).

Methods Used by SHBs to Integrate into *Apis mellifera* Nests

The SHB gained significance in the mid 1990s, being largely benign throughout its native range of sub-Saharan Africa (Lundie 1940). In its introduced range of North America and Australia, the SHB can cause devastation to even strong colonies, and there is evidence that it may transmit bee pathogens mechanically (Neumann and Elzen 2004, Neumann and Ellis 2008, Eyer et al. 2009a, b, Schäfer et al. 2010a).

Morphological

There are few morphological adaptations reported for SHBs that would enable them to integrate into bee colonies. One adaptation occurs in the larval stage, wherein their bodies are covered with a series of dorsal and lateral protuberances. Lundie (1940) hypothesized that these protuberances prevent the larvae from being covered in honey while feeding. The adult SHB's hard exoskeleton likely enables them to resist stings by bees as well (Neumann et al. 2001). However, neither of these hypotheses has been investigated quantitatively.

Behavioral

The majority of SHB integrative adaptations are behavioral and include passive defense, hiding, behavioral mimicry, and clustering. Upon entrance into a honey bee colony, a SHB often is met with aggression (Schmolke 1974, Elzen et al. 2001). To avoid being stung and/or removed from the colony, the SHB retracts its appendages beneath its body, leaving nothing extended for bees to grasp, which is similar to what Salt (1929) proposed for the corylophid *Cleidostethus meliponae* in nests of *Melipona alinderi* (Schmolke 1974, Neumann et al. 2001).

Some SHBs do not retract their appendages upon entering the hive, rather they use an alternative strategy of running quickly to an area of the hive inaccessible to host bees, such as a crack or crevice on the periphery (Schmolke 1974, Neumann et al. 2001). If the SHB is on a frame or wall of a colony and is pursued by a bee, it may drop to the bottom of the hive, which is a common defensive strategy employed by beetles and insects generally (Schmolke 1974, Dill et al. 1990, Leschen 2000, Neumann and Elzen 2004, Ohno and Miyatake 2007). Regardless, the SHB must find a place inaccessible to the bees to avoid removal. Once the SHB arrives in the space, the bees may station guards around the area (confinement sites), keeping the SHBs from escaping and reproducing in the colony (Neumann et al. 2001, Ellis et al. 2003b, Ellis 2005).

Within the confinement sites, the SHBs survive longer than would be expected without resources (Pettis and Shimanuki 2000, Ellis et al. 2002a). Survival is achieved through a form of behavioral mimicry, wherein the SHBs antennate the guarding bees' mandibles thereby inducing them to regurgitate a droplet of food, which is consumed by the confined SHBs (Ellis et al. 2002b).

Finally, during the cold months following peak autumn infestations (de Guzman et al. 2010), and once the SHBs are "established" within the colony, they regulate their body temperature by entering the thermoregulatory cluster of bees surrounding the brood (Pettis and Shimanuki 2000, Ellis et al. 2003a, Neumann and Elzen 2004). In this way, they are able to survive the cold weather experienced in temperate regions of the United States and Australia.

Chemical

Host location and recruitment are two interrelated chemical strategies used by SHBs to integrate into honey bee colonies. The SHB is attracted to components of honey bee colonies (Elzen et al. 1999, Suazo et al. 2003, Torto et al. 2005, 2007a, b, Graham et al. 2011), specifically components of honey bee alarm pheromone such as isopentyl acetate. The SHB can detect these compounds at lesser quantities than are detectable by the bees themselves (Torto et al. 2007a). This high degree of sensitivity explains the SHB's attraction to stressed colonies (Wenning 2001), as well as strong colonies under "normal" conditions (Neumann and Elzen 2004).

The second chemical method of integration involves recruitment of other SHB individuals to the hive. Once SHBs have infested a colony, they cause a low level of stress to the colony. This stress leads to the release of alarm volatiles (e.g. isopentyl acetate) (Breed et al. 2004). This, in turn, attracts more SHBs to the colony. Furthermore, as the SHBs feed on bee-collected pollen in the colony, a commensal yeast on their body, i.e. *Kodamaea ohmeri* (Etchells & T.A. Bell),

invades the pollen and produces isopentyl acetate, thereby attracting more SHBs (Torto et al. 2007a). In both the beetle and yeast recruitment chemical cues, there is a positive feedback mechanism that leads to the rapid collapse of the colony.

Introduction to the Research Presented in this Dissertation

Many arthropod species have been found in honey bee colonies and scavenge or prey on the inhabitants. However, only a few have a close, protracted relationship with honey bees. The SHB exhibits a variety of interactions with honey bees, including clustering among and being fed by the host bees (Pettis and Shimanuki 2000, Ellis et al. 2002b, Ellis et al. 2003a, Neumann and Elzen 2004). These interactions demonstrate SHB's high level of integration into honey bee colonies. A series of experiments was performed to investigate how the SHB is able to integrate successfully into honey bee colonies. The majority of the experiments (Chapters 2, 3, 4, 6) were comparative, allowing me to gain insight into the origins of particular traits as well as their adaptive value through comparing traits among beetles exhibiting various levels of integration within honey bee colonies. Such levels of integration were assigned based on the species' frequency of occurrence within honey bee nests, with higher frequencies of occurrence believed to reflect a higher level of integration by a beetle species. The SHB is hypothesized to use morphological, behavioral, and chemical adaptations to integrate into honey bee colonies (Wilson 1971, Kistner 1979). Collectively, the data will enable generalizations and predictions to be made concerning the attributes necessary to become a symbiont in social bee nests.

Before invading a colony, symbionts first must find the nest. In Chapter 2, results are presented from an investigation involving the degree of attraction of various beetle species representing three levels of colony integration toward honey bee hive odors. A hypothesis was proposed that the most highly integrated beetle species (SHB) would be more attracted to hive odors than beetles marginally associated or not associated at all with honey bee colonies.

Morphological adaptations are important to the success of symbionts in entering social insect colonies. An investigation of leg morphology in SHB and other related nitidulid beetles was performed to determine physical features that may aid in colony integration (Chapter 3). Modifications in the femora and tibiae, as well as in tarsal setation were expected in the SHB, but not the other beetles. Modifications exist in other beetle species known to inhabit social insect colonies, providing them with a way to survive host attacks (Attygalle et al. 2000, Eisner and Aneshansley 2000).

The nest entrance is where beetles are presented with the first round of nest defense by the bees. Therefore, a comparison of guard bee behavior toward SHBs and other beetles of varying levels of integration was performed. The other beetles included beetles found in honey bee colonies previously, and those never found in bee colonies (Chapter 4). A hypothesis was proposed that highly integrated SHBs would be treated more aggressively by bees than other beetles due to their status as a colony pest.

The intruder's cuticular chemical profile also is important for successful entrance into the hive (Breed et al. 2004). This profile can be altered through diet. Therefore, a hypothesis was proposed that, although SHBs may be treated more aggressively than other beetle species (Chapter 4), SHBs provided a diet of honey bee products will have a chemical profile that enhances their acceptance by guard bees at the hive entrance (Chapter 5).

Once beetle invaders successfully navigate bee defenses at the colony entrance, they enter the colony and seek cracks/crevices to hide from attacking bees. In Chapter 6, an investigation was performed to test the hypothesis that a beetle's ability to find confinement sites within a bee colony will vary according to the beetle's level of integration within bee colonies. Also tested

was that previous SHB occupation of confinement sites predisposed those sites to hosting invading SHBs that were never before exposed to honey bee colonies.

Despite the complicated interaction between SHBs and honey bees at confinement sites, the defensive system deteriorates during winter when bees cluster tightly in the nest to keep warm. In Chapter 7, the temperature at which the beetles leave confinement sites to enter the bee cluster, and the temperature at which they leave the cluster and return to their confinement sites was evaluated.

The research presented herein contributes to an understanding of the attributes that make the SHB successful at integrating into honey bee nests and provides several interesting lines of research for further study.

Table 1-1. Arthropods found in nests of non-*Apis mellifera* social bees and their means of integrating into the nests.

Symbiont	Host	Integrative characteristic	Reference
		Morphology	
Acari			
Acaridae			
Acarus farris (Oudemans)	*Bombus terrestris* (L.), *Megabombus argillaceus* Scopoli, *Megabombus zonatus* (Smith), *Pyrobombus niveatus* Kriechbaumer	reduced mouthparts in phoretic deutonymph	Aytekin et al. 2002
Histiostoma halictonida	*Halictus rubicundus*	caudal suckers for attachment to pupae	Woodring 1973
Kuzinia laevis	*Bombus* spp.	anal and ventral suckers for phoretic attachment	Alford 1975, Schwarz and Huck 1997
Uropodidae			
Fuscuropoda marginata	*Bombus* spp.	pedicel secreted from glands for phoretic attachment	Alford 1975
Pseudoscorpionida			
Chernetidae			
Corosoma spp., *Dasychernes* spp.	*Melipona* spp.	disguise themselves with vestitural setae	Salt 1929, Roubik 2006, Gonzalez et al. 2007
Collembola			
Cyphoderidae			
Paracyphoderus spp., *Cyphoderus* spp., *Partamora* spp.	*Melipona* spp.	tough cuticle	Roubik 2006
Pseudocyphoderus melittophilous	*Trigona testacea*	passive defense, well-sclerotized dorsal cuticle, heavy scales, enlarged mesothorax, lateroventrally-expanded meso- and metathorax, shortened legs	Mutt 1977
Coleoptera			
Leiodidae			
Parabystus spp.,	*Melipona* spp.	toungue-and-groove	Roubik and

Table 1-1. Continued.

Symbiont	Host	Integrative characteristic	Reference
Scotocryptodes spp., *Synaristers* spp.		mechanism to lock elytra together, notched mandibles to grasp setae on hosts' corbiculae for phoresy, blind, lacking hindwings	Wheeler 1982, Bezerra et al. 2000
Scotocryptus spp.	*Melipona* spp.	strongly convex dorsum, can fit head, antennae, and legs under body, larvae have flattened body and heavy dorsal setation, toungue-and-groove mechanism to lock elytra together, notched mandibles to grasp setae on hosts' corbiculae for phoresy, blind, lacking hindwings	Salt 1929, Roubik and Wheeler 1982, Wheeler 1985, Bezerra et al. 2000, Davis and Gonzalez 2008
Corylophidae			
Cleidostethus meliponae	*Melipona alinderi*	fused elytra and pronotum flattened at margins to conceal reduced head and antennae, shortened legs, tibiae fold within femora, which fit into grooves in the body, interlocking device to make body rigid	Salt 1929, Bowestead et al. 2001
Monotomidae			
Crowsonius spp.	*Trigona* spp.	reduced eyes, wingless	Pakaluk and Ślipiński 1993
Ripiphoridae			
Ripiphoris spp.	*Halictus* spp., *Lasioglossum* spp., *Augochlora* spp., *Augochlorella* spp.	attach to host wings with terminal abdominal segment and tarsal pulvilli that have been modified into suckers, flattened,	Falin 2002, Tomlin and Miller 1989

Table 1-1. Continued.

Symbiont	Host	Integrative characteristic	Reference
		heavily-sclerotized bodies	
Diptera			
Phoridae			
Melittophora salti Brues	*Trigona amalthea* Olivier	head is close to thorax to protect junction between them, modified scutellum protects junction between thorax and abdomen, heavily-sclerotized ovipositor, able to fit legs under body	Salt 1929
Hymenoptera			
Perilampidae			
Ecthrodape africana	*Braunsapis* spp.	larvae have setae which give support tactile mimicry	Michener 1969
	Behavioral		
Acari			
Acaridae			
Acarus spp.	*Bombus* spp., *Megabombus* spp., *Pyrobombus* spp.	phoretic deutonymphs	Frison 1926, Richards and Richards 1976, Schwarz et al. 1996, Huck et al. 1998, Aytekin et al. 2002
Glycyphagus spp., *Tyroglyphus* spp.	*Melipona* spp.	feed on pollen	Roubik 2006
Kutzinia laevis	*Bombus terrestris*	phoretic, predators of other nest invaders	Allen et al. 2007, Alford 1975,
Tyrophagus spp.	*Melipona* spp.	feed on fungi	Roubik 2006
Ameroseiidae			
Garmamilla spp., *Proctolaelaps* spp.	*Bombus* spp., *Megabombus* spp., *Pyrobombus* spp.	phoretic deutonymphs, predators of other nest invaders	Frison 1926, Alford 1975, Richards and Richards 1976, Schwarz et al. 1996, Huck et al. 1998, Aytekin et

Table 1-1. Continued.

Symbiont	Host	Integrative characteristic	Reference
			al. 2002
Neocypholaelaps spp.	*Melipona* spp.	feed on pollen	Roubik 2006
Anoetidae			
Histiostoma halictonida	*Lasioglossum zephyrum, Megalopta* spp.	phoretic on wings of emerging adults	Woodring 1973, Engel and Fain 2003
Chaetodactylidae			
Sennertia alfkeni (Oudemans)	*Xylocopa* spp.	phoretic in acarinaria	Okabe and Makino 2002, Kawazoe et al. 2008
Gamasidae			
Urozercon melittophiles Silvestri	*Trigona cupira* Smith	Phoretic	Salt 1929
Hemileiidae			
Hemileius spp.	*Melipona* spp.	feed on fungi	Roubik 2006
Hypoaspididae			
Hypoaspis spp.	*Bombus* spp., *Megabombus* spp., *Pyrobombus* spp.	phoretic deutonymphs, predators of other nest invaders	Frison 1926, Alford 1975, Richards and Richards 1976, Schwarz et al. 1996, Huck et al. 1998, Aytekin et al. 2002
Laelapidae			
Hypoaspis spp., *Meliponaspis* spp.	*Melipona* spp., *Meliponula* spp., *Trigona* spp.	parasites of host larvae	Salt 1929, Roubik 2006
Laelapsoides spp.	*Megalopta* spp.	feeds on fungus on host brood	Biani et al. 2009
Laelaspoides ordwayae Eickwort	*Augochlorella* spp.	phoretic on emerging in spring	Eickwort 1966
Neohypoaspis ampliseta Delfinado-Baker, Baker, & Roubik	*Melipona* spp.	predacious on mites in nests	Roubik 2006
Pneumolaelaps spp.	*Bombus* spp.	phoretic on foragers, feed on honey and pollen	Hunter and Husband 1973
Tropilaelaps clarae	*Apis cerana*	parasites of drone brood	Eickwort 1997

Table 1-1. Continued.

Symbiont	Host	Integrative characteristic	Reference
Tropilaelaps koenigirum Delfinado-Baker & Baker	*Apis dorsata* (F.), *A. florea* (F.)	parasites of host brood	Eickwort 1997
Macrochelidae			
Macrocheles spp.	*Melipona* spp.	feed on fungi	Roubik 2006
Trigonholaspis spp.	*Melipona* spp., *Meliponula* spp., *Trigona* spp.	parasites of host larvae, feed on fungi	Salt 1929, Roubik 2006
Parasitidae			
Parasitellus fucorum de Geer, *Parasistus* spp.	*Bombus* spp., *Megabombus* spp., *Pyrobombus* spp.	phoretic deutonymphs, predators of other nest invaders	Frison 1926, Alford 1975, Richards and Richards 1976, Goldblatt and Fell 1984, Schwarz et al. 1996, Huck et al. 1998, Aytekin et al. 2002
Podapolipidae			
Locustacarus buchneri	*Bombus* spp.	feed and reproduce within tracheae, overwinter with queens	Husband and Sinha 1970, Shykoff and Schmid-Hempel 1991, Otterstatter and Whidden 2004, Yoneda et al. 2008
Podocinidae			
Lasiodeius spp.	*Melipona* spp.	feed on pollen	Roubik 2006
Pyemotidae			
Parapygmephous spp.	*Agapostemon* spp., *Dialictus umbripennis* (Ellis)	Phoretic	Eickwort and Eickwort 1969, 1971, Woodring 1973
Pyemotes spp.	*Melipona* spp.	feed on pollen	Roubik 2006
Scutacaridae			
Imparipes eickworti Mahunka	*Dialictus umbripennis*	Phoretic	Eickwort and Eickwort 1969, 1971, Woodring 1973
Scutacarus acarorum	*Bombus* spp., *Parasistus* spp.	phoretic deutonymphs, use	Richards and Richards 1976,

Table 1-1. Continued.

Symbiont	Host	Integrative characteristic	Reference
	attached to *Bombus* spp.	flowers to transfer between bee hosts	Schwarz and Huck 1997
Tarsonemidae			
Acarapis woodi	*Apis cerana, Apis dorsata*	feed and reproduce within tracheae	Eickwort 1997
Tydeidae			
Neotydeolus spp.	*Melipona* spp.	feed on fungi	Roubik 2006
Uropodidae			
Fuscuropoda marginata	*Bombus* spp., *Volucella bombylans* within *Bombus* nests	phoretic deutonymphs, scavenger	Frison 1926, Alford 1975
Varroidae			
Euvarroa sinhai Delfinado & Baker	*Apis dorsata, Apis florea*	parasites of host brood	Eickwort 1997
Varroa jacobsoni, Varroa underwoodi	*Apis cerana*	parasites of drone brood	Eickwort 1997
Pseudoscorpionida			
Chernetidae			
Dasychernes inquilinus Chamberlin	*Melipona* spp.	phoretic	Bezerra et al. 2000, Gonzalez et al. 2007, Davis and Gonzalez 2008
Collembola			
Entomobryidae			
Pseudosinella pettersoni Borner	*Lasioglossum zehyrum, Augochlora* spp.	feed on pollen and fecal material	Batra 1965, Eickwort and Eickwort 1972
Dermaptera			
Forficulidae			
Forficula auricularia L.	*Bombus* spp.	feed on host brood	Alford 1975
Hemiptera			
Coccidae			
Cryptostigma spp.	*Schwarzula* spp.	phoretic, secrete honeydew and wax	Camargo and Pedro 2002, Roubik 2006
Coleoptera			
Cryptophagidae			
Antherophagus spp.	*Bombus* spp.	phoretic, attach to legs and proboscis at	Frison 1921, 1926, Wheeler

Table 1-1. Continued.

Symbiont	Host	Integrative characteristic	Reference
		flowers, feed and reproduce within nest debris	1928, Free and Butler 1959 Chavarria 1994, Gonzalez et al. 2004
Silvanidae			
Silvanis trivialis Grouvelle	*Melipona* spp., *Trigona* spp.	feed on nest debris	Lea 1910, Salt 1929
Leiodidae			
Parabystus spp., *Scotocryptodes* spp., *Scotocryptus melittophilus* Reitter, *Synaristus* spp.	*Cephalotrigona* spp., *Melipona* spp., *Partamona* spp.	phoretic, feed on pollen, fungi, and fecal debris	Bezerra et al. 2000, Roubik 2006, Gonzalez et al. 2007, Davis and Gonzalez 2008
Nitidulidae			
Brachypeplus auritus Murray, *B. basalis* Erichson, *Epuraea luteola* Erichson	*Melipona* spp., *Trigona* spp.	feed on nest debris	Lea 1910, Salt 1929
Glischrochilus fasciatus (Olivier)	*Bombus* spp.	scavenger	Frison 1926, Alford 1975
Ripiphoridae			
Ripiphorous smithi	*Lasioglossum* spp.	phoretic 1st instar larvae attach to host bees at flowers, feed on host larvae	Tomlin and Miller 1989, Majka et al. 2006
Scarabaeidae			
Onthophagous hecate (Panzer)	*Bombus* spp.	scavenger	Frison 1926, Alford 1975
Staphylinidae			
Belonuchus mordens Erichson	*Melipona* spp.	predacious	Wheeler 1928
Tenebrionidae			
Tenebrio obscurus F., *T. tenebrioides* Beauv.	*Bombus* spp.	scavenger	Frison 1926, Alford 1975
Diptera			
Metopiidae			
Brachycoma devia (Fallén), *B. sarcophagina* (Townsend)	*Bombus* spp.	larviposit in or on larval cells, parasitic larvae consume host larvae	Frison 1926, Free and Butler 1959

Table 1-1. Continued.

Symbiont	Host	Integrative characteristic	Reference
Michiliidae	*Melipona capixaba* Moure & Camargo	feed on fecal debris	Melo 1996, Roubik 2006
Muscidae			
Fannia canicularis L.	*Bombus* spp.	scavenger	Frison 1926, Alford 1975
Phoridae			
Gymnoptera vitripennis (Meigen)	*Bombus* spp.	scavenger	Frison 1926, Alford 1975
Phalacrotophora halictorum Melander & Brues	*Agapostemon* spp.	oviposit in brood cells, parasitic larvae consume host larvae	Eickwort and Eickwort 1969
Pseudohypocera kerteszi Enderlein	*Melipona beecherii* B.	scavenger at low levels, larvae are predators of host brood at high levels	Robroek et al. 2003, Roubik 2006
Pseudohypocera nigrofascipes Borgmeier & Schmitz	*Melipona* spp., *Trigona* spp.	feed on nest debris	Lea 1910, Salt 1929
Syrphidae			
Volucella spp.	*Bombus* spp.	oviposit on or in host larval cells, parasitic larvae consume host larvae, oviposit immediately if killed	Wheeler 1928, Free and Butler 1959
Lepidoptera			
Oecophoridae			
Endrosis sarcitrella L., *Hofmannophila pseudospretella* (Stainton)	*Bombus* spp.	scavenger	Frison 1926, Alford 1975
Pyralidae			
Achroia grisella, *Galleria colonella*	*Melipona* spp.	feed on nest debris	Salt 1929, Cepeda-Aponte et al. 2002
Aphomia sociella	*Bombus* spp.	feed on food stores and brood	Frison 1926, Wheeler 1928
Ephestia kühniella	*Bombus* spp.	feed on food stores	Frison 1926, Wheeler 1928, Alford 1975, Schmid-Hempel 2001
Galleria mellonella	*Apis cerana, A.*	feed on food stores	Williams 1997

Table 1-1. Continued.

Symbiont	Host	Integrative characteristic	Reference
	dorsata, A. florea		
Vitula edmandsii	*Bombus* spp.	feed on food stores	Frison 1926, Wheeler 1928, Alford 1975, Schmid-Hempel 2001
Hymenoptera			
Braconidae			
Apanteles spp., *Aspilota* spp., *Orthostigma pumilum* (Nees)	*Bombus* spp.	parasites of other nest invaders	Frison 1926, Alford 1975
Blacus paganus Haliday, *Hysteromerus mystacinus* Wesmael, *Stenocyptus* spp.	*Bombus* spp.	parasitize *Antherphagus* spp.	Alford 1975
Habrobracon juglandis	*Bombus* spp.	parasitize *Vitula edmandsii*	Frison 1926
Eulophidae			
Melittobia spp.	*Bombus* spp.	oviposit on or in host larval cells, parasitic larvae consume host larvae	Frison 1926, Free and Butler 1959
Formicidae			
Acromyrmex octospinosus	*Bombus pullatus*	builds fungus gardens within abandoned cells	Chavarria 1996
Cryptocerus elongata	*Trigona mosquito*	consume host colony's resources, establish colony within host nest	Salt 1929
Lasius niger (L.), *Solenopsis molesta* (Say)	*Bombus* spp.	scavenger	Frison 1926, Alford 1975
Oecophylla smaragdina F.	*Apis cerana, A. florea*	inhabit host nest	Fell 1997
Ichneumonidae			
Stilpnus gagates	*Bombus* spp.	parasite of other nest invaders	Alford 1975
Mutillidae			
Mutilla europaea	*Bombus* spp.	parasites of host brood	Brothers et al. 2000

Table 1-1. Continued.

Symbiont	Host	Integrative characteristic	Reference
Myrmilla capitata (Lucas) Vespidae	*Lasioglossum malachurum* (Kirby)	parasites of host brood	Polidori et al. 2009
Vespa tropica L.	*Apis florea*	predate of host bees in nest	Fell 1997

Table 1-2. Arthropods found in nests of *Apis mellifera* and their means of integrating into the nests.

Symbiont	Integrative characteristic	Reference
	Morphology	
Acari		
Ameroseidae		
Afrocypholaelaps africana	sucker-like ambulacral pads to attach to bees at flowers	Seeman and Walter 1995
Laelapidae		
Tropilaelaps clarae	elongated body for moving between hairs of bee and large legs with claws for attachment	Rath et al. 1991, Sammataro et al. 2000
Tarsonemidae		
Acarapis woodi	adults have tarsal claws and leg setae to accommodate attachment to bee hairs, larvae have reduced claws and enlarged pulvillar pads to allow for movement around tracheae, distally-oriented leg setation to allow for measurement of tracheae, modified mouthparts for piercing integument	Eickwort 1997, Ochoa et al. 2005
Coleoptera		
Nitidulidae		
Aethina tumida	larval protuberances to protect from drowning in honey, adults have thick cuticles to protect from sting	Lundie 1940, Neumann et al. 2001
Diptera		
Braulidae		
Braula coeca	wingless, pretarsal claws with modified combs for attaching to bee hairs	Sammataro 1997
Lepidoptera		
Sphingidae		
Acherontia atropos	thick cuticles to protect from sting	Moritz et al. 1991
	Behavior	
Acari		
Acaridae		
Acarus immobilis Griffiths, *Acarus siro* L., *Forcellinia galleriella* (Womersley), *Tyrophagus longior* (Gervais),	feed on comb, fungi, and debris	Abrol et al. 1994, Eickwort 1997

Table 1-2. Continued.

Symbiont	Integrative characterisitic	Reference
Tyrophagus palmarum Oudemans, *Tyrophagus putrescentiae* (Schrank)		
Ascidae		
Blattisocius spp., *Lasoseius* spp., *Melichares* spp., *Proctolaelaps* spp.	feed on other arthropods	Eickwort 1997
Carpoglyphidae		
Carpoglyphus lactis (L.)	feed on comb, fungi, and debris	Eickwort 1997
Cheletidae		
Cheyletus spp.	feed on other arthropods	Eickwort 1997
Glycyphagidae		
Glycyphagus domesticus de Geer	feed on comb, fungi, and debris	Eickwort 1997
Laelapidae		
Melittiphis spp.	feed on other arthropods	Eickwort 1997
Tropilaelaps clarae	phoretic on adults, feed on brood	Eickwort 1997, Brown et al. 2002
Macrochelidae		
Macrocheles spp.	feed on other arthropods	Eickwort 1997
Parasitidae		
Parasistus spp.	feed on other arthropods	Eickwort 1997
Pyemotidae		
Pyemotes spp.	feed on brood	Eickwort 1997
Scutacaridae		
Imparipes spp., *Scutacarus* spp.	feed on comb, fungi, and debris	Eickwort 1997
Tarsonemidae		
Acarapis dorsalis, *Acarapis externus*	feed on adults	Eickwort 1997
Acarapis woodi	phoretic	Eickwort 1997
Tarsonemus spp.	feed on comb, fungi, and debris	Eickwort 1997
Varroidae		
Varroa destructor	phoretic, feed on adults and brood	Eickwort 1997, Rosenkranz et al. 2010
Pseudoscorpiones		
Cheliferidae		
Chelifer cancroides, *Ellingsensis* spp.	feed on *Galleria mellonella* and *Varroa destructor*	Caron 1997b, Donovan and Paul 2005
Atemnidae		
Paratemnus minor	feed on adults and brood	Caron 1997b
Aranae		
Salticidae		
Salticus spp.	reside in silken cases on inner	Caron 1997b

Table 1-2. Continued.

Symbiont	Integrative characterisitic	Reference
	cover, feed on passing bees	
Thysanura		
Lepismatidae		
Ctenolepisma lineate F.	scavengers	Caron 1997a
Blattodea		
Blattidae		
Blatta orientalis L., *Periplaneta americana* (L.)	feed on wax, honey, and pollen	Caron 1997a
Rhinotermitidae		
Heterotermes tenuis	feed on hive components	Caron 1997a
Termitidae		
Microcerotermes arboreus (Emerson), *Nasutitermes costalis* (Holmgren)	feed on hive components	Caron 1997a
Dermaptera		
Forficulidae		
Forficula auricularia	scavengers	Alford 1975, Caron 1997a
Psocoptera		
Liposcelidae		
Liposcelis divinatorius (Müller)	scavengers	Caron 1997a
Trogiidae		
Trogium pulsatorium (L.)	scavengers	Caron 1997a
Hemiptera		
Pyrrhocoridae		
Pyrrhocoris apterus (L.)	feed on adults and brood	Caron 1997a
Coleoptera		
Cleridae		
Trichodes apiaries (L.)	feed on adults and brood	Caron 1997a
Cryptophagidae		
Cryptophagous hexagonalis Tournier, *Cryptophagus scanius* L.	scavengers	Caron 1997a, Haddad et al. 2008
Dermestidae		
Dermestes lardarius, *Dermestes vulpinus* F.	feed on hive components	Caron 1997a
Trogoderma glabrum (Herbst)	feed on wax, honey, and pollen	Caron 1997a
Trogoderma ornatum (Say)	feed on adults and brood, feed on hive components	Caron 1997a
Meloidae		
Meloe spp.	feed on adults and brood	Caron 1997a
Nitidulidae		
Aethina tumida	retract appendages when	Schmolke 1974, Pettis and

Table 1-2. Continued.

Symbiont	Integrative characterisitic	Reference
	attacked, hide, drop when provoked, take food from bee mouthparts, induce regurgitation from host, enter thermoregulatory clusters during winter	Shimanuki 2000, Neumann et al. 2001, Ellis et al. 2002b, Ellis 2003a, Neumann and Elzen 2004
Brachypeplus basalis, *Brachypeplus blandus* Murray, *Brachypeplus inquilinus* Lea, *Cychramus luteus* (F.), *Epuraea luteola*, *Glischrochilus fasciatus*, *Lobiopa insularis* (Castelnau deLaPorte)	scavengers	Lea 1910, 1912, Caron 1997a, Neumann and Ritter 2004, Ellis et al. 2008
Carpophilus dimidiatus	feed on pollen stores	Ellis et al. 2008
Epuraea corticina	feed on supplemental protein patties	Ellis et al. 2008
Ptinidae		
Ptinus fur (L.), *Ptinus raptor* Sturm	feed on wax, honey, and pollen	Caron 1997a
Pselaphidae		
Mesoplatus spp.	scavengers	Caron 1997a
Scarabaeidae		
Anomola dimidiate Hope, *Protaetia impavida* (Janson), *Torynorrhina opalina* (Hope)	feed on stored pollen	Caron 1997a
Copris lunaris L., *Potosia opaca* (F.)	feed on honey	Caron 1997a
Euphoria lurida (F.), *Euphoria sepulcralis*	feed on food stores	Caron 1997a, Woodruff 2006
Macrocyphonistes kolbeanus Ohaus, *Potosia hungarica* Herbst, *Rhyzoplatys auriculatus* (Burmeister)	feed on adults and brood	Caron 1997a
Oplostoma fulgeneus, *Pachnoda sinuata flaviventris* (Gory & Percheron)	feed on brood, honey, and pollen	Donaldson 1989, Torto et al. 2010
Staphylinidae		
Polylobus quadratipennis Lea	scavengers	Caron 1997a
Tenebrionidae		
Bradymerus sp., *Platybolium alvearium* Blair	scavengers	Caron 1997a
Tenebrio madens Charpentier	feed on stored pollen	Caron 1997a
Diptera		

Table 1-2. Continued.

Symbiont	Integrative characterisitic	Reference
Bombyliidae		
Anthrax spp.	parasitize honey bee larvae	Sammataro 1997
Braulidae		
Braula coeca	phoretic on adults, take food from bee mouth, induce regurgitation from host	Sammataro 1997
Drosophilidae		
Drosophila busckii	larvae feed on honey stores	Sammataro 1997
Phoridae		
Pseudohypocera kerteszi	feed on adults and brood	Sammataro 1997
Stratiomyidae		
Hermetia illucens	larvae feed on food stores	Sammataro 1997
Syrphidae		
Tenthredomyia australis	larvae feed on food stores	Sammataro 1997
Lepidoptera		
Oecophoridae		
Endrosis sarcitrella	scavengers	Williams 1997
Pyralidae		
Achroia grisella, Vitula edmandsii	feed on wax, honey, pollen	Williams 1997
Galleria mellonella	feed on wax, honey, pollen, evades detection by remaining very still	Nielson and Brister 1977, Eischen et al. 1986
Plodia interpunctella (Hübner)	scavengers	Williams 1997
Aphomia sociella	feed on brood, wax, honey, pollen	Williams 1997
Sphingidae		
Acherontia atropos	steal nectar, disorients guard bees by making squeaking noise	Moritz et al. 1991, Williams 1997
Hymenoptera		
Braconidae		
Apanteles galleriae, Bracon hebetor	parasitize *Achroia grisella* and *Galleria mellonella*	Williams 1997, Dweck et al. 2010
Crabronidae		
Bembex handlirschella Ferton	parasitize *Anthrax* spp.	Sammataro 1997
Eulophidae		
Melittobia acasta Walk	parasitize honey bee larvae	Fell 1997
Formicidae		
Camponotus spp.	feed on food stores and destroys hive materials	Fell 1997
Crematogaster spp., *Dorylus* spp., *Eciton* spp., *Formica* spp.,	feed on adults, brood, and food stores	Fell 1997

Table 1-2. Continued.

Symbiont	Integrative characterisitic	Reference
Mutillidae		
Iridomyrmex spp., *Solenopsis* spp.		
Mutilla europaea	lays eggs in pupal cells, larvae feed on bee pupae, adults feed on honey stores	Alford 1975
Vespidae		
Dolichovespula spp., *Vespula* spp.	steal nectar	Fell 1997
Vespa spp.	feed on adults	De Jong 1990, Fell 1997
	Chemical	
Acari		
Varroidae		
Varroa destructor	have chemical profiles that mimic honey bees	Nation et al. 1992, Martin et al. 2001
Coleoptera		
Nitidulidae		
Aethina tumida	detect pheromones at low concentrations to find host, recruit conspecifics through multitrophic interaction	Elzen et al. 1999, Suazo et al. 2003, Torto et al. 2005, Torto et al. 2007a
Lepidoptera		
Sphingidae		
Acherontia atropos	have chemical profiles that mimic honey bees	Moritz et al. 1991
Hymenoptera		
Formicidae		
Linepithema humilis	lack identifying odors to escape detection	Spangler and Taber 1970
Vespidae		
Vespa mandarinia japonica	use pheromonal markings to assist in mass attacks	Fell 1997

CHAPTER 2
ATTRACTION OF MULTIPLE BEETLE SPECIES TO HONEY BEE HIVE ODORS

SHBs locate host honey bee colonies by detecting volatile chemicals, including those emitted by honey bee workers and bee-collected pollen (Suazo et al. 2003, Torto et al. 2005). Specifically, volatiles of honey bee alarm pheromone, including isopentyl acetate (IPA), are highly attractive to the SHB which can detect IPA at lower quantities than the bees themselves (Torto et al. 2007a). Furthermore, SHBs carry a symbiotic yeast (*Kodamaea ohmeri***)** which, when mixed with bee-collected pollen, produces IPA and other components of the alarm pheromone that attracts SHBs (Torto et al. 2007a, b). Therefore, the SHB exhibits a high degree of adaptation for finding host colonies.

The SHB is not the only nitidulid beetle that occurs in honey bee colonies. Other nitidulids reported in honey bee colonies include *Cychramus luteus* (Neumann and Ritter 2004), *Lobiopa insularis*, *Carpophilus dimidiatus*, *Glischrochilus fasciatus*, *Epuraea corticina* (Ellis et al. 2008), and *E. luteola* (personal observation). Although the SHB exhibits a high degree of integration into honey bee colonies (Ellis and Hepburn 2006), it remains unclear if other nitidulids share a similar relationship with honey bees. No damage has been reported from the presence of these nitidulids in honey bee colonies, and their ecological niche in honey bee colonies is likely that of a facultative scavenger.

Cychramus luteus is a saproxylic species found in Europe (Kaila et al. 1994). In 2003, this species was found inhabiting two healthy honey bee colonies. However, this beetle's inability to reproduce on hive components suggests that they are accidental inhabitants (Neumann and Ritter 2004). *Lobiopa insularis* usually is found in agricultural settings feeding on fruits, especially strawberries (Parsons 1938, Williams and Salles 1986). The species also has been found in colonies in Georgia, USA, but shows no evidence of integration beyond seeking shelter (Ellis et

al. 2008). *Carpophilus dimidiatus* is found in tropical and temperate climates worldwide (Parsons 1943) and often is found in agricultural settings feeding on corn (Connell 1975), over-ripe fruits, stored products, and a variety of fermenting substrates (see Hinton 1945). Several individuals were collected from a single colony in Georgia, USA, and were assumed to be accidentals (Ellis et al. 2008). *Glischrochilus fasciatus* is found throughout North America, predominately in woodlots (Parsons 1943, Blackmer and Phelan 1995, Majka and Cline 2006, Price and Young 2006). This species is a pest of raspberries, strawberries, tomatoes, and corn (Williams et al. 1981), and typically visits most fermenting substrates. The species also was discovered in several honey bee colonies in Georgia, USA but upon further investigation, did not reproduce successfully on honey bee colony components (Ellis et al. 2008). *Epuraea corticina* is found throughout the United States and is associated commonly with sap flows and oak wilt mats (Parsons 1969, Cease and Juzwik 2001). This species has been found in honey bee colonies in association with supplemental protein patties given to bees and is considered accidental (Ellis et al. 2008). Finally, a single specimen of *E. luteola* was found in a honey bee colony in Florida (personal observation). However, the presence of this species is assumed to be accidental.

Herein, I tested the hypothesis that beetle species highly integrated into honey bee colonies will be more attracted to honey bee colony odors than beetles less integrated into honey bee colonies. To test this hypothesis, I recognized three distinct categories of beetle integration into colonies (Wheeler 1910). Synecthrans/symphiles (= highly integrated) are the most highly integrated beetles. This category includes species that are treated with hostility by hosts while they feed on host nest parts (synechthrans) and those species that are accepted to varying degree, and even fed by hosts occasionally (symphiles) (Wheeler 1910, Ellis and Hepburn 2006). The SHB was the highly integrated species used in this study due to its high level of integration into

honey bee colonies and its known ability to solicit food from worker honey bees (Ellis 2005, Ellis and Hepburn 2006). Accidental species occur in colonies, but more often are found outside of colonies (Smith 1886). Included in this category were *L. insularis* and *E. luteola*, which have been found in honey bee colonies (Ellis et al. 2008, personal observation). Finally, non-integrated beetles included *Carpophilus hemipterus* (L.), *C. humeralis* (F.) (Coleoptera: Nitidulidae), and *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae) that have never been captured in honey bee colonies. Beetle species were chosen based on their level of integration and availability in the study area. Furthermore, *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae) was the preferred non-nitidulid (also used in Chapters 4 and 6), but *O. surinamensis* was substituted in this experiment as *T. castaneum* was unable to walk in the olfactometer.

Materials and Methods

Beetles

Adult *O. surinamensis* were obtained from *in vitro* rearing colonies at the USDA-ARS facility in Gainesville, Florida (29.64° N, 82.35° W). Adult SHBs were captured from experimental honey bee colonies maintained at the University of Florida Bee Biology and Research Unit (BBRU) in Gainesville, Florida (29.63° N, 82.36° W). The SHBs were reared in an incubator (25°C; 80% relative humidity; constant darkness) on a diet of honey, pollen, and Brood Builder™ (Dadant and Sons, Inc., Hamilton, IL) in a ratio of 1:1:2 respectively (Ellis et al. 2008, 2010) at the University of Florida Department of Entomology and Nematology (29.64° N, 82.36° W). All other nitidulids were captured on rotting cantaloupe, *Cucumis melo* L., at the University of Florida Plant Science Research and Education Unit (PSREU) in Citra, Florida (29.41° N, 82.17° W) and reared in an incubator (24°C; 40% relative humidity; constant darkness) on a tomato and prune-based diet developed by Peng and Williams (1990) for several

generations prior to the experiment. All nitidulids were lab-reared and 2 – 4 weeks old at the time of the experiment. *Oryzaephilus surinamensis* were lab-reared but were of unknown ages at the time of the experiment. All beetles were given water only for 24 hours prior to the experiment.

Honey Bees

Honey bees were of Russian honey bee descent and were established in a 5 frame nucleus (nuc) hive of standard Langstroth dimensions at the PSREU before being taken to the BBRU one week prior to experimentation. The hive body of the nuc had a 1.25 cm hole drilled into the front and rear panels to accommodate air flow. The colony contained honey, pollen, brood, worker bees, and a mature egg-laying queen. During the experiment, cracks around the hive were sealed with duct tape so that airflow into and out of the colony was possible only through the drilled holes (see Graham et al. 2011).

Olfactometer

A 4-way olfactometer was used to determine beetle attractiveness to honey bee colony volatiles (Vet et al. 1983, Graham et al. 2011). Glass traps designed to capture beetles were attached at each of the four arms of the test arena (per Graham et al. 2011). The traps were attached to corrugated FEP tubing (1.15 cm inside diameter × 1.27 cm outside diameter, Cole-Parmer, Vernon Hills, IL). A tube from one of the traps connected to the hole at the front of the nuc while the tubes from the remaining three traps were connected to a carboloy air flow regulator (Aalborg Instruments and Controls, Inc., Orangeburg, NY) set at 0.5 liter/min. The flow regulators were attached via corrugated FEP tubing to a portable filtered-air pump developed by the USDA-ARS, Gainesville, Florida. The rear of the nuc also was attached to the air flow regulator (0.5 liters/min) and then to the pump. The bottom of the arena had an insect inlet port which was modified to accommodate the introduction of beetles. This consisted of a

~10 cm piece (diameter = 0.32 cm) and a ~4 cm piece (diameter = 0.64 cm) of industrial tubing (Cole-Parmer, Vernon Hills, IL). The port was connected to corrugated FEP tubing, which connected to another air flow regulator set at 2 liter/min (Graham et al. 2011). The air flow regulator was connected to the house vacuum at USDA-ARS by way of Master flex® Tygon® tubing (Cole-Parmer, Vernon Hills, IL). Nylon mesh was attached at the inlet port and metal mesh was built into the glass trap ends distal to the arena to prevent beetle escape.

Choice Bioassay

The choice tests were conducted in November 2009 under red-light conditions over two consecutive nights between 18:30 and 02:30 the next morning. They were performed at the USDA-ARS in Gainesville, Florida in a temperature-controlled room held at approximately 31°C and 65% relative humidity. Each replicate consisted of five consecutive, separate conspecific beetle introductions. Beetle sex was not determined. The bioassay was replicated eight times per beetle species (four replicates per beetle species per night). Species order and port assignments were randomized between replicates. Three ports delivered filtered air (control) and one delivered air pushed through the honey bee nuc. For each introduction, a beetle was placed in the insect inlet port and observed. A choice was made when a beetle completely entered one of the arms of the olfactometer. A beetle was given 10 minutes to make a choice, after which it was removed and a new beetle was introduced if no choice had been made (per Graham et al. 2011). Beetles were used only once and non-choices were not included in the analysis.

Statistical Analysis

Control means were averaged for each replicate (= number of beetles choosing control ports / 3 control ports = avg. # beetles going to a single control port). Beetle attraction to the olfactometer ports was tested recognizing treatment (filtered air or air from a honey bee colony) and beetle species (6 species) or level of integration (3 levels of integration) as main effects

using a two-way factorial ANOVA. Within each treatment (filtered air and air from a honey bee colony), a one-way ANOVA was used to test beetle attraction to olfactometer ports by the main effects (1) beetle species and (2) beetle level of integration. Where necessary, means were compared using Tukey-Kramer tests and all analyses were conducted using JMP software (JMP 2008).

Results

Since there was a significant beetle species × treatment interaction ($F = 13.08$; df = 5, 84; $P < 0.01$) and level of integration × treatment interaction ($F = 21.68$; df = 2, 90; $P < 0.01$), beetle attraction to the olfactometer ports was analyzed separately by beetle species and level of integration (Table 2-1). SHBs were significantly more attracted to hive odors than to filtered air (Table 2-1). *Carpophilus humeralis* and *O. surinamensis* exhibited no preference to ports emitting either filtered air or air from a honey bee colony (Table 2-1). *Carpophilus hemipterus* and *E. luteola* were more attracted to the filtered air or were repelled by the hive odor, whereas *L. insularis* consistently chose ports emitting filtered air in every assay (Table 2-1). When beetle species were grouped according to level of integration within honey bee colonies, the most highly integrated species (SHB) was attracted to hive odors more than to filtered air (Table 2-1). However, accidental species were more attracted to filtered air (or repelled by hive odors) than to hive odors (Table 2-1) while the non-integrated beetle species showed no preference to odors emanating from ports releasing either type of air (Table 2-1).

SHBs, *C. humeralis*, and *O. surinamensis* did not differ in their level of attraction to air from a honey bee colony (Table 2-1). However, SHBs were significantly more attracted to air from honey bee colonies than *C. hemipterus*, *E. luteola*, and *L. insularis*, whose level of attraction to honey bee colony odors did not differ significantly from one another. Reciprocally, *L. insularis*, *E. luteola*, and *C. hemipterus* were attracted to the filtered air (or repelled by air

from the honey bee colony) more often than *C. humeralis* and SHBs (Table 2-1). Concerning the levels of integration, the highly integrated species (SHB) was more attracted to air from a honey bee colony than were the non-integrated and accidental species, which decreased in their level of attraction to air from a honey bee colony respectively and significantly. That said, exactly the opposite relationship existed between beetles in the three levels of integration with respect to their attraction to filtered air with accidental species being most attractive to filtered air (or repelled by air from a honey bee colony) followed by non-integrated species and highly integrated species respectively (Table 2-1).

Discussion

In general, there was a clear relationship between a beetle's level of integration into honey bee colonies and its preference for hive odors, though the relationship was different from that which I hypothesized originally. Although the highly integrated species (SHB) was more attracted to colony odors than beetles from the other two integration levels, I expected accidental species to be somewhat attracted to hive odors due to their known occurrence in honey bee colonies. This, however, was not the case. In fact, collectively, accidental species were less attracted to (more repelled by) hive odors than non-integrated species that had never been reported from honey bee colonies.

The SHB is well known to be highly integrated into honey bee colonies, and is attracted to honey bee nests and, more specifically, to components of honey bee alarm pheromone (Suazo et al. 2003, Torto et al. 2005, 2007a, b, Graham et al. 2011). Therefore, the preference of SHBs for hive odors as demonstrated in this study was expected and is consistent with previous results (Suazo et al. 2003, Torto et al. 2005, 2007a, b, Graham et al. 2011). Because the SHB represents the only beetle species known to be highly integrated in honey bee colonies in this study, then

this olfactory affinity for hive odors may be a key factor to its success at integrating into bee nests.

Though SHBs were attracted to odors from honey bee colonies, no other beetle species in the study demonstrated a similar propensity. *Epuraea luteola* and *L. insularis*, the accidental associates, were the species least attracted to (or most repelled by) odors emanating from the hive. Both species have been documented in honey bee colonies (Ellis et al. 2008, personal observation) so a lack of attraction to the hive odors in this study is somewhat perplexing. This result may be interpreted as an adaptation of these beetles reflecting their level of integration into honey bee colonies. Repulsion from the scent of alerted bees may be an adaptive strategy used by beetle species that do not possess other adaptive traits. Highly integrated species, such as the SHB, possess adaptive traits that allow them to be attracted to honey bee colonies while defending themselves against bee attacks. For example, the SHB possesses the ability to retract its appendages beneath its body (Neumann and Elzen 2004), thus lessening the likelihood that a bee can grab, sting, and/or remove it from a colony. In contrast, accidental species such as *E. luteola* lack such adaptations and defend themselves by fleeing from stressed or angered bees.

Alternatively, accidental invaders may be attracted to odors from nest debris, pollen, or some other hive component that were not detectable in the odors emanating from the nuc used in this study. The suite of odors (e.g. brood stages and pollen reserves) may not be present in the nuc at the particular time of year the study was carried out (i.e. November). Their observed repulsion may not be in response to pheromones produced by alerted bees, but rather to other odors associated with danger or unfavorable habitats.

Overall, the non-integrated beetle species were neither attracted to nor repelled by odors present in the nucleus colony. *Oryzaephilus surinamensis*, a non-integrated beetle species, is a

stored grain pest. Despite a preference for stored grains, this species may be attracted to honey bee colony odors for three reasons. First, the species has been found in pollen traps on bee colonies (Leonard 1983). Second, it is attracted to fungi-produced volatiles, (Pierce et al. 1991) and fungi are present in honey bee colonies (Gilliam and Vandenberg 1997). Specifically, *O. surinamensis* is attracted to 3-methyl-1-butanol (Pierce et al. 1991, Collins et al. 2008), which is a component of both fungi volatiles and honey bee alarm pheromone (Wager and Breed 2000). Third, it has been suggested that ancestral stored product beetles thrived on seeds that had accumulated in nests of rodents, birds, and social insects (Cox and Collins 2002), thus possibly predisposing some stored product beetles to attraction to honey bee nests. For example, *Tribolium myrmecophilum*, a close relative of the pest species *T. castaneum*, has been found in nests of ants and stingless bees (Lundie 1940, Angelini and Jockusch 2008). Despite these reasons to expect an attraction to hive odors by *O. surinamensis*, the results indicated no such preference.

The other non-integrated beetle species, *C. hemipterus* and *C. humeralis*, also are attracted to 3-methyl-1-butanol (Phelan and Lin 1991, Nout and Bartelt 1998) just as with *O. surinamensis*. However, *C. hemipterus* was repelled by hive odors and *C. humeralis* was neither attracted nor repelled by hive odors. There are no records of these species occurring in honey bee colonies, but the related *C. dimidiatus* has been found in pollen cells within the hive (Ellis et al. 2008). Therefore, multiple *Carpophilus* species may forage occasionally inside honey bee colonies.

Performing assays of the behavioral responses of the accidental beetle species to different components of the hive, such as pollen, honey, brood, wax, and adult bees, would likely yield interesting far-reaching results. The presence of *E. luteola* and *L. insularis* in hives suggests that

some colony component is attractive to these species even though their overall response to odors in this study was one of repulsion or avoidance.

In conclusion, the results from this study suggest that the most highly integrated beetle species, i.e. the SHB, was the species most highly attracted to the hive odors. Those beetle species exhibiting an intermediate level of integration into honey bee colonies were less attracted to hive odors or even repelled by them.

Table 2-1. Beetle attraction to hive odors. Values are mean ± SE (*n*) number of beetles attracted to a port. The number of beetles attracted to filtered air was divided by three to account for multiple ports. Columnar means followed by the same capital letter are not different at $\alpha = 0.05$. Row means followed by the same lower-case letter are not different at $\alpha = 0.05$. For level of integration, beetle species were categorized together by level of integration into honey bee colonies using the terms highly integrated (HI), accidental (A), or non-integrated (NI) (Smith 1886, Wheeler 1910). df = 1, 14 unless otherwise indicated.

	Treatment			
Beetle species (level of integration)	Honey bee colony air	Filtered air	ANOVA	
A. tumida (HI)	2.250 ± 0.453 (8)A a	0.875 ± 0.125 (8)C b	F = 8.56	P = 0.01
E. luteola (A)	0.125 ± 0.125 (8)C D b	1.583 ± 0.055 (8)A B a	F = 114.33	P < 0.01
L. insularis (A)	0 (8)D b	1.667 (8)A a	all beetles in every replication went only to the ports emitting filtered air	
C. hemipterus (NI)	0.375 ± 0.263 (8)B C D b	1.542 ± 0.088 (8)A B a	F = 17.70	P < 0.01
C. humeralis (NI)	1.750 ± 0.491 (8)A B a	1.083 ± 0.164 (8)C a	F = 1.66	P = 0.22
O. surinamensis (NI)	1.500 ± 0.378 (8)A B C a	1.167 ± 0.126 (8)B C a	F = 0.70	P = 0.42
ANOVA→	F = 8.1; df = 5, 42; P < 0.01	F = 9.0; df = 5, 42; P < 0.01		
	Treatment			
Level of integration	Air from a honey bee colony	Filtered air	ANOVA	
Highly integrated	2.250 ± 0.453 (8)A a	0.875 ± 0.125 (8)C b	F = 8.56	P = 0.01
Accidental	0.063 ± 0.063 (16)C b	1.625 ± 0.028 (16)A a	F = 517.64; df = 1,30; P < 0.01	
Non-integrated	1.208 ± 0.248 (24)B a	1.264 ± 0.083 (24)B a	F = 0.05; df = 1,46; P = 0.83	
ANOVA→	F = 13.4; df = 2, 45; P < 0.01	F = 14.6; df = 2, 45; P < 0.01		

CHAPTER 3
SHB ADAPTIVE LEG MORPHOLOGY

Inquilinism is a ubiquitous lifestyle of many arthropods associated with social insect nests. Life with social insects provides inquilines a stable source of food that may be obtained either directly or indirectly from the host, as well as a means of protection from the environment (Gullan and Cranston 2005). The most diverse social insect inquilines are beetles, which display a variety of integrative adaptations (Kistner 1982). Many of these adaptations are morphological, providing inquilines defensive postures or structures which effectively negate biting, stinging and/or removal tactics used by the social insect host to defend against the inquiline.

There are many examples of beetles that possess adaptive morphology that permits them to integrate into social insect colonies. These include histerids, leiodids, chrysomelids, corylophids, cybocephalids, and nitidulids among others. Histerid beetles frequently are found in ant nests (Kistner 1982, Kovarik and Caterino 2001, Caterino and Vogler 2002, Kovarik and Tishechkin 2004, Caterino and Tishechkin 2008). Inquilinous histerids generally are considered to possess four morphological modifications associated with inquilinism, including: 1) a hard smooth exoskeleton; 2) a convex and often vaulted body; 3) a retractable head; and 4) short legs that can be retracted within body grooves (Reichensperger 1924, cited in Kistner 1982). Additionally, the myrmecophilous (ant inquilines) histerid *Psiloscelis* spp. have flattened and expanded tibiae (Kistner 1982).

Myrmecophilous leiodids have proportionately shorter legs with reduced contiguous tarsal segments as well as more compact antennae than their free-living counterparts (Kistner 1982). Melittophilous (bee inquilines) leiodids in the genus *Scotocryptus* are able to retract their head, antennae, and legs beneath a hemispheric body, making them difficult to grasp (Salt 1929, Peck 2003). Ventral grooves on the head accommodate the antennae and caniculate femora receive the

tibiae (Roubik and Wheeler 1982) during retraction of the appendages. These beetles possess elytra that are held together tightly by a tongue-and-groove mechanism, thereby protecting the relatively soft, lightly sclerotized tergites from attack (Roubik and Wheeler 1982).

The melittophilous corylophid *Cleidostethus* Arrow has similar adaptations to those of *Scotocryptus* (Bowstead et al. 2001, Salt 1929). The head is small and can be retracted along with the antennae between the pronotum and prosternum, the margins of which are flattened and explanate. The legs are small in proportion to the body, and the tibiae retractable within the femora, which subsequently fit into grooves on the body proper. The elytra are fused, and the only moveable region of the major body segments is between the prothorax and mesothorax. However, even this region can be made rigid, allowing the beetle to firmly grasp the substratum (Salt 1929).

The chrysomelid *Hemisphaerota cyanea* (Say) can be found on palmetto plants in the southeastern United States (Woodruff 1965) and possesses a number of morphological features that allow it to survive ant attacks. The beetle has evolved highly modified tarsi that are covered with branched setae and pores that secrete sticky oils. Upon attack by ants, the beetle presses its tarsi firmly to the plant's surface, creating a higher area of contact between the hairs and sticky oil with the substratum. Additionally, *H. cyanea* has a hemispherical, glabrous, convex body that is difficult for ants to grasp with their mandbiles (Attygalle et al. 2000, Eisner and Aneshansley 2000). Ant removal of the beetle from the palmetto consequently becomes difficult or impossible to achieve.

Nitidulidae contains many examples of inquilinism and the phenomenon purportedly has arisen independently at least four times within the subfamily Nitidulinae (Cline 2005). *Cychramptodes murrayi* Reitter is a predator of the waddle tick scale, *Cryptes baccatus*

(Maskell) (Coccidae). These scales are tended by ants, and the nitidulid beetles possess morphological adaptations suited for passive defense. These include a highly convex and glabrous body, ventrally-projecting hypomera and epipleura that conceal the underside of the beetle including the legs, broad flattened tibiae, and caniculate femora. These leg structures allow the beetles to retract their legs and lay flat against a surface (Kirejtshuk and Lawrence 1992, Leschen 2000). Species of *Amphotis* Erichson are associated commonly with ants (Parsons 1943, Hölldobler 1968, Audisio 1993) and have a broad, flat body which conceals the legs if attacked (Cline 2005). *Amborotubus* Leschen & Carlton is a recently described nitidulid genus that has been hypothesized to be a social insect inquiline based solely on its morphology, which includes flattened femora and tibiae and a shielded appearance. *Cylindroramus* Kirejtshuk and Lawrence has a similar body shape as *Amborotubus*, but its legs are hidden in lateral view, unlike *Amborotubus* (Leschen and Carlton 2004). *Cylindroramus* (Cline 2005) and *Arhina* Murray (A. R. Cline, pers. comm.) are postulated as social insect inquilines based solely on their morphology.

Herein, I tested the hypothesis that the SHB exhibits several inquilinous morphological adaptations specific to legs, and that other related species have a decreasing number of these structures based on their degree of integration into honey bee colonies. Specifically, sap beetles that have been found in colonies on occasion ("accidentals") are expected to have fewer morphological leg adaptations than SHBs, but more than those beetles which have not been found in colonies. To test this hypothesis, the leg morphology of the SHB was compared to that of five bee colony accidentals (*Aethina villosa* Reitter, *L. insularis*, *G. fasciatus*, *C. dimidiatus*, and *E. luteola*) and four nitidulid species which have not been found in bee colonies, including

Stelidota geminata (Say), *Amphicrossus ciliatus* (Olivier), *Carpophilus hemipterus* L., and *C. humeralis* (F.).

Materials and Methods

SHBs were collected from a honey bee colony at the University of Florida Bee Biology Research Unit in Gainesville, Florida (29.63° N, 82.36° W). *Aethina villosa* specimens were supplied from the Andrew R. Cline Collection (ARCC), which currently is housed at the California State Collection of Arthropods (CSCA) in Sacramento, California. Individuals from all other beetle species were collected at the University of Florida Plant Science Research and Education Unit (29.41° N, 82.17° W) in Citra, Florida on rotting cantaloupes, *Cucumis melo* L. Beetle sex was not determined. Scanning electron micrographs were prepared according to standard protocols (Eisner and Aneshansley 2000) at the Division of Plant Industry in Gainesville, Florida using a JEOL JSM-5510LV SEM.

Species were scored based on presence or absence of the following morphological traits: caniculate femora (i.e. *Scotocryptus* spp. in Roubik and Wheeler 1982), flattened/expanded tibiae (= width ≥ 1/3 length) (i.e. *Psiloscelis* spp. in Kistner 1982), undulate tarsal setation (i.e. *Cantharis fusca* in Beutel and Gorb 2001), and dense tarsal setation (i.e. *Hemisphaerota cyanea* in Eisner and Aneshansley 2000). These characters were chosen to represent adaptation to an inquilinous lifestyle (Kistner 1982). Scores were based on visual inspection of SEM images.

Results

SHBs exhibit all four leg morphological characters that were investigated in this study (Table 3-1, Figures 3-1, 3-2). All species except *L. insularis*, *G. fasciatus*, and *E. luteola* have dense setal pads on the ventral surface of the tarsomeres (Table 3-1, Figure 3-2). Also, all species except *S. geminata*, *A. ciliatus*, *C. dimidiatus*, and *E. luteola* possess undulate tarsal setae (Table 3-1, Figure 3-2). The *Carpophilus* species have broad flattened tibiae (Table 3-1, Figure 3-1).

Discussion

The data may suggest that the presence of caniculate femora is correlated with inquilinous species since the SHB was the only species to possess this morphological characteristic (Table 3-1). That said, the SHB was the only beetle species to possess all of the studied leg characters. In sharp contrast, *E. luteola* exhibited none of the morphological features investigated in this study though the beetle has been found in honey bee colonies before (personal observation). Consequently, the data do not support the tested hypothesis that the number of leg morphological adaptations a beetle possesses is directly related to the degree of integration into honey bee colonies (i.e. *E. luteola* exhibits a moderate level of integration - it is an "accidental" species – though it possesses none of the studied leg characteristics).

The caniculate femora possessed by SHBs, which accommodate the broad flattened tibiae, allow the beetle to retract its legs underneath the body when encountering an aggressive bee (Neumann et al. 2001, Neumann and Elzen 2004). Furthermore, the SHB's dense, undulate tarsal setae presumably allow it to grasp a substrate, similar to the phenomenon found in the chysomelid *Hemisphaerota cyanea* (Eisner and Aneshansley 2000), though this has not been tested, nor has the presence of oil on the tarsi of SHBs. A secure substrate attachment would prohibit bees from flipping the beetle over and stinging and/or removing them from the nest – a feature which Schmolke (1974) detected in his behavioral observations. These leg adaptations accompany other defense-providing morphological features that the SHB possesses, including a hardened exoskeleton and overlapping body regions (Kistner 1979, Neumann and Elzen 2004), all of which allow the beetle to invade, colonize, and reproduce within honey bee colonies.

The trophic habits of the beetles used in this study, and nitidulids generally, likely predispose them to possessing at least some of the morphological adaptations that would increase their ability to infest bee nests. Many nitidulids are associated with fungal substrates in detritus,

rotting fruits, subcortical spaces, and fungal fruiting bodies (Parsons 1943, Cline 2005). These conditions often favor flat to relatively flattened body forms. *Aethina villosa, L. insularis, G. fasciatus, A. ciliatus, Carpophilus* spp., and *E. luteola* not only are known from sap flows and fungal substrata but may also occur in flowers as well. Thus, their bodies should be somewhat compact and/or flattened so that they can enter small cavities such as a developing flower bud (Parsons 1943, 1972, Vogt 1950, Nadel and Pena 1994, Majka and Cline 2006). *Stelidota geminata* occurs mainly in leaf litter but also at sap flows and overripe fruits (hence, the common name "strawberry sap beetle") (Parsons 1943, Loughner et al. 2007). Therefore, it must be able to access small spaces as well. Furthermore, males of several beetle species exhibit dense tarsal setation used to grasp the female during copulation (A. R. Cline, pers. comm.). However, the specimens used in this study were not sexed, so such a correlation between sex and setation density could not be surmised.

Other species of *Aethina* are associated with decaying plant matter (including fruits) and flowers (Kirejtshuk 1997, Kirejtshuk and Lawrence 1999), as well as occur within leaf litter (A. R. Cline, pers. comm.). Therefore, the general body form of *Aethina* should enable them to enter concealed places, including the small crevices of a bee nest. This hiding behavior is common in SHBs (Lundie 1940, Schmolke 1974, Neumann et al. 2001, Neumann and Elzen 2004, Ellis 2005). Also, some bee hive odors are identical to those associated with decay (Phelan and Lin 1991, Pierce et al. 1991, Nout and Bartelt 1998, Wager and Breed 2000, Collins et al. 2008, Graham 2009), so attraction to volatiles emanating from decomposition might predispose SHBs to attraction to honey bee nests. However, the causative mechanism underlying bee nest-seeking behavior in SHB ancestors remains unclear.

The striking differences between the leg characters of the two *Aethina* species investigated in this study provide further evidence that the SHB is well-adapted for penetrating and persisting within honey bee nests. For example, the ability to retract appendages beneath the body is not crucial to the survival of the free-living species *A. villosa*, but it is for the inquilinous SHBs. Thus, despite the two species' close phylogenetic relationship, only the SHB displays caniculate femora and flattened tibiae. Also, the fact that the two species share dense, undulate tarsal setae suggests that this character is an exaptation which was adopted by SHBs for use within honey bee nests. These tarsal setation characters may have helped *A. villosa* enter the honey bee colony in which it was found (A. R. Cline, pers. comm); however the absence of other morphological and behavioral adaptations may have precluded this species from becoming fully integrated into the hive environment.

The degree of morphological adaptation in SHBs may suggest a long association with honey bee nests. This idea is supported when considered in conjunction with other characters the SHB displays. These include (1) behavioral mimicry, wherein a beetle which is otherwise trapped within the colony is able to solicit food from bee "prison-guards" trophallactically (Ellis et al. 2002b, Ellis 2005), (2) the beetle's ability to detect and cue into honey bee volatiles (Suazo et al. 2003, Torto et al. 2005), and (3) the harboring of a yeast (*Kodamaea ohmeri*) which, when mixed with bee-collected pollen, produces volatiles attractive to other conspecifics (Torto et al. 2007a). These characters act synergistically to accommodate SHB entrance and integration within honey bee colonies.

Table 3-1. Presence/absence of hypothesized inquilinous morphological characters in selected nitidulid species.

	Leg character			
Species	Femora caniculate	Tibiae flattened/expanded	Tarsi Undulate setae	Dense setae
Aethina tumida	X	X	X	X
Aethina villosa			X	X
Stelidota geminata				X
Lobiopa insularis			X	
Glischrochilus fasciatus			X	
Amphicrossus ciliatus				X
Carpophilus dimidiatus		X		X
Carpophilus hemipterus		X	X	X
Carpophilus humeralis		X	X	X
Epuraea luteola				

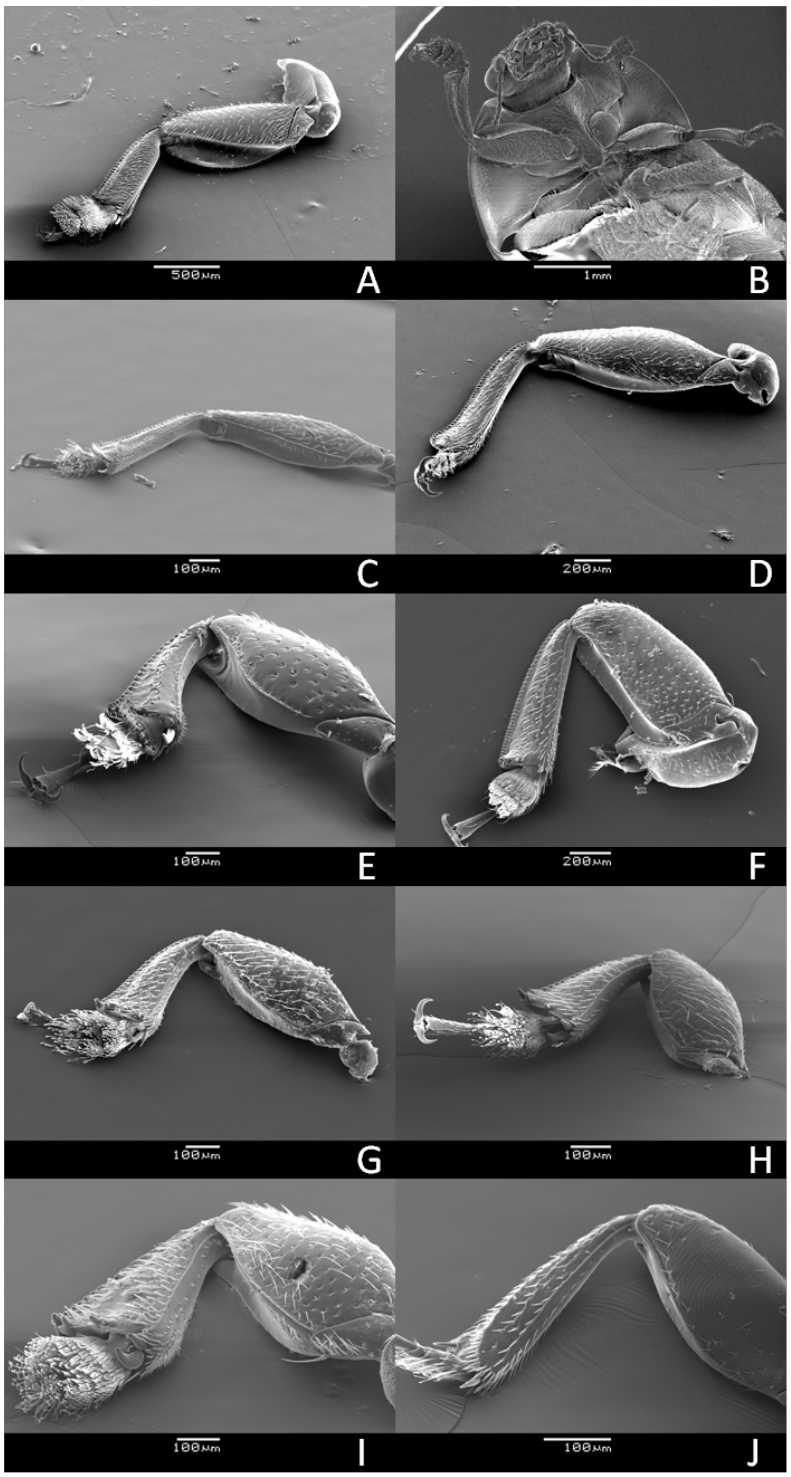

Figure 3-1. SEM images of entire legs. The species are as follows: A) *Aethina tumida*, B) *A. villosa*, C) *Stelidota geminata*, D) *Lobiopa insularis*, E) *Glischrochilus fasciatus*, F) *Amphicrossus ciliatus*, G) *Carpophilus dimidiatus*, H) *C. hemipterus*, I) *C. humeralis*, J) *Epuraea luteola*. All images are the left proleg, except for J, which is the left mesoleg. *C. humeralis* (I) received a puncure in the femur during preparation.

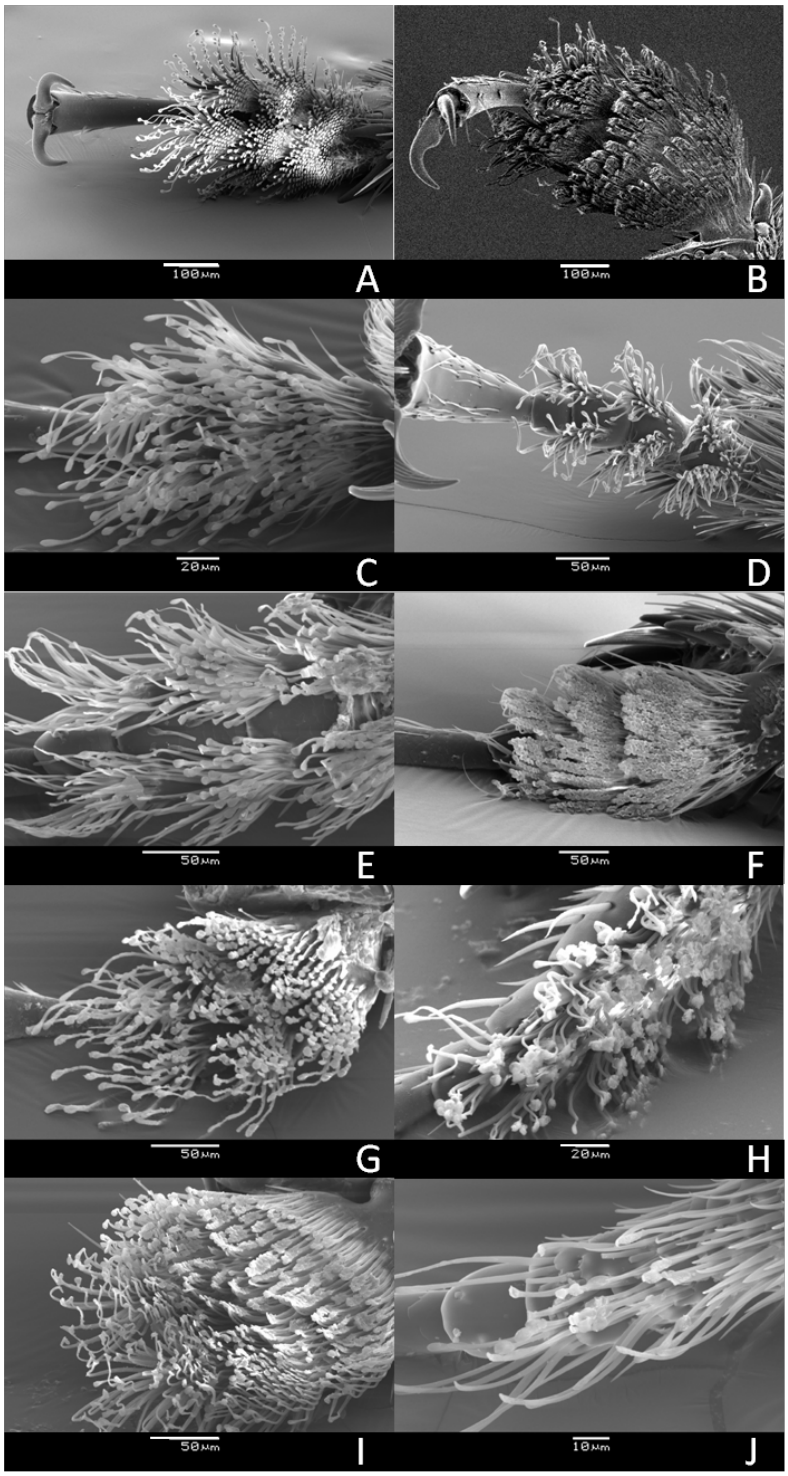

Figure 3-2. SEM images of tarsi. The species are as follows: A) *Aethina tumida*, B) *A. villosa*, C) *Stelidota geminata*, D) *Lobiopa insularis*, E) *Glischrochilus fasciatus*, F) *Amphicrossus ciliatus*, G) *Carpophilus dimidiatus*, H) *C. hemipterus*, I) *C. humeralis*, J) *Epuraea luteola*. A, E, F, and H are images are the right mesotarsi; B is the right protarsi; C, D, G, and I are the left protarsi; and J is the right metatarsi.

CHAPTER 4
ADAPTIVE BEHAVIOR OF HONEY BEES TOWARD BEETLE INVADERS EXHIBITING VARIOUS LEVELS OF COLONY INTEGRATION

Social insect colonies host many inquilinous species exhibiting various levels of integration (see Kistner 1982, Chapter 1). This is true particularly of ant (Hymenoptera: Formicidae) and termite (Blattodea) nests. In contrast, bees seem to host fewer guests, and those that are present exhibit fewer adaptations (Wilson 1971, Kistner 1982). Some potential explanations for this have been offered, although few have been tested directly. One explanation offered by Wilson (1971) is the tendency of bees to nest in arboreal locations. This presents an obstacle that must be overcome by would-be invaders as they must first locate and invade an arboreal colony. However, ground-nesting bees are common, and researchers conducting a survey of parasites in wasp and bee nests found no significant difference in parasite loads between ground and tree nests (Wcislo 1996). Although discounted by Wilson (1971) as a potential explanation, Kistner (1982) suggests that the effectiveness of defense likely is part of the explanation for this apparent phenomenon. In ants, he points out, those with effective stings and other defenses (e.g. *Solenopsis* sp.) host fewer guests than do species with less-prominent defenses (e.g. army ants).

Western honey bees have particularly well-defended nest entrances, so colony integration by would-be invaders begins at the colony entrance where honey bees station guard bees to keep out intruders. When a potential invader attempts to enter the nest, guard bees antennate the subject to determine whether it is a nestmate or an intruder (Breed et al. 2004). If the guard determines the invader is a threat, it either attacks the invader itself or, if the intruder is particularly large or aggressive, it recruits more guards to the area (Breed et al. 2004). Thus, few arthropod species are successful at penetrating the nest entrance. Thus, intrusion into the hive by the SHB is the exception and not the rule for bee hive invasion.

The SHB is considered a highly integrated invader of honey bee colonies, and possesses many adaptations that allow it to enter and thrive within host colonies (Ellis and Hepburn 2006). When approached by a honey bee, the SHB retracts its appendages beneath the body, thus inhibiting guard bees from grasping it to sting and/or remove it from the hive (Neumann and Elzen 2004). SHB leg morphology complements this appendage retraction behavior, i.e. the femora are grooved to accommodate broad flattened tibiae during leg retraction (Chapter 3). SHBs quickly find hiding places where honey bees cannot reach them upon host colony invasion. However, this inhibits beetle escape nonetheless due to host bees confining them to these areas (Neumann et al. 2001, Ellis 2005). While honey bees can keep the SHBs confined in the hiding places indefinitely at low to moderate infestation levels, SHBs survive in these "prisons" through a form of behavioral mimicry, wherein they solicit food from honey bee guards (Ellis et al. 2002b, Ellis 2005).

To begin to address honey bee responses to beetles, I performed an experiment to ascertain if bees guarding colony entrances exhibit differential behavioral responses to beetles that are integrated in honey bee colonies at various levels. Specifically, I looked at four different integration levels (Chapter 2). The first is synechthrans/symphiles (= highly integrated), which includes species that prey upon host colonies while being treated with hostility by the hosts (synechthrans) as well as those which are accepted to some degree by their hosts, possibly being fed by them (symphiles) (Wheeler 1910, Ellis and Hepburn 2006). The second group includes the accidentals (defined above). The third group includes non-integrated species that have not been found in colonies previously. Five species of nitidulid beetles (Coleoptera: Nitidulidae), one tenebrionid beetle (Coleoptera: Tenebrionidae), and one glass bead (control) were used to test the hypothesis that guard honey bees will exhibit varying defensive responses toward invaders at the

nest entrance in relation to the invaders' degree of integration. The test subjects included the SHB (highly integrated), *L. insularis* (accidental), *E. luteola* (accidental), *Carpophilus humeralis* Fabr. (non-integrated), *C. hemipterus* L. (non-integrated), *Tribolium castaneum* (non-integrated), and a small black jewelry bead as a control.

Materials and Methods

Beetles

Adult SHBs and *T. castanaeum* were obtained from *in vitro* rearing colonies at the USDA-ARS, Gainesville, Florida (29.64° N, 82.35° W) and were used to initiate rearing programs at the University of Florida Department of Entomology and Nematology (29.64° N, 82.36° W). Adults of the other nitidulid species were collected from rotting cantaloupe, *Cucumis melo* L., at the University of Florida Plant Science Research and Education Unit (PSREU) in Citra, Florida (29.41° N, 82.17° W) and used to initiate rearing programs. All species, with the exception of SHBs and *T. castaneum*, were reared in an incubator (24°C; 40% relative humidity; constant darkness) on a tomato and prune-based diet developed by Peng and Williams (1990). SHBs, *E. luteola*, *C. humeralis*, and *C. hemipterus* were 2 – 4 weeks old at the time of the experiment. Due to limited availability, *L. insularis* used during the study were obtained from rearing programs or field collections. As such their age at the time of the experiment was unknown. Though *T. castaneum* were lab-reared, their age also was unknown at the time of the experiment. Beetle sex was not determined.

Observation Hives

Four observation hives were created from previously established honey bee colonies of mixed European origin located at the PSREU. The observation colonies were given three frames containing pollen, capped honey, brood of all ages, worker bees, and an egg-laying queen. The observation hive structure (Figure 4-1) accommodated three, 23.0 × 42.6 cm (L × W) wooden

frames. The entrance corridor to the hives contained a 10 × 20 cm (L × W) test arena with delineated 1 cm squares, and could be closed at both ends with Plexiglass doors containing holes to accommodate ambient airflow (Figure 4-1). The arena was used for the behavioral assays and included a side entrance where test beetles could be introduced directly into the arena.

Behavioral Assays

All trials were conducted in October 2009 and under red lights between 18:30 and 02:30 the following morning as the SHB has been found to be more active within the hive during the evening (Ellis et al. 2003a). For each trial, one beetle or a 60 mg black bead (Darice, Inc., Strongsville, OH, USA) tethered to a 15 cm piece of monofilament fishing line (Zebco, Tulsa, OK, USA) was placed through the side entrance into the test arena. Beads and fishing line were auto-claved before the trials. Prior to introducing the beetle or bead, the test arena was closed at both ends to "trap" guard bees in the arena. Once the beetle or bead was introduced, the guard bees' responses to the beetle or control bead were recorded for 60 s. Three potential guard bee responses were recognized (described by Elzen et al. 2001): ignore (a bee's head comes within 5 mm of the subject, but there is no contact); contact (the bee makes physical non-defensive contact with the subject); and defend (the bee attempts to sting and/or remove the subject). Because some beetles, particularly SHBs, were able to escape from the arena despite efforts to limit this, only trials in which the beetles remained in the arena for ≥30 s were counted. Trials were run simultaneously in the four observation hives with four different observers and one individual from a given beetle species or bead being inserted randomly into colonies (completely randomized design blocked on colony, N = 6 beetle species and 1 control (black bead) × 15 individuals × 4 observation colonies)). All observers were trained prior to the experiment to reduce observer bias. The sliding Plexiglas doors on either side of the test arenas were opened briefly (≥10s) between trials to allow honey bee movement into/out of the central nest area and

reduce guard bee agitation. Also, the used bead was removed or the beetles were allowed to exit the test arenas at this time. Individual beetle and beads were used only once.

Statistical Analysis

Bee responses to beetles or beads were converted to proportion data due to the variable number of guard bees trapped in the test arena for each trial. This is not the proportion of bees performing a given response but rather the proportion of all responses that were either ignore, contact, or defend as a single bee may have demonstrated all of these behaviors multiple times during the 60 s observation period. All proportion response data (i.e. dependent variables) were transformed with arc-sine √ transformations prior to analyses to stabilize the variance. For each type of response (ignore, contact, defend), the transformed proportions were analyzed using a two way ANOVA (JMP 2008) recognizing colony (A-D) and either invader type (6 beetle species and 1 control bead) or level of integration (highly integrated, accidental, non-integrated, control bead) as main effects and colony × invader type or level of integration as the interaction term. If the interaction of colony × invader and colony × level of integration was significant, then these variables were analyzed within colony. When the ANOVA detected significant effects, means were compared using Tukey-Kramer tests, accepting differences at $P \leq \alpha = 0.05$. Untransformed means are reported in this chapter.

Results

The proportion of bee responses to invaders that were ignore (invader type: $F = 6.1$; $df = 3, 391$; $P < 0.01$; level of integration: $F = 4.6$; $df = 3, 403$; $P < 0.01$) and contact ($F = 5.4$; $df = 3, 391$; $P < 0.01$; level of integration: $F = 3.8$; $df = 3, 403$; $P = 0.01$) varied significantly by colony. In general bees from colony C (0.52 ± 0.02, 105) or D (0.48 ± 0.03, 105) ignored invaders more so than bees from colony A (0.37 ± 0.04, 105). Bees in colony B (0.42 ± 0.02, 104) ignored invaders at a level equal to that of bees in all of the other colonies (data are mean ± SE

proportion of responses that were “ignore”, *n* beetles). Regarding contacting nest invaders, guard bees from colony B (0.48 ± 0.02, 104) contacted nest invaders more than bees from colony D (0.35 ± 0.02, 105). Bees in colonies A (0.41 ± 0.03, 105) and C (0.40 ± 0.02, 105) contacted invaders at a rate equal to bees in colonies B and D (data are mean ± SE proportion of responses that were “contact”, *n*). In contrast to bee ignore and contact responses, the proportion of responses that were defend did not vary significantly by colony (invader type: F = 1.4; df = 3, 391; P = 0.23; level of integration: F = 1.7; df = 3, 403; P = 0.15). Colonies A (0.16 ± 0.03, 105), B (0.10 ± 0.01, 104), C (0.08 ± 0.01, 105), and D (0.13 ± 0.02, 105) exhibited defensive responses equally (data are mean ± SE proportion of responses that were “defend”, N).

Overall, bees differed in their level of ignore and contact responses to the different nest invaders, as well as to the different levels of integration of the invaders (Table 4-1). Guard bees ignored *T. castaneum* and *E. luteola* more than they ignored *C. hemipterus*, *L. insularis*, SHBs, and the control bead. Bees ignored *C. humeralis* more than they ignored the SHB and the control bead. When invaders were grouped by level of integration, guard bees ignored accidental and non-integrated species moreso than highly integrated species or the control bead (Table 4-1).

In general, the control bead was contacted more by guard bees than were the six beetle species. Bees contacted *L. insularis* more than *T. castaneum* and *E. luteola* while their contact response to *C. humeralis*, *C. hemipterus*, and the SHB did not differ significantly from the other beetles. When considering invader level of integration, the control bead was contacted more by guard bees than any other invader group (Table 4-1).

Guard bee ignore (invader type: F = 0.8; df = 18, 391; P = 0.65; level of integration: F = 0.8; df = 9, 403; P = 0.63) and contact (invader type: F = 0.68; df = 18, 391; P = 0.84; level of integration: F = 0.7; df = 9, 403; P = 0.72) responses were not affected by the interaction

between colony and invader type or level of integration. However, the defend response was affected by the interaction (invader type: F = 2.3; df = 18, 391; P = 0.01; level of integration: F = 2.2; df = 9, 403; P = 0.02). In colony A, the proportion of responses directed at SHBs that were defensive was higher than those toward any other species with the exception of *C. hemipterus*, which itself was not treated more defensively than the other species. Also, the highly integrated species, i.e. the SHB, was treated more defensively than groups at other levels of integration. In colony B, the SHB was treated defensively by bees more than any other species except for the two *Carpophilus* species. Bees in colony B responded to all non-SHB beetles with equal defensive responses. Regarding level of integration, bees in colony B treated the highly integrated species, i.e. the SHB, more defensively than any other group. Also, the non integrated species were treated more defensively than the control bead (Table 4-2). In colony C, the SHB elicited defensive responses from bees more than the control bead or any other beetle species. Also, the SHB was the sole representative of the highly integrated species and received more defensive responses from the bees (proportionally) than beetle groups at other levels of integration and the control bead. A similar pattern was seen in colony D except that bees responded to SHBs and the control bead with equal defensive responses (Table 4-2). All non-SHB beetle species elicited similar defensive responses from guard honey bees, and all beetles except for *T. castaneum* were treated with the same level of defense as the bead. When grouping beetles by level of integration in colony D, the highly integrated species was treated more defensively than the accidental and non-integrated species. The control bead was treated similarly as compared to all other groups.

Discussion

Overall, the most integrated species, i.e. the SHB, was ignored the least and treated more defensively by guard bees than beetle groups representing all other levels of integration. These

findings contrast with those of previous findings by Elzen et al. (2001) who demonstrated that European subspecies of honey bees did not treat SHBs and a control push pin differently (either ignore, contact, or defend). There are multiple possible reasons that these results differed from those of Elzen et al. (2001). First, Elzen et al. (2001) conducted their study in wooden hoarding cages, which may affect bee/beetle behavior unpredictably (i.e. an artificial bioassay). Secondly, the study herein was conducted nearly one decade later, possibly suggesting evolving bee development of defensive adaptations toward SHBs over time. Finally, Elzen et al. (2001) collected bees randomly from colonies, whereas in this study, bees already guarding the colony entrance were sequestered. While one would expect bees guarding colony entrances (e.g. this study) to be defensive (Breed et al. 2004), the same is not intuitive when randomly collecting bees from a colony (e.g. Elzen et al. 2001). Nurse bees, wax builders, etc. all would be sampled and are known to express reduced defensive behavior relative to guard bees (Breed et al. 1992a). Regardless, guard bees in this study were more defensive toward the SHB, the highly integrated species, than they were toward all other beetle groups at other levels of integration.

The heightened defensive response by guard bees toward the SHB while exhibiting a consistently lower defensive response toward invaders at all other levels of integration may be an effort by bees to maximize energy efficiency. Like many other organisms, honey bees are known to engage in behaviors that are energetically conserved (see Dedej and Delaplane 2005). For example, this has been documented for honey bee foraging behavior, where honey bees are driven to nectar larceny to increase net energy profit (Dedej and Delaplane 2005). Though honey bee defensive behavior was not quantified energetically, one could see how expending energy to attack only those intruders known to threaten colony health (i.e. SHBs) while ignoring or minimizing defensive responses toward all other species is an effort to maximize energy

conservation. If energy conservation is important in bee defensive responses to nest invaders at the colony entrance, then these data are consistent with the superorganism theory of social insect colony organization. Individual organisms, including insects and mammals, are known to initiate immune responses to invading pathogens at an energy cost (e.g. Freitak et al. 2003, Simmons and Roney 2009, Cutrera et al. 2010). Consequently, one could argue that guard bee behavior at the nest entrance is initiated conservatively yet efficiently to limit invasion by organisms (i.e. "pathogens") threatening the entire colony (or "superorganism").

The SHB is integrated more into honey bee colonies than the other beetle species tested. As such, one may hypothesize that the SHB has mechanisms to reduce defensive responses by guard honey bees at the colony entrance. Such mechanisms, usually chemical or morphological (Wilson, 1971; Kistner, 1979), in highly-integrated invaders of other social insects such as ants and termites are common (Dettner and Liepert 1994). The SHB employs an entire suite of morphological adaptations that allow it to penetrate honey bee colonies. These include a limuloid body form, the ability to retract its appendages beneath its body (Neumann and Elzen 2004, Ellis and Hepburn 2006), and flattened tibiae and grooved femora to accommodate retraction, (Chapter 3).

In this study, guard bees displayed an interesting behavior toward nest invaders that may represent an integral defensive response. This behavior began with a honey bee approaching the invader, turning around so the abdomen faced the subject, and then kicking the metathoracic legs backwards while fanning the wings. Bees employed this behavior regularly (>50% of all introductions) against *T. castaneum* (less so with other invaders). This behavior has been documented in honey bees as a defense against ants (Spangler and Taber 1970) and other small

insects (Yang et al. 2010), and may be advantageous to include an analysis of this behavior in future studies of arthropod invasion into honey bee colonies.

Bee responses to nest invaders seemed to increase in intensity after initial bee contact with any potential invader. There are two possible explanations for increased intensity of responses during the observation period. First, a honey bee guard, when disturbed, may release pheromones from her sting gland that subsequently excite surrounding bees and heighten the overall defensive response toward the invader (Breed et al. 2004). Second, guard bees may "mark" intruders with 2-heptanone, an alarm pheromone component that is produced in the mandibular glands. This marking indicates an "invader status" to other guard bees (Breed et al. 2004). Thus, heightened bee defensive responses toward an invader after initial contact is likely advantageous for colonies, and subsequently decreases the chance that a "marked" invader will infiltrate a colony successfully.

In contrast to defensive responses toward beetle invaders, guard bee contact responses toward invaders were not significantly different among the three levels of colony integration. This constant guard bee response is consistent with the regular mode of intruder discrimination performed by guard bees at the nest entrance. Guard bees antennate all intruding arthropods to the hive and then subsequently decide to accept or attack the invader (Breed et al. 2004).

Bee responses to the control bead were interesting. Guard bees ignored the control bead less than any beetle species except SHBs, and they contacted it significantly more than any beetle species. Perhaps guard bees were "curious" toward the foreign object, which did not release any biologically based volatiles. Such a behavior would be adaptive if the guard bee encountered invaders that emit low-to-undetectable (by bees) amounts of volatiles. For example, the argentine ant, *Linepithema humile* (Mayr), emits relatively low volatile titers compared to

other ant invaders. Spangler and Taber (1970) hypothesized that this enables the ant to enter honey bee colonies easily. Alternatively, bees may have recognized the bead as refuse and were assessing the size of the object to determine whether it could be removed effectively from the colony. A difference in bead size assessment between colonies may explain why the bead was treated defensively in colony D (Table 4-2). These guards may have determined that the bead was an appropriate size to be expelled, and the expulsion attempts were recorded as defensive responses. Furthermore, the bead may have adsorbed odors as a result of observer handling, thereby eliciting curiosity from the bees, but not defense.

The four colonies used in the study differed significantly with respect to the level they expressed ignore and contact responses toward invaders, thereby suggesting that some degree of genetic variation for these behaviors exists. Variation is important for bee populations to be able to adapt to environmental stresses, including would-be invaders. As such, variations in these traits (ignore and contact responses toward invaders) may be influenced by selection pressures, thus enabling colonies to adapt to invader pressures over times. In contrast to colony ignore and contact behavior, colonies did not differ in their pooled defensive response toward invaders.

The SHB remains one of the most highly integrated insects in honey bee colonies and social bee colonies in general (Ellis and Hepburn 2006) despite heightened defensive responses by honey bees toward SHBs. These defensive responses include increased defensive activity at the nest entrance (this chapter), overall aggression (Elzen et al., 2001), and confinement behavior (Ellis 2005). The SHB accomplishes its high level of integration through morphological (Chapter 3), behavioral (Ellis et al. 2002b), and potentially chemical adaptations. The data presented herein demonstrate that beetle invasion into honey bee colonies is met by bees in the form of a heightened defensive response.

Table 4-1. Proportion of honey bee guard responses that were "ignore" and "contact" during the observation period. Data are mean ± SE (*n*) proportion of total responses that were either ignore or contact. Columnar means followed by the same letter are not different at $\alpha = 0.05$. Means were separated using the Tukey-Kramer method. For "Invader type", bee responses to each beetle species and the control bead were analyzed. For "Level of integration", beetle species were grouped together by their level of integration into honey bee colonies.

	Invader type	
Species ↓	Ignore	Contact
A. tumida	0.314 ± 0.028 (60)c d	0.368 ± 0.018 (60)b c
L. insularis	0.392 ± 0.036 (59)b c	0.489 ± 0.036 (60)b
E. luteola	0.613 ± 0.039 (60)a	0.279 ± 0.032 (60)c
C. humeralis	0.510 ± 0.031 (60)a b	0.395 ± 0.030 (60)b c
C. hemipterus	0.436 ± 0.041 (60)b c	0.362 ± 0.033 (60)b c
T. castaneum	0.643 ± 0.031 (60)a	0.292 ± 0.026 (60)c
Control	0.220 ± 0.022 (60)d	0.693 ± 0.024 (60)a
ANOVA→	F = 18.2; df = 6, 391; P < 0.01	F = 18.7; df = 6, 391; P < 0.01
	Level of integration	
Level ↓	Ignore	Contact
Highly integrated	0.314 ± 0.028 (60)b	0.368 ± 0.018 (60)b
Accidental	0.503 ± 0.029 (119)a	0.383 ± 0.026 (119)b
Non-integrated	0.529 ± 0.021 (180)a	0.350 ± 0.018 (180)b
Control bead	0.220 ± 0.022 (60)b	0.693 ± 0.024 (60)a
ANOVA→	F = 21.1; df = 3, 403; P < 0.01	F = 26.5; df = 3, 403; P < 0.01

Table 4-2. Species × colony interaction on the proportion of guard bee responses that were "defend" during the observation period. Data are mean ± SE (*n*) proportion of total responses that were defend. Columnar means followed by the same letter are not different at $\alpha = 0.05$. Means were separated using the Tukey-Kramer method. For "Invader type", bee responses to each beetle species and the control bead were analyzed. For "Level of integration", beetle species were grouped together by their level of integration into honey bee colonies.

	Invader type			
Species↓	Colony A	Colony B	Colony C	Colony D
A. tumida	0.439 ± 0.062 (15)a	0.216 ± 0.031 (15)a	0.291 ± 0.037 (15)a	0.329 ± 0.054 (15)a
L. insularis	0.144 ± 0.069 (15)b	0.055 ± 0.012 (14)b c	0.040 ± 0.012 (15)b	0.032 ± 0.019 (15)b c
E. luteola	0.059 ± 0.047 (15)b	0.069 ± 0.015 (15)b c	0.045 ± 0.016 (15)b	0.127 ± 0.043 (15)b c
C. humeralis	0.042 ± 0.025 (15)b	0.109 ± 0.022 (15)a b	0.049 ± 0.016 (15)b	0.116 ± 0.038 (15)b c
C. hemipterus	0.217 ± 0.094 (15)a b	0.135 ± 0.026 (15)a b	0.081 ± 0.028 (15)b	0.111 ± 0.048 (15)b c
T. castaneum	0.119 ± 0.059 (15)b	0.079 ± 0.019 (15)b c	0.036 ± 0.012 (15)b	0.026 ± 0.024 (15)c
Control	0.084 ± 0.034 (15)b	0.030 ± 0.009 (15)c	0.053 ± 0.023 (15)b	0.181 ± 0.063 (15)a b
ANOVA→	F = 5.7; df = 6, 98; P < 0.01	F = 7.8; df = 6, 97; P < 0.01	F = 11.6; df = 6, 98; P < 0.01	F = 6.6; df = 6, 98; P < 0.01
	Level of integration			
Level ↓	Colony A	Colony B	Colony C	Colony D
Highly integrated	0.439 ± 0.062 (15)a	0.216 ± 0.031 (15)a	0.291 ± 0.037 (15)a	0.329 ± 0.054 (15)a
Accidental	0.102 ± 0.042 (30)b	0.062 ± 0.009 (29)b c	0.042 ± 0.010 (30)b	0.080 ± 0.025 (30)b
Non-integrated	0.126 ± 0.039 (45)b	0.108 ± 0.013 (45)b	0.055 ± 0.011 (45)b	0.084 ± 0.022 (45)b
Control bead	0.084 ± 0.034 (15)b	0.030 ± 0.009 (15)c	0.053 ± 0.023 (15)b	0.181 ± 0.063 (15)a b
ANOVA→	F = 9.2; df = 3, 101; P < 0.01	F = 14.2; df = 3, 100; P < 0.01	F = 23.2; df = 3, 101; P < 0.01	F = 10.1; df = 3, 101; P < 0.01

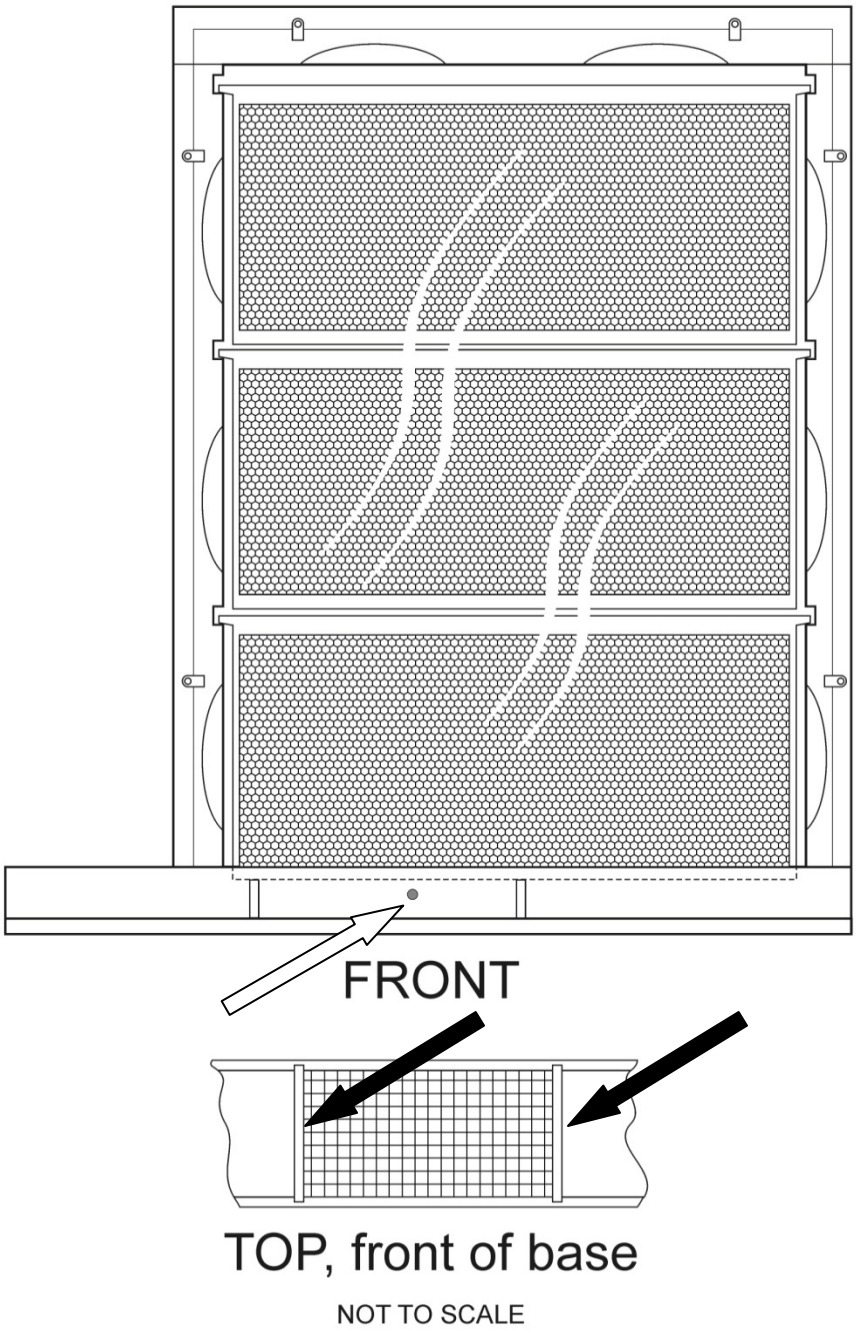

Figure 4-1. Diagram of observation hive. The white arrow indicates the location where beetle species or control beads were introduced into the test arena. The black arrows indicate the location of the Plexiglass doors that captured guard bees in the test arena.

CHAPTER 5
DIETARY EFFECTS ON THE CUTICULAR PROFILE OF SHBS AND ITS EFFECT ON BEHAVIORAL TREATMENT BY HONEY BEES

Nestmate recognition is a feature common to most, if not all, eusocial insect colonies (Breed 2003). This type of recognition is the ability of an insect to discriminate between conspecifics that are part of the colony and those that are not. Nestmate recognition is often based on the specific blend of hydrocarbons found on the insect's exoskeleton. These hydrocarbons can be of a glandular source, as occurs in the ant *Cataglyphis niger* (Andre) (Hymenoptera: Formicidae) (Soroker et al. 1994), which is secreted on the exoskeleton surface. Hydrocarbons also can be cuticle-derived, either resulting from endogenous chemicals from internal sources or diet based Diet based cues occur in the ant *Linepithema humile*, which acquires its chemical profile from insect prey (Liang and Silverman 2000). Additionally, these hydrocarbons can be externally-derived, being acquired by contacting another source of the hydrocarbons which "rub off" onto the insect. This occurs in honey bees and also in combination with other cues (e.g. genetic) (Breed 1983, Breed et al. 1995, Breed 1998, Downs and Ratnieks 1999).

Though social insects can recognize nestmates, and by application non-nestmates as well, other insects occasionally succeed at entering social insect colonies. There are three main methods by which arthropods can invade/integrate into social insect nests: (1) adaptations in body form, (2) behavior, and (3) chemical use (Wilson 1971, Kistner 1979). While not observable without scientific equipment, chemical adaptations are well-known among arthropods invading termite, wasp, ant, and bee nests.

In honey bee colonies there are a few examples of invading arthropod use of chemical mimicry to invade/integrate into the colony (Chapter 1). *Varroa destructor* has a hydrocarbon profile similar to its host (Nation et al. 1992). Furthermore, this profile changes with the host as

it undergoes different developmental stages, thereby indicating a high degree of chemical adaptation (Martin et al. 2001).

The death's head hawkmoth *Acherontia atropos* invades honey bee colonies and subsequently feed on honey within. This is, in part, accommodated by its thick cuticle, which cannot be penetrated easily by stings. Once inside the hive, the moth can move about the colony rather unnoticed and with impunity. This occurs because the moth chemically mimicks the honey bee hydrocarbon profile and, thus, the odor of the host colony (Moritz et al. 1991).

The SHB is able to enter honey bee colonies due to several adaptive morphological features (Chapter 3). The SHB is able to retract its appendages underneath its body and firmly grasp the substrate, thereby making it difficult for guard bees to eject the invader (Lundie 1940, Neumann et al. 2001). Once the guards cease aggression, the SHBs hide in cracks and crevices around the colony, often without further aggression from the bees. Bees detect the hiding SHBs and react by stationing guards around them, imprisoning them. The SHBs do not starve in these prisons. Occasionally, they are able to solicit trophallaxis from their guards, thus allowing them to survive longer and increasing their likelihood of successful reproduction within the colony (Neumann et al. 2001, Ellis et al. 2002b).

The mechanism that allows the SHBs to initiate trophallaxis with bees is not well-understood. One possible method is via tactile stimulation of the bee. However, this is unlikely, as honey bees have a highly developed nestmate recognition system based largely, if not entirely, on cuticular hydrocarbons (Breed et al. 1995, Breed 1998, Downs and Ratnieks 1999). Therefore, it is quite possible, especially given the wealth of recent literature on the subject, that SHBs use chemical means to aid in successful integration into honey bee colonies and subsequently solicit food from their hosts. There is preliminary chemical evidence to support this

assertion (B. Torto, pers. comm). Schmolke (1974), observed that SHBs newly introduced into hives are treated more aggressively than SHBs that have been in the colony for some time. In this study, I hypothesized that the SHBs acquire cuticular hydrocarbons through diet on which they feed while in colonies, thus affording them a higher probability of acceptance into bee colonies.

Materials and Methods

Beetles

Adult SHBs were captured from experimental honey bee colonies maintained at the University of Florida Bee Biology and Research Unit (BBRU) in Gainesville, Florida (29.63° N, 82.36° W). SHBs were reared in an incubator (25°C; 80% relative humidity; constant darkness) on a diet of honey, pollen, and Brood Builder™ (Dadant and Sons, Inc., Hamilton, IL) in a ratio of 1:1:2 respectively (Ellis et al. 2008, 2010b) at the University of Florida Department of Entomology and Nematology (29.64° N, 82.36° W). Wandering larvae were captured and put onto soil to pupate. From the resulting adult beetles, three groups (~150 beetles per group) were used for chemical and behavioral analyses. 1) The first group was unfed and collected 12 hours after eclosion. 2) A second group was fed nothing but sugar-water (1:1) for 14 days post eclosion. 3) The third group was fed the honey-pollen-Brood Builder™ diet described above for 14 days post eclosion. Beetles used for chemical analyses were frozen immediately at -80°C until needed. For the behavioral trials, one additional group was created. This group consisted of beetles taken directly from managed honey bee colonies at the BBRU (not one of the test colonies) ~3 hours prior to the behavioral assays.

Observation Hives

Four observation hives were created from previously established honey bee colonies of mixed European origin located at the University of Florida Plant Science Research and Education Unit in Citra, Florida (29.41° N, 82.17° W). The observation colonies were given

three frames containing pollen, capped honey, brood of all ages, worker bees, and a laying queen. The observation hive structure (Figure 5-1) accommodated three, 23.0 × 42.6 cm (L × W) wooden frames. The entrance corridor to the hives contained a 10 × 20 cm (L × W) test arena with delineated 1 cm squares, and could be closed at both ends with Plexiglas doors containing holes to accommodate normal airflow (Figure 5-1). The arena was used for the behavioral assays and included a side entrance where test beetles could be introduced directly into the arena.

Behavioral Assays

All trials were conducted per Chapter 4 under red lights between 18:30 and 02:30 the following morning. Trials were run simultaneously in the four observation hives with four different observers who were trained in order to reduce observer bias. As a further measure to reduce bias, the observers were only given coded containers and the treatments were unknown to them. For each trial, one beetle or an auto-claved 60 mg black bead (Darice, Inc., Strongsville, OH, USA) tethered to an auto-claved 15 cm piece of monofilament fishing line (Zebco, Tulsa, OK, USA) was placed through the side entrance into the test arena. The test arena was closed at both ends to "trap" guard bees in the arena prior to introducing the subjects. Following their introduction, guard bees' responses to the subject were recorded for 1 min. The responses were determined to be either ignore (a bee's head comes within 5 mm of the subject, but there is no contact), contact (the bee makes physical non-defensive contact with the subject), or defend (the bee attempts to sting and/or remove the subject). Only trials in which the beetles remained in the arena for ≥30 s were counted. The design was a completely randomized design blocked on colony (4 colonies). The replicate schedule was beetles from 4 diet groups and 1 control (black bead) × 15 individuals × 4 observation colonies. To allow honey bee movement through the entrance and to reduce guard bee agitation, the sliding Plexiglas doors on either side of the test arenas were opened briefly (≥10s) between trials. The used bead was removed or the beetles

were allowed to exit the test arenas at this time. Individual beetles and beads were used only once.

Chemical Analysis

Ten frozen beetles (unsexed) from each of the three diet groups were used in chemical analyses. Each individual beetle was submerged in 1 ml of pentane for 10 minutes. Five µg of tetracosane (C_{24}) were added to the samples as internal standard. Samples were analyzed at the USDA-ARS, Gainesville, Florida (29.64° N, 82.35° W) using a HP-6890 gas chromatograph (GC, Hewlett Packard, Palo Alto, CA) equipped with a HP-1 column (30 m × 0.25 µm, Agilent, Palo Alto, CA). For chemical identification, the column was linked to a HP 5973 mass spectrometer operated in the electron impact mode (70 eV, Agilent, Palo Alto, CA). Helium was used as a carrier gas at a linear flow velocity of 18 cm/s. The GC oven temperature began at 35° C for the first min and then increased at 5° C per min to 200° C and held constant for 5 min. Then, the temperature was increased at 15° C per min up to 300° C and held constant for 10 min. The transfer line for the mass spectrometer was held at 280° C. Samples also were analyzed using a gas chromatograph-flame ionization detector (HP 6890) using the same method as above to quantify amounts of compounds. The flame ionization detector was held at 250 ° C.

Statistical Analysis

Bee responses to beetles or beads were converted to proportion data. All proportion response data were transformed with arc-sine √ transformations prior to analyses. The transformed proportions were analyzed using a two way ANOVA (JMP 2008) recognizing colony and diet as main effects and colony × diet type as the interaction term. Data for the chemical assays were √ transformed to adjust for normality, and the means were analyzed using a one way ANOVA (JMP 2008). Where appropriate, means were compared using Tukey-Kramer tests, accepting differences at $P \leq \alpha = 0.05$. Untransformed means are reported in this chapter.

Results

Overall, bees at the colony entrance did not differ in their level of responses to the SHB groups (Tables 5-1, 5-2, 5-3). The interaction of colony × diet type was significant for the all three responses (ignore, contact, and defend), so these variables were analyzed within colony. Concerning the ignore response, only in Colony C was there a significant effect, wherein SHBs from all of the diet groups, except for those fed sugar-water, were ignored more than were the control beads (Table 5-1). Guard bees in Colonies B, C, and D contacted the control bead more than the SHBs (Table 5-2). In Colony D, SHBs that were newly-eclosed or collected from the honey bee colony were contacted more than the SHBs fed honey, pollen, and Brood Builder™ (Table 5-2). Finally, bees in Colonies B, C, and D treated all SHBs more defensively than they did the control bead. Bees in Colony D treated SHBs fed honey, pollen, and Brood Builder more defensively than those from the honey bee colony (Table 5-3).

The most prevalent hydrocarbons found on the adult SHBs were saturated and monounsaturated C_{23}, C_{25}-C_{29} (Figures 5-2, 5-3). Saturated and monounsaturated C_{26} and C_{28} are omitted from Figure 5-3 due to their relatively small abundance and insignificant differences between treatments (Table 5-4). Sugar-water fed SHBs had all other detected hydrocarbons (saturated and monounsaturated C_{23}, C_{25}, C_{27}, and C_{29}) present on their cuticles in higher amounts than those of newly-eclosed SHBs (Table 5-4). Furthermore, they had higher levels of all of these hydrocarbons except for saturated C_{25} and C_{27} than did the cuticles of SHBs fed honey, pollen, and Brood Builder™ (Table 5-4). Finally, SHBs fed honey, pollen, and Brood Builder™ had higher amounts of monounsaturated C_{23} and saturated C_{23}, C_{25}, and C_{27} than did newly-eclosed SHBs (Table 5-4).

Discussion

SHBs that were fed sugar-water for 14 days differed in their cuticular profiles markedly and consistently from those fed honey, pollen, and Brood Builder™ for 14 days and from newly-emerged SHBs. The former had a more pronounced profile. However, based on the behavioral assays, this apparently did not alter their treatment by guard bees at the nest entrance. Reducing, or under-producing, one's cuticular profile could be beneficial to a SHB trying to survive within a dark honey bee nest, where the majority of cues are chemical (Seeley 1998). SHBs that are in bee hives feed on pollen, honey, and brood (Lundie 1940). If such a diet were to reduce a SHB's cuticular chemical signature compared to a sub-optimal diet (like the sugar water in this study), as the results suggest, such a change can be considered adaptive, as in the ponerine ant *Ectatomma ruidum* (Roger) (Hymenoptera: Formicidae) (Jeral et al. 1997). To determine if this is the case in SHBs, behavioral assays would be better done using younger bees within the nest where attack thresholds are higher than those of the guard bees at the nest entrance (Breed et al. 1992a). Furthermore, since confined beetles are fed by the bees trophallactically (Ellis et al. 2002b), it would be interesting to 1) determine the specific contents of the liquid food and 2) measure bee responses to SHBs fed this substance.

All of the compounds found on the SHB cuticle have also been found associated with honey bees. Although the configurations were not determined directly in this experiment, most alkenes on the insect cuticle are in the (Z) configuration and have the double bond in the 9 position (Blomquist 2010). Breed (1998) found that (Z)-9-tricosene ($C_{23:1}$), which is a sex attractant in the housefly, *Musca domestica* L. (Diptera: Muscidae) (Carlson et al. 1971), is used in honey bee nestmate recognition while tricosane (C_{23}), pentacosane (C_{25}), and nonacosane (C_{29}) yielded negative results (i.e. bees treated with the compounds were not treated differently by bees than untreated controls), suggesting they are not used in nestmate recognition (Breed and

Stiller 1992). However, all of these are present on the honey bee cuticle, as are Δ1 pentacosene ($C_{25:1}$), Δ1 heptacosene ($C_{27:1}$), heptacosane (C_{27}), and Δ1 nonacosene ($C_{29:1}$) (Blomquist et al. 1980). Furthermore, Arnold et al. (1996, 2000) found several alkanes and alkenes between C_{21} and C_{33} to be associated with subfamilies (i.e. sister groups with the same drone father) within a honey bee colony, which may be used for kin recognition via contact cues. Schmitt et al. (2007) found tricosene, tricosane, pentacosene, pentacosane, heptacosane, and nonacosane in the headspace above foraging honey bees, suggesting that they may provide volatile cues. Similarly, Thom et al. (2007) found that returning foragers release (Z)-9-tricosene, tricosane, (Z)-9-pentacosene, and pentacosane while recruiting more foragers.

In addition to cuticular chemical composition possibly being affected by diet, it would be interesting to determine if SHBs also can adsorb colony odors onto their cuticles from their host nests. This occurs in the myrmecophilous beetle *Myrmecaphodius excavaticollis* (Blanchard) (Coleoptera: Scarabaeidae) within nests of *Solenopsis* spp. (Vander Meer and Wojcik 1982) as well as the thief ant species *Ectatomma ruidum* which, as discussed above, also reduces its cuticular profile (Breed et al. 1992b, Jeral et al. 1997). If a reduced cuticular profile aids SHB integration into honey bee colonies, this would be a novel case of adaptive diet-mediated cuticular chemical reduction.

Table 5-1. Diet × colony interaction on the proportion of guard bee responses that were "ignore" during the observation period for newly-eclosed beetles ("new"), beetles that have fed for 14 days on a diet of honey, pollen, and Brood Builder™ (1:1:2) ("diet"), beetles that have fed for 14 days on sugar-water, beetles that are from a colony ("colony"), or a control bead. Data are mean ± SE (*n* beetles) proportion of total responses that were ignore. Columnar means followed by the same letter are not different at $\alpha = 0.05$. Means were compared using the Tukey-Kramer method.

Diet↓	Colony A	Colony B	Colony C	Colony D
new	0.314 ± 0.064 (15)	0.422 ± 0.064 (15)	0.369 ± 0.030 (15)a	0.154 ± 0.030 (13)
diet	0.429 ± 0.061 (15)	0.435 ± 0.031 (15)	0.438 ± 0.033 (15)a	0.233 ± 0.031 (14)
sugar-water	0.496 ± 0.061 (15)	0.374 ± 0.042 (14)	0.319 ± 0.036 (15)a b	0.196 ± 0.027 (12)
colony	0.346 ± 0.044 (15)	0.419 ± 0.046 (15)	0.378 ± 0.039 (15)a	0.316 ± 0.055 (13)
control	0.503 ± 0.077 (15)	0.363 ± 0.045 (15)	0.228 ± 0.037 (15)b	0.194 ± 0.020 (15)
ANOVA→	F = 2.2; df = 4, 70; P = 0.08	F = 0.4; df = 4, 70; P = 0.78	F = 5.3; df = 4, 70; P < 0.01	F = 2.1; df = 4, 62; P = 0.10

Table 5-2. Diet × colony interaction on the proportion of guard bee responses that were "contact" during the observation period for newly-eclosed beetles ("new"), beetles that have fed for 14 days on a diet of honey, pollen, and Brood Builder™ (1:1:2) ("diet"), beetles that have fed for 14 days on sugar-water, beetles that are from a colony ("colony"), or a control bead. Data are mean ± SE (*n* beetles) proportion of total responses that were contact. Columnar means followed by the same letter are not different at $\alpha = 0.05$. Means were compared using the Tukey-Kramer method.

Diet↓	Colony A	Colony B	Colony C	Colony D
new	0.398 ± 0.064 (15)	0.321 ± 0.030 (15)b	0.286 ± 0.033 (15)b	0.309 ± 0.036 (13)b
diet	0.300 ± 0.030 (15)	0.293 ± 0.026 (15)b	0.345 ± 0.029 (15)b	0.123 ± 0.032 (14)c
sugar-water	0.311 ± 0.043 (14)	0.367 ± 0.034 (14)b	0.342 ± 0.023 (15)b	0.243 ± 0.045 (12)b c
colony	0.343 ± 0.037 (15)	0.282 ± 0.029 (15)b	0.335 ± 0.026 (15)b	0.272 ± 0.032 (13)b
control	0.373 ± 0.054 (15)	0.632 ± 0.042 (15)a	0.760 ± 0.038 (15)a	0.710 ± 0.021 (15)a
ANOVA→	F = 0.7; df = 4, 70; P = 0.57	F = 16.7; df = 4, 70; P < 0.01	F = 35.6; df = 4, 70; P < 0.01	F = 34.8; df = 4, 62; P < 0.01

Table 5-3. Diet × colony interaction on the proportion of guard bee responses that were "defend" during the observation period for newly-eclosed beetles ("new"), beetles that have fed for 14 days on a diet of honey, pollen, and Brood Builder™ (1:1:2) ("diet"), beetles that have fed for 14 days on sugar-water, beetles that are from a colony ("colony"), or a control bead. Data are mean ± SE (*n* beetles) proportion of total responses that were defend. Columnar means followed by the same letter are not different at $\alpha = 0.05$. Means were compared using the Tukey-Kramer method.

Diet↓	Colony A	Colony B	Colony C	Colony D
new	0.288 ± 0.061 (15)	0.257 ± 0.046 (15)a	0.345 ± 0.041 (15)a	0.537 ± 0.049 (13)a b
diet	0.271 ± 0.062 (15)	0.272 ± 0.036 (15)a	0.217 ± 0.025 (15)a	0.643 ± 0.055 (14)a
sugar-water	0.193 ± 0.052 (15)	0.259 ± 0.039 (14)a	0.339 ± 0.031 (15)a	0.561 ± 0.049 (12)a b
colony	0.310 ± 0.060 (15)	0.299 ± 0.042 (15)a	0.287 ± 0.036 (15)a	0.412 ± 0.053 (13)b
control	0.123 ± 0.035 (15)	0.006 ± 0.006 (15)b	0.016 ± 0.006 (15)b	0.097 ± 0.018 (15)c
ANOVA→	F = 2.1; df = 4, 70; P = 0.08	F = 26.4; df = 4, 70; P < 0.01	F = 31.5; df = 4, 70; P < 0.01	F = 25.1; df = 4, 62; P < 0.01

Table 5-4. Amounts (in µg) of various hydrocarbons present on the cuticles of newly-eclosed beetles ("new"), beetles that have fed for 14 days on a diet of honey, pollen, and Brood Builder™ (1:1:2) ("diet"), and beetles that have fed for 14 days on sugar-water. Data are mean ± SE (*n* beetles) ng of the each hydrocarbon. Columnar means followed by the same letter are not different at $\alpha = 0.05$. Means were compared using the Tukey-Kramer method.

Diet↓	$C_{23:1}$	C_{23}	$C_{25:1}$	C_{25}	$C_{26:1}$	C_{26}	$C_{27:1}$	C_{27}	$C_{28:1}$	C_{28}	$C_{29:1}$	C_{29}
new	5 ± 3 (9)c	8 ± 3 (9)c	7 ± 4 (9)b	11 ± 6 (9)b	1 ± 1 (9)	2 ± 2 (9)	2 ± 2 (9)b	22 ± 13 (9)b	2 ± 2 (9)	9 ± 5 (9)	0 ± 0 (9)b	57 ± 11 (9)b
diet	21 ± 4 (8)b	26 ± 3 (8)b	44 ± 11 (8)b	114 ± 30 (8)a	5 ± 3 (8)	0 ± 0 (8)	13 ± 6 (8)b	61 ± 8 (8)a	9 ± 4 (8)	60 ± 28 (8)	0 ± 0 (8)b	88 ± 18 (8)b
sugar-water	152 ± 26 (10)a	60 ± 12 (10)a	477 ± 91 (10)a	133 ± 34 (10)a	2 ± 2 (10)	4 ± 4 (10)	116 ± 23 (10)a	110 ± 21 (10)a	18 ± 7 (10)	13 ± 6 (10)	56 ± 13 (10)a	274 ± 71 (10)a
ANOVA→	F = 46.8; df = 2, 24; P < 0.01	F = 18.7; df = 2, 24; P < 0.01	F = 60.5; df = 2, 24; P < 0.01	F = 18.8; df = 2, 24; P < 0.01	F= 1.3; df = 2, 24; P = 0.29	F = 0.4; df = 2, 24; P = 0.65	F = 43.8; df = 2, 24; P < 0.01	F = 14.9; df = 2, 24; P < 0.01	F = 1.9; df = 2, 24; P = 0.17	F = 2.5; df = 2, 24; P = 0.10	F = 73.5; df = 2, 24; P < 0.01	F = 7.7; df = 2, 24; P < 0.01

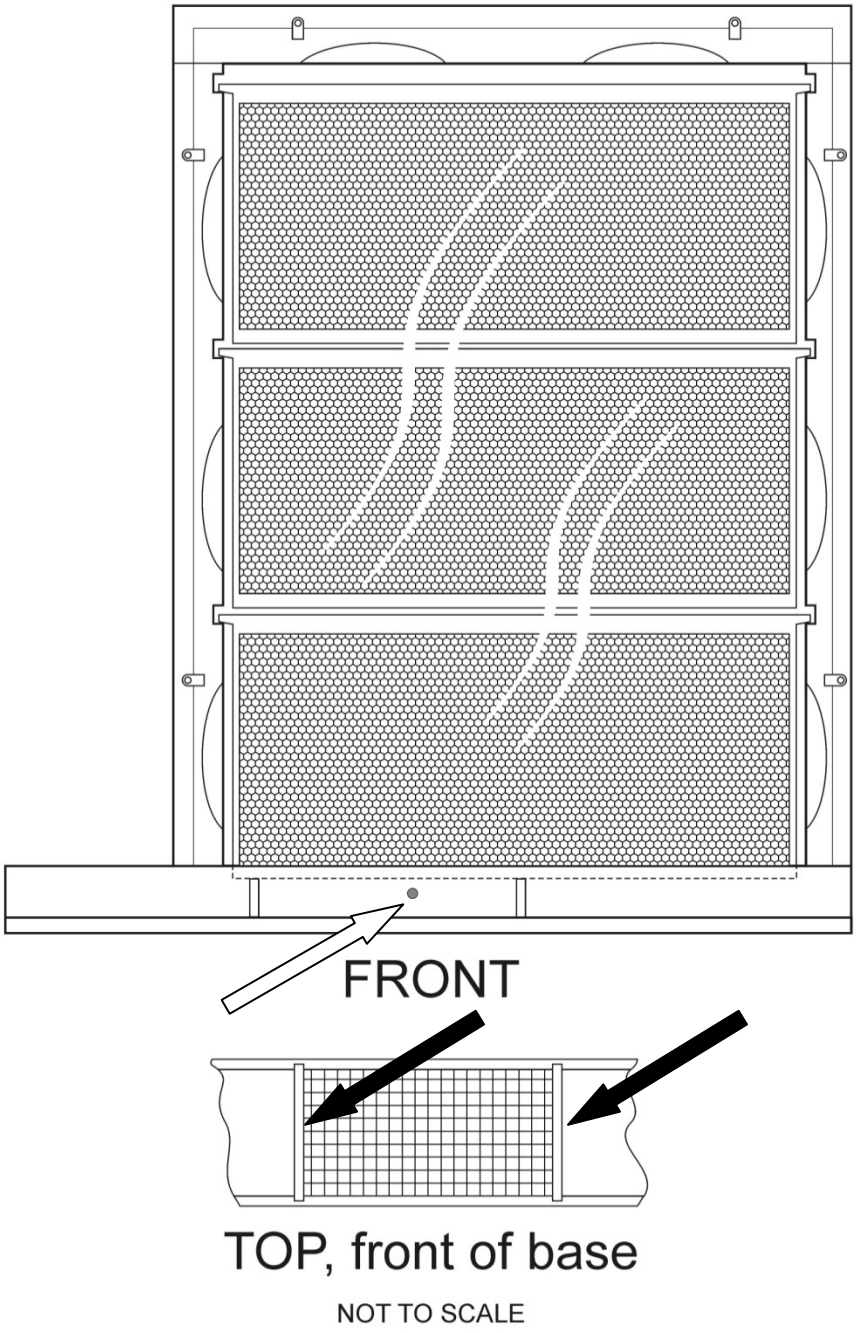

Figure 5-1. Diagram of observation hive. The entrance to the test arena is denoted by the white arrow. The Plexiglass doors are denoted by the black arrows.

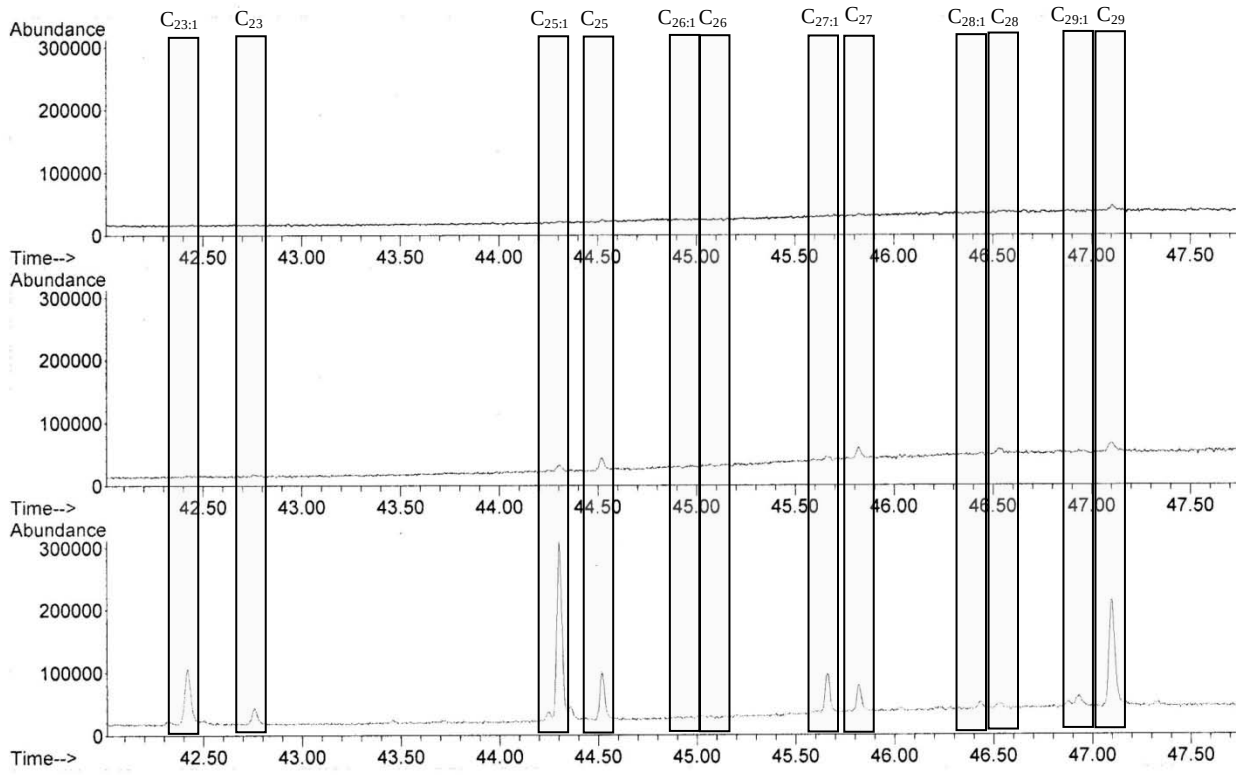

Figure 5-2. Chromatograms from cuticle of newly-eclosed (top), 14 day old beetles fed a diet of honey, pollen, and BroodBuilder™ (middle), and 14 day old beetles fed a diet of sugar and water (bottom).

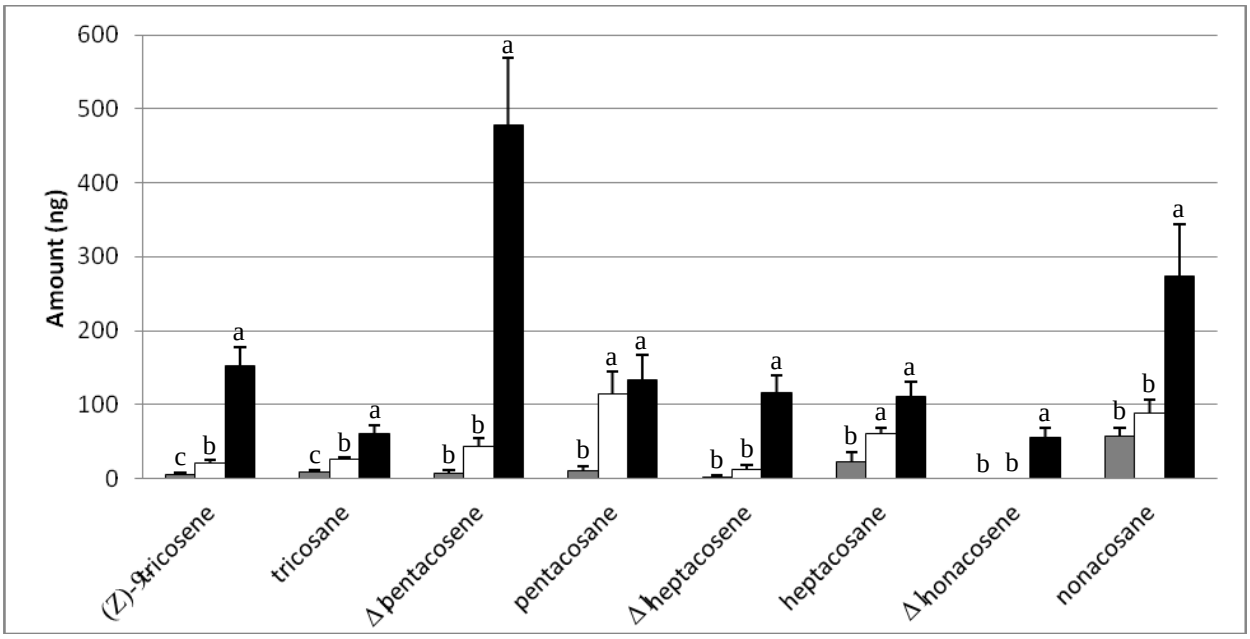

Figure 5-3. Relative amounts of various hydrocarbons in newly-eclosed (gray), 14 day old beetles fed a diet of honey, pollen, and BroodBuilder™ (white), and 14 day old beetles fed a diet of sugar and water (black).

CHAPTER 6
DISTRIBUTION OF MULTIPLE BEETLE SPECIES INTRODUCED INTO HONEY BEE COLONIES

Honey bees employ defensive behaviors to prevent would-be invaders from entering the colony. Many of these defensive tactics occur at the colony's entrance and are expressed by guard bees (Breed et al. 2004, Chapter 4). Honey bees also have colony-level defensive behaviors for nest invaders once they penetrate the nest. For example, honey bees use confinement behavior against SHBs (Neumann et al. 2001, Ellis 2005). This behavior involves confining the SHBs to peripheral cracks and crevices away from the colony's resources by stationing guard bees around the confinement sites in which SHBs hide naturally (Neumann and Elzen 2004, Ellis and Hepburn 2006). Such hiding behavior is important to the SHB's success, as it allows the beetle to avoid its honey bee hosts. Also, it is critical to the hosts' success, as it allows the bees to confine the SHBs away from valuable resources.

Peculiarly, the behavior is present in SHB-naïve European races of honey bees even though they had not been exposed to the beetle prior to the mid 1990s. Seemingly, bees do not exhibit this behavior toward other nest intruders (Ellis et al. 2003a). As such, the purpose of the current study was to determine how honey bees react to non-SHB species once they enter colonies in an effort to better understand the origins of confinement behavior. I hypothesized that the presentation of confinement behavior by honey bees may be linked to the invader's level of colony integration.

SHBs are considered highly-integrated into honey bee colonies because they are able to endure defensive attacks from bees (Elzen et al. 2001, Chapter 4), exhibit a high degree of morphological specialization (Chapter 3), and can induce their hosts to feed them trophallactically (Ellis et al. 2002b). Other Nitidulidae beetles have been found in honey bee colonies, but their respective degrees of integration remain unknown. However, they are

assumed to be no more than innocuous shelter-seeking visitors or facultative scavengers (Ellis et al. 2008) and their presence in bee colonies provides one the ability to test multiple aspects of confinement behavior. These beetle species include *Cychramus luteus* (Neumann and Ritter 2004), *Lobiopa insularis*, *Carpophilus dimidiatus*, *Glischrochilus fasciatus*, *Epuraea corticina* (Ellis et al. 2008), and *E. luteola* (personal observation). These species are considered accidental within colonies, because they are not obligate inquilines and occur more frequently in other habitats (Smith 1886).

I conducted two experiments to address the hypothesis that the presentation of confinement behavior by honey bees may be linked to the invader's level of colony integration. In the first experiment, I compared intra-colonial responses of honey bees toward beetles expressing varying degrees of colony integration to determine if 1) highly integrated species were more likely to find confinement sites than less integrated ones, 2) if there is a temporal pattern associated with confinement site location, and 3) whether confinement behavior is a general response toward all invaders (i.e. not specific to SHBs). Beetle species used in this experiment included: SHBs (highly-integrated), *L. insularis* and *E. luteola* (accidental); and *Carpophilus humeralis, C. hemipterus*, and *Tribolium castaneum* (non-integrated). In the second experiment, I determined if previous SHB occupation of confinement sites predisposed those sites to hosting invading SHBs never before exposed to honey bee colonies.

Materials and Methods

Beetles

Adult SHBs and *T. castanaeum* were obtained from *in vitro* rearing colonies at the USDA-ARS, Gainesville, Florida (29.64° N, 82.35° W) and were used to initiate rearing programs at the University of Florida Department of Entomology and Nematology (29.64° N, 82.36° W). Adults from the other nitidulid species were collected from rotting cantaloupe, *Cucumis melo* L., in July

2009 at the University of Florida Plant Science Research and Education Unit (PSREU) in Citra, Florida (29.41° N, 82.17° W) and used to initiate rearing programs. All species, with the exception of SHBs and *T. castaneum*, were reared in an incubator (24°C; 40% relative humidity; constant darkness) on a tomato and prune-based diet developed by Peng and Williams (1990). SHBs were reared on a diet of honey, pollen, and Brood Builder™ (Dadant and Sons, Inc., Hamilton, IL) in a ratio of 1:1:2 respectively (Ellis et al. 2008, Ellis et al. 2010) while *T. castaneum* were reared on flour. SHBs, *E. luteola*, *C. humeralis*, and *C. hemipterus* were 2 – 4 weeks old at the time of the experiment. Due to limited availability, the *L. insularis* used during the study were obtained from rearing programs or field collections and, as such, their age at the time of the experiment was unknown. Though *T. castaneum* were lab-reared, their age also was unknown at the time of the experiment. No adult beetle used during the experiment had ever been in contact with honey bees or bee colonies prior to the study.

Observation Hives

In September 2009, four observation hives were created from previously established honey bee colonies of mixed European origin located at the PSREU. The observation colonies were given three frames collectively containing pollen, capped honey, brood of all ages, worker bees, and an egg-laying queen. The observation hive structure (Figure 6-1) accommodated three, 23.0 × 42.6 cm (L × W) wooden frames. I further modified each observation hive by cutting 8 semicircular grooves (~10 cm^2 total area) on the periphery of both sides of the hives, totaling 16 grooves per hive (Figure 6-1). The grooves provided confinement sites for invading beetles. I drilled a small hole (1 cm diameter) into the side of the colony entrance to introduce beetles into the colony (Figure 6-1). The entrance corridor to the hives could be closed at both ends using Plexiglas doors.

Experiment 1: Hiding Behavior of Multiple Beetle Species

Because the study was conducted in an area where SHBs are present naturally, I removed existing SHBs from each observation colony ~24 hours before each trial. For each trial, conducted in October 2009, 25 beetles from a single species were introduced through the side of the entrance corridor of an observation hive at 21:00 hours. The Plexiglas door distal to the colony was closed to limit beetle escape from the colony. Once the beetles were introduced, I recorded the location of the beetles 5 min, 15 min, 30 min, 1 h, 2 h, 6 h, 12 h, and 24 h after their introduction into the colony. After conducting the 1 hour observation, I removed the Plexiglass door from the colony to allow for natural bee movement (1 h was presumed adequate to limit any initial beetle escape from the hive). At each observation period, the beetles could be one of four places: (1) hiding where bees could not reach them (in the confinement sites), (2) in or on the comb, (3) roaming freely on the interior walls of the observation hive (interior walls), or (4) not present in the colony or hiding where they could not be seen by the observer (missing). The procedure was repeated until all beetle species were introduced into each of the four observation hives. Introductions into individual colonies were separated by 48 h.

Experiment 2: SHB Hiding Behavior

To account for the possibility that SHBs are attracted to confinement sites because other SHBs occupied the sites previously, I conducted a separate experiment in December 2009 using only SHBs and following the procedure outlined in experiment 1 with one modification. In this experiment, the observation hives were opened before the trials and the surfaces of the grooved confinement sites were wiped with a cloth soaked with acetone to remove potential beetle pheromone or other marking residues. The outside surface of the glass side panels of the observation hives also were cleaned with acetone and rotated such that the cleaned outside surface was now the inside surface, having never been exposed to SHBs previously.

Statistical Analysis

For both experiments, I analyzed the data separately for each time period. For each beetle species in experiment one, the number of beetles at each location (confinement site, colony wall, missing) was converted into proportions of the total number of beetles observed in each colony. No beetles were found on the comb during the first experiment so this location was excluded from the analysis. Within each time period for the first experiment, I analyzed the proportion of each beetle species found at the three locations separately by location using a one way ANOVA. Where necessary, individual means were compared using the Student's t method for multiple pairs. For the second experiment, I analyzed the proportion of SHBs found at each location (confinement site, colony wall, comb, or missing) within "washed" colonies (with the confinement sites cleaned with acetone) or "unwashed" colonies (the main effect). Prior to all analysis, the proportion data were arc-sine $\sqrt{}$ transformed. Analyses were conducted using JMP (2008).

Results

Experiment 1: Hiding Behavior of Multiple Beetle Species

At every recorded observation period, there were significantly more SHBs found in confinement sites than any other beetle species (Table 6-1, Figure 6-2). When not considering SHBs, few individuals of the other beetle species were present at confinement sites. That said, some beetle species were present at confinement sites during some time periods more so than were other beetle species, though no general trends in confinement site distribution of non-SHB species existed (Table 6-1, Figure 6-2). In general, the same proportions of all beetle species were found on the interior walls of the colony at all but one time period (1 hour – Table 6-1). Finally, at all time periods$\geq$ 15 min there were significantly fewer SHBs m issing than there were other beetles which could not be found.

Experiment 2: SHB Hiding Behavior

At every observation period through 1 h, there were significantly more SHBs found in unwashed confinement sites than in washed ones (Table 6-2, Figure 6-3). At 2 hours, 12 hours, and 24 hours, there were significantly more beetles found on the combs in washed colonies than in unwashed colonies. At 5 minutes, 6 hours, 12 hours, and 24 hours there were significantly more beetles found on the interior walls in washed colonies than in unwashed ones. At 15 minutes, 30 minutes, and 1 hour, there were significantly more beetles missing in washed than unwashed colonies (Table 6-2, Figure 6-3).

Discussion

The ability to navigate small spaces is a general characteristic of nitidulid beetles because many nitidulid species spend much of their adult lives under tree bark, in flowers, in fungus-inhabited fruits, or in other similar decaying matter (Parsons 1943). Although not all nitidulid species tested in this study have been found in honey bee colonies previously, I expected that individuals from all species would run from bee aggression and hide in the confinement sites around the colony perimeter. The generated data did not support this assertion.

In the first study, more SHBs could be found in confinement sites than at other locations for all time periods. This behavior was unique to SHBs as only a few individuals from the other beetle species were observed hiding in confinement sites. Not only did the other beetle species not hide in the confinement sites, but >90% had left the colony within the first 1 hour of the observation period. Therefore, there was a strong pattern relating the level of beetle integration into honey bee colonies to beetle ability to find confinement sites when considering highly-integrated species (SHB) versus all other levels of integration. However, I did not observe any temporal patterns related to beetle ability to find confinement sites.

There are five possible reasons that SHBs were confined by honey bees while the other beetle species largely were not. First, it is possible that the honey bees were able to remove non-SHB beetles from the colonies better than they were able to remove SHBs. There are morphological data that support this assertion as SHBs possess adaptations on their legs that allow them to avoid being flipped or lifted from a surface, making them more difficult to remove from a colony (Chapter 3). These morphological adaptations are absent in the other beetle species tested in this study. Despite this, other data (Chapter 4) suggest that the non-SHB beetle species tested in this study usually are not treated defensively by bees, and often are ignored, at the nest entrance, and I did not observe any non-SHB removals. This observation would lead one to believe that the non-SHB beetle species tested in this study can access a colony freely, thus predisposing them to being confined by the honey bees within as a second line of defense against colony intrusion. In the current study, I show that this is not the case.

The second possible reason that SHBs were confined by honey bees while the other beetle species largely were not may rely on other data which show that of the beetle species tested in this study, only the SHB is attracted to bee colony odors (Chapter 2). This may suggest that the non-SHB beetles are more likely to leave the nest rather than willingly seek shelter within. This theory is strengthened by the observation that the beetles are not met with aggression at the colony entrance and could enter if they desired to do so. While in earlier work (Chapter 2) *C. humeralis* did not show any attraction to or repulsion from the colony, the other beetles tested in the current study have been shown to be repelled by colony odors, possibly suggesting a reason they were not found in confinement sites. Interestingly, *C. humeralis* occurred at confinement sites at a greater rate than did the other non-SHB species at all times except for 24 hours and they were shown in Chapter 2 to not be repelled by bee colony odors. That the accidental species were

not found in the colonies in nontrivial numbers is somewhat perplexing, as others have found *L. insularis* (Ellis et al. 2008) and *E. luteola* (personal observation) within colonies on multiple occasions. However, they are not found in colonies nearly as often as SHBs, suggesting that they may enter colonies under specific conditions (weakened or stressed colonies, specific floral resources, etc.), which may not have been met in the four colonies used in this study.

Third, confinement behavior in honey bees might be a specific defense against SHBs rather than against all colony intruders in general. However, this is unlikely given that the race of honey bee used in the study was first exposed to the beetle only in the mid-1990s (Hood 2000) and the behavior is identical to that exhibited by African races of honey bees toward SHBs. Recent evidence suggests that *A. mellifera* originated in Africa (Whitfield et al. 2006). If true, ancestors of European races may have been exposed to the SHB or its ancestors in their evolutionary history, thus expressing confinement behavior as a vestige. However, the SHB association with honey bees may have occurred after European honey bee races evolved, thus erasing an ancestral link between the two.

Fourth, confinement behavior may be an exaptation of drone corralling, which occurs when resources diminish in the fall when the drones become an expense to the colony (Free and Williams 1975). When this occurs, honey bees force the drones to the outside frames, then to the walls, then to the bottom board, where they are ultimately expelled from the colony, and not allowed to re-enter (Leventes 1956). Thus, SHBs may induce drone corralling behavior, which becomes expressed as confinement. Drones are larger than workers and would not, therefore, be able to "hide" from them as the SHB does.

The final possible reason I discuss for honey bee confinement of SHBs but not other beetle invaders could be that confinement behavior is an adaptation by the SHB producing favorable

responses from their host. Upon entering a colony, beetles find hiding places quickly to escape aggressive bees (Neumann and Elzen 2004, Ellis and Hepburn 2006). However, they are unable to live much longer than a week without food resources (Pettis and Shimanuki 2000, Ellis et al. 2002a), and leaving the hiding areas may result in fatal encounters with honey bees. As such, it is possible that SHBs elicit confinement behavior from adult bees (perhaps chemically) so that they can be tended while in cracks/crevices around the nest. Here the beetles are safe from attack and can induce the attracted bees to regurgitate liquid, which the beetles consume, thereby surviving for a longer period of time (Ellis et al. 2002b).

Further support for beetle-mediated confinement is offered in Chapter 4, wherein I observed that SHBs were treated defensively by honey bees more often than any of the other beetles observed. This may be due to the rapid movements of SHBs when placed in the entrance, as no other beetle moved as rapidly as that species. Honey bees are attracted to objects that are moving rapidly and are dark (though this study was carried out under red light conditions) (Breed et al. 2004). Furthermore, the SHB vectors a yeast (*Kodamaea ohmeri*) which, when mixed with bee-collected pollen, produces honey bee alarm pheromone, which is very attractive to honey bees (Breed et al. 2004, Torto et al. 2007a). However, this likely would not explain their treatment at the colony entrance since they have not yet accessed pollen at that point.

Attracting defensive guard bees at the colony entrance is fatal for most potential nest invaders. However, SHBs have morphological and behavioral characters which allow them to survive attacks by honey bees. For example, reportedly they have a hardened exoskeleton which allows them to resist honey bee stings and makes them difficult to grasp, although this has not been quantified (Lundie 1940, Schmolke 1974, Neumann et al. 2001). Also, they have grooved femora and flattened tibiae to accommodate their retracting behavior, wherein they pull all of

their appendages beneath their body inaccessible to honey bees (Neumann et al., 2001, Chapter 3). Furthermore, they have dense tarsal setation which may allow them to grasp to the substrate while their legs are beneath their body and resist being flipped by honey bees (Chapter 3), similar to *Hemisphaerota cyanea*, which uses its dense tarsal setae to avoid being moved by ants (Eisner and Aneshansley 2000). Such characters would enable the SHB to resist defensive bees.

There are several examples of parasites altering the behavior of their animal hosts to their own benefit (Moore 1995, Schmid-Hempel 1995, 1998). For example, the trematode *Diplostomum spathaceum* (Rudolphi) causes diminished predator avoidance behavior in its intermediate fish host, thereby increasing the chance that it will be consumed by and infect its primary avian hosts (Seppälä 2005). Similarly, the ant *Leptothorax nylanderi* (Förster) (Hymenoptera: Formicidae) becomes lethargic relative to unaffected workers when it becomes infected by the cestode *Anomotaenia brevis* (Clerc). The ant is the intermediate host for the cestode, which must be consumed by the primary host, which is a woodpecker (Plateaux 1972, cited in Moore 1995). Also, the fungus *Entomophthora myrmecophaga* Turian & Wuest infects ants of the genus *Formica*. When infected, ants leave their colonies in the evening and climb leaves of grass to which they become affixed with threads of fungus. From here, the fungal spores are able to disperse by air from the infected, dead ant (Balazy and Sokolowski 1977, Schmid-Hempel 1998). If SHBs do initiate confinement behavior, as proposed here, they would be acting like a parasite within a superorganismal host (see Ellis and Hepburn 2006), altering the host's behavior to increase its own fitness.

Host-finding behavior in SHBs is facilitated by their attraction to hive odors as well as attraction to volatiles produced by a yeast, *Kodamaea ohmeri* (Suazo et al. 2003, Torto et al. 2005, Torto et al. 2007a). When the yeast mixes with bee-collected pollen, it produces

components of honey bee alarm pheromone, a pheromone that is highly attractive to adult SHBs (Torto et al. 2007a). Though SHBs are attracted to components of honey bee alarm pheromone, this attraction likely does not explain their attraction to confinement sites within the colony, especially sites that have hosted SHBs previously as the sites typically do not contain pollen or associated debris. Other nitidulid species are known to produce aggregation pheromones (Bartelt et al. 1991, 1992, 1994, 1995, 2004, Dowd and Bartelt 1993, Williams et al. 1993, Nardi et al. 1996, Cosse and Bartelt 2000), and the occurrence of such pheromones in the SHB has been suggested (Neumann and Elzen 2004). Though investigators in at least one study failed to show that SHBs produce aggregation pheromones (Torto et al. 2007a), the data suggest that such pheromones may exist and be important in the hiding behavior of SHBs within honey bee colonies. Interestingly, over time the proportion of SHBs in confinement sites of both colony types (washed and unwashed confinement sites) converged, thus suggesting that free roaming SHBs were able to locate confinement sites easier once other SHBs found and occupied them.

Confinement behavior is one of the most intriguing aspects of the honey bee – SHB relationship. This study suggests that many beetles simply do not enter colonies, so there are no opportunities for confinement of those species to occur. However, there are no known examples of confinement behavior displayed by honey bees toward other pests, or in other social insects in general. Therefore, it appears to be specific to SHBs, though what mediates this behavior remains unknown.

Table 6-1. Proportions of different beetle species found in confinement sites, the interior walls of the colony, or missing altogether at different time periods following their introduction into observation honey bee colonies. Within each time period and location, columnar means followed by different letters are significantly different at $\alpha = 0.05$. Means were compared using the Student's t method for multiple pairs.

		Location mean ± SE (*n*) proportion of beetles		
Time	Beetle species	Confinement site	Interior walls	Missing
5 min	*A. tumida*	0.570 ± 0.148 (4)a	0.010 ± 0.010 (4)	0.420 ± 0.155 (4)
	C. humeralis	0.100 ± 0.048 (4)b	0.170 ± 0.081 (4)	0.730 ± 0.060 (4)
	L. insularis	0.020 ± 0.020 (4)b c	0.300 ± 0.127 (4)	0.680 ± 0.121 (4)
	T. castaneum	0.010 ± 0.010 (4)b c	0.340 ± 0.145 (4)	0.650 ± 0.150 (4)
	C. hemipterus	0.010 ± 0.010 (4)b c	0.120 ± 0.054 (4)	0.870 ± 0.053 (4)
	E. luteola	0.000 ± 0.000 (4)c	0.100 ± 0.038 (4)	0.900 ± 0.038 (4)
	ANOVA	F = 13.7; df = 5, 18; P <0.01	F = 2.4; df = 5, 18; P = 0.08	F = 2.4; df = 5, 18; P = 0.08
15 min	*A. tumida*	0.600 ± 0.121 (4)a	0.020 ± 0.012 (4)	0.380 ± 0.132 (4)b
	C. humeralis	0.060 ± 0.038 (4)b	0.030 ± 0.019 (4)	0.910 ± 0.025 (4)a
	L. insularis	0.040 ± 0.023 (4)b	0.210 ± 0.117 (4)	0.750 ± 0.131 (4)a
	T. castaneum	0.020 ± 0.020 (4)b	0.120 ± 0.107 (4)	0.860 ± 0.101 (4)a
	C. hemipterus	0.020 ± 0.020 (4)b	0.050 ± 0.038 (4)	0.930 ± 0.041 (4)a
	E. luteola	0.000 ± 0.000 (4)b	0.110 ± 0.044 (4)	0.890 ± 0.044 (4)a
	ANOVA	F = 15.0; df = 5, 18; P < 0.01	F = 1.3; df = 5, 18; P = 0.31	F = 4.7; df = 5, 18; P < 0.01
30 min	*A. tumida*	0.590 ± 0.125 (4)a	0.040 ± 0.028 (4)	0.370 ± 0.150 (4)b
	C. humeralis	0.100 ± 0.050 (4)b	0.000 ± 0.000 (4)	0.900 ± 0.050 (4)a
	L. insularis	0.040 ± 0.023 (4)b c	0.190 ± 0.111 (4)	0.770 ± 0.100 (4)a
	T. castaneum	0.000 ± 0.000 (4)c	0.100 ± 0.087 (4)	0.900 ± 0.087 (4)a
	C. hemipterus	0.010 ± 0.010 (4)b c	0.020 ± 0.012 (4)	0.970 ± 0.019 (4)a
	E. luteola	0.010 ± 0.010 (4)b c	0.040 ± 0.028 (4)	0.950 ± 0.038 (4)a
	ANOVA	F = 16.5; df = 5, 18; P < 0.01	F = 1.9; df = 5, 18; P = 0.14	F = 6.3; df = 5, 18; P < 0.01
1 hr	*A. tumida*	0.580 ± 0.141 (4)a	0.040 ± 0.028 (4)a b	0.380 ± 0.158 (4)b
	C. humeralis	0.170 ± 0.104 (4)b	0.000 ± 0.000 (4)b	0.830 ± 0.104 (4)a
	L. insularis	0.030 ± 0.019 (4)b c	0.120 ± 0.080 (4)a	0.850 ± 0.072 (4)a
	T. castaneum	0.010 ± 0.020 (4)c	0.020 ± 0.012 (4)a b	0.970 ± 0.019 (4)a
	C. hemipterus	0.020 ± 0.012 (4)c	0.000 ± 0.000 (4)b	0.980 ± 0.012 (4)a
	E. luteola	0.010 ± 0.020 (4)c	0.100 ± 0.053 (4)a	0.890 ± 0.062 (4)a
	ANOVA	F = 12.0; df = 5, 18; P < 0.01	F = 3.0; df = 5, 18; P = 0.04	F = 6.4; df = 5, 18; P < 0.01
2 hr	*A. tumida*	0.630 ± 0.127 (4)a	0.000 ± 0.000 (4)	0.370 ± 0.127 (4)b
	C. humeralis	0.070 ± 0.030 (4)b	0.010 ± 0.010 (4)	0.920 ± 0.028 (4)a
	L. insularis	0.060 ± 0.038 (4)b c	0.040 ± 0.016 (4)	0.900 ± 0.053 (4)a
	T. castaneum	0.000 ± 0.000 (4)c	0.010 ± 0.010 (4)	0.990 ± 0.010 (4)a
	C. hemipterus	0.010 ± 0.020 (4)b c	0.000 ± 0.000 (4)	0.990 ± 0.010 (4)a
	E. luteola	0.000 ± 0.000 (4)c	0.010 ± 0.010 (4)	0.990 ± 0.010 (4)a

Table 6-1. Continued.

Time	Beetle species	Location mean ± SE (*n*) proportion of beetles Confinement site	Interior walls	Missing
2 hr	ANOVA	F = 20.9; df = 5, 18; P < 0.01	F = 2.1; df = 5, 18; P = 0.11	F = 16.6; df = 5, 18; P < 0.01
6 hr	*A. tumida*	0.640 ± 0.146 (4)a	0.010 ± 0.010 (4)	0.350 ± 0.143 (4)c
	C. humeralis	0.090 ± 0.025 (4)b	0.000 ± 0.000 (4)	0.910 ± 0.025 (4)b
	L. insularis	0.030 ± 0.030 (4)b c	0.010 ± 0.010 (4)	0.960 ± 0.028 (4)a b
	T. castaneum	0.010 ± 0.010 (4)b c	0.010 ± 0.010 (4)	0.980 ± 0.012 (4)a b
	C. hemipterus	0.010 ± 0.010 (4)b c	0.000 ± 0.000 (4)	0.990 ± 0.010 (4)a b
	E. luteola	0.000 ± 0.000 (4)c	0.000 ± 0.000 (4)	1.000 ± 0.000 (4)a
	ANOVA	F = 18.2; df = 5, 18; P < 0.01	F = 0.6; df = 5, 18; P = 0.70	F = 18.1; df = 5, 18; P < 0.01
12 hr	*A. tumida*	0.710 ± 0.172 (4)a	0.010 ± 0.010 (4)	0.280 ± 0.169 (4)b
	C. humeralis	0.040 ± 0.016 (4)b	0.000 ± 0.000 (4)	0.960 ± 0.016 (4)a
	L. insularis	0.030 ± 0.019 (4)b	0.010 ± 0.010 (4)	0.960 ± 0.016 (4)a
	T. castaneum	0.010 ± 0.010 (4)b	0.010 ± 0.010 (4)	0.980 ± 0.012 (4)a
	C. hemipterus	0.010 ± 0.010 (4)b	0.000 ± 0.000 (4)	0.990 ± 0.010 (4)a
	E. luteola	0.000 ± 0.000 (4)b	0.000 ± 0.000 (4)	1.000 ± 0.000 (4)a
	ANOVA	F = 14.5; df = 5, 18; P < 0.01	F = 0.6; df = 5, 18; P = 0.70	F = 14.9; df = 5, 18; P < 0.01
24 hr	*A. tumida*	0.750 ± 0.144 (4)a	0.000 ± 0.000 (4)	0.250 ± 0.144 (4)b
	C. humeralis	0.010 ± 0.010 (4)b	0.000 ± 0.000 (4)	0.990 ± 0.010 (4)a
	L. insularis	0.010 ± 0.010 (4)b	0.000 ± 0.000 (4)	0.990 ± 0.010 (4)a
	T. castaneum	0.000 ± 0.000 (4)b	0.000 ± 0.000 (4)	1.000 ± 0.000 (4)a
	C. hemipterus	0.010 ± 0.010 (4)b	0.000 ± 0.000 (4)	0.990 ± 0.010 (4)a
	E. luteola	0.000 ± 0.000 (4)b	0.000 ± 0.000 (4)	1.000 ± 0.000 (4)a
	ANOVA	F = 33.2; df = 5, 18; P < 0.01	No beetles found on interior walls	F = 33.2; df = 5, 18; P < 0.01

Table 6-2. Proportions of *A. tumida* found in confinement sites, on the combs, on the interior walls, or missing altogether at multiple time periods after their introduction into observation honey bee colonies. Significant effects were determined using a one-way ANOVA. Within each time period and location, columnar means followed by different letters are significantly different at $\alpha = 0.05$. Confinement sites were either washed with acetone or unwashed prior to releasing *A. tumida* into the colonies.

		Location mean ± SE (*n*) proportion of beetles			
Time	Condition of confinement site	Confinement sites	Combs	Interior walls	Missing
5 min	Unwashed	0.570 ± 0.148 (4)a	0.000 ± 0.000 (4)	0.010 ± 0.010 (4)b	0.420 ± 0.155 (4)
	Washed	0.076 ± 0.046 (4)b	0.089 ± 0.053 (4)	0.093 ± 0.044 (4)a	0.742 ± 0.108 (4)
	ANOVA	F = 11.0; df = 1, 6; P = 0.02	F = 5.5; df = 1, 6; P = 0.06	F = 7.4; df = 1, 6; P = 0.03	F = 3.0; df = 1, 6; P = 0.13
15 min	Unwashed	0.600 ± 0.121 (4)a	0.000 ± 0.000 (4)	0.020 ± 0.012 (4)	0.380 ± 0.132 (4)b
	Washed	0.056 ± 0.044 (4)b	0.000 ± 0.000 (4)	0.057 ± 0.035 (4)	0.887 ± 0.047 (4)a
	ANOVA	F = 19.4; df = 1, 6; P < 0.01	No beetles found on comb	F = 0.8; df = 1, 6; P = 0.39	F = 13.6; df = 1, 6; P = 0.01
30 min	Unwashed	0.590 ± 0.125 (4)a	0.000 ± 0.000 (4)	0.040 ± 0.028 (4)	0.370 ± 0.150 (4)b
	Washed	0.038 ± 0.026 (4)b	0.000 ± 0.000 (4)	0.062 ± 0.014 (4)	0.900 ± 0.016 (4)a
	ANOVA	F = 23.0; df = 1, 6; P < 0.01	No beetles found on comb	F = 1.4; df = 1, 6; P = 0.28	F = 12.6; df = 1, 6; P = 0.01
1 hr	Unwashed	0.580 ± 0.141 (4)a	0.000 ± 0.000 (4)	0.040 ± 0.028 (4)	0.380 ± 0.158 (4)b
	Washed	0.029 ± 0.018 (4)b	0.019 ± 0.019 (4)	0.089 ± 0.020 (4)	0.864 ± 0.030 (4)a
	ANOVA	F = 20.5; df = 1, 6; P < 0.01	F = 1.0; df = 1, 6; P = 0.36	F = 2.8; df = 1, 6; P = 0.14	F = 7.3; df = 1, 6; P = 0.04
2 hr	Unwashed	0.630 ± 0.127 (4)	0.000 ± 0.000 (4)b	0.000 ± 0.000 (4)	0.370 ± 0.127 (4)
	Washed	0.220 ± 0.111 (4)	0.070 ± 0.025 (4)a	0.081 ± 0.057 (4)	0.629 ± 0.052 (4)
	ANOVA	F = 5.5; df = 1, 6; P = 0.06	F = 8.6; df = 1, 6; P = 0.03	F = 2.6; df = 1, 6; P = 0.16	F = 3.4; df = 1, 6; P = 0.11
6 hr	Unwashed	0.640 ± 0.146 (4)	0.000 ± 0.000 (4)	0.010 ± 0.010 (4)b	0.350 ± 0.143 (4)
	Washed	0.276 ± 0.126 (4)	0.063 ± 0.051 (4)	0.152 ± 0.057 (4)a	0.510 ± 0.060 (4)
	ANOVA	F = 3.7; df = 1, 6; P = 0.10	F = 2.3; df = 1, 6; P = 0.18	F = 11.3; df = 1, 6; P = 0.02	F = 1.2; df = 1, 6; P = 0.32
12 hr	Unwashed	0.710 ± 0.172 (4)	0.000 ± 0.000 (4)b	0.010 ± 0.010 (4)b	0.280 ± 0.169 (4)
	Washed	0.248 ± 0.165 (4)	0.149 ± 0.036 (4)a	0.074 ± 0.029 (4)a	0.529 ± 0.137 (4)

Table 6-2. Continued.

Time	Condition of confinement site	Location mean ± SE (*n*) proportion of beetles Confinement sites	Combs	Interior walls	Missing
12 hr	ANOVA	F = 4.4; df = 1, 6; P = 0.08	F = 59.7; df = 1, 6; P < 0.01	F = 8.9; df = 1, 6; P = 0.02	F = 1.8; df = 1, 6; P = 0.23
24 hr	Unwashed	0.750 ± 0.144 (4)	0.000 ± 0.000 (4)b	0.000 ± 0.000 (4)b	0.250 ± 0.144 (4)
	Washed	0.553 ± 0.139 (4)	0.194 ± 0.086 (4)a	0.123 ± 0.042 (4)a	0.130 ± 0.076 (4)
	ANOVA	F = 1.2; df = 1, 6; P = 0.31	F = 17.0; df = 1, 6; P < 0.01	F = 24.1; df = 1, 6; P < 0.01	F = 1.0; df = 1, 6; P = 0.35

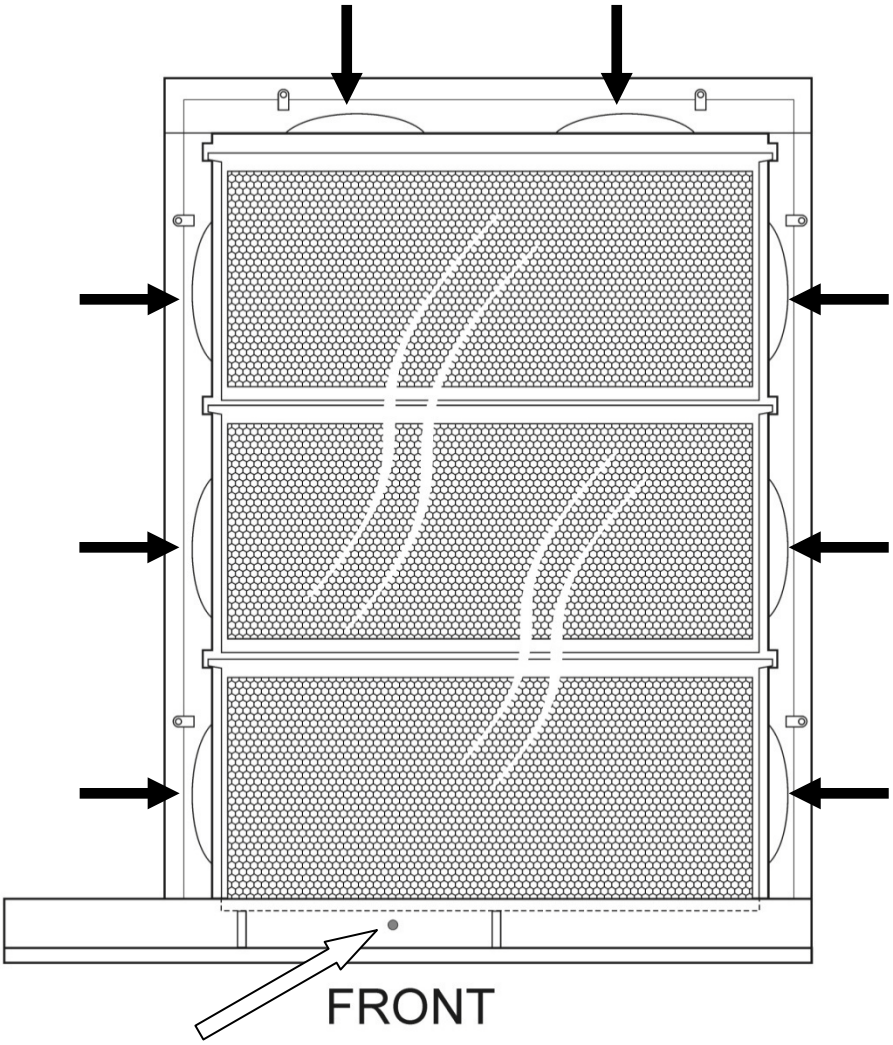

Figure 6-1. Diagram of experimental observation hive. The white arrow indicates the location where beetles were introduced into the observation hive while the black arrows indicate the location of the eight grooves (confinement sites) located on the periphery of the observation hive. The confinement sites were present on both sides, totaling 16 sites.

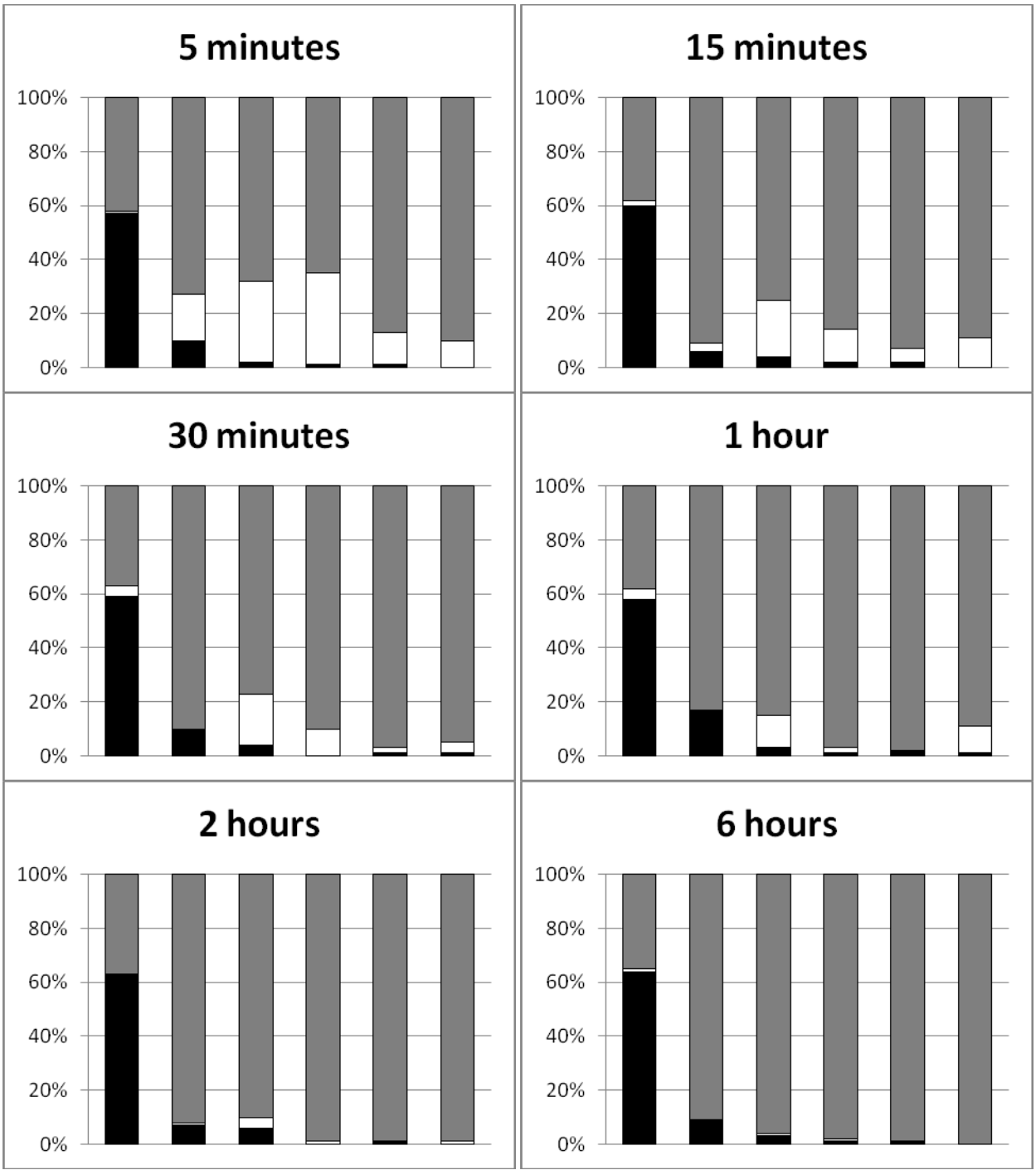

Figure 6-2. Percentage distribution of each beetle species found in confinement sites (black), on the interior walls of the hive (white), or missing (gray) at each time period.

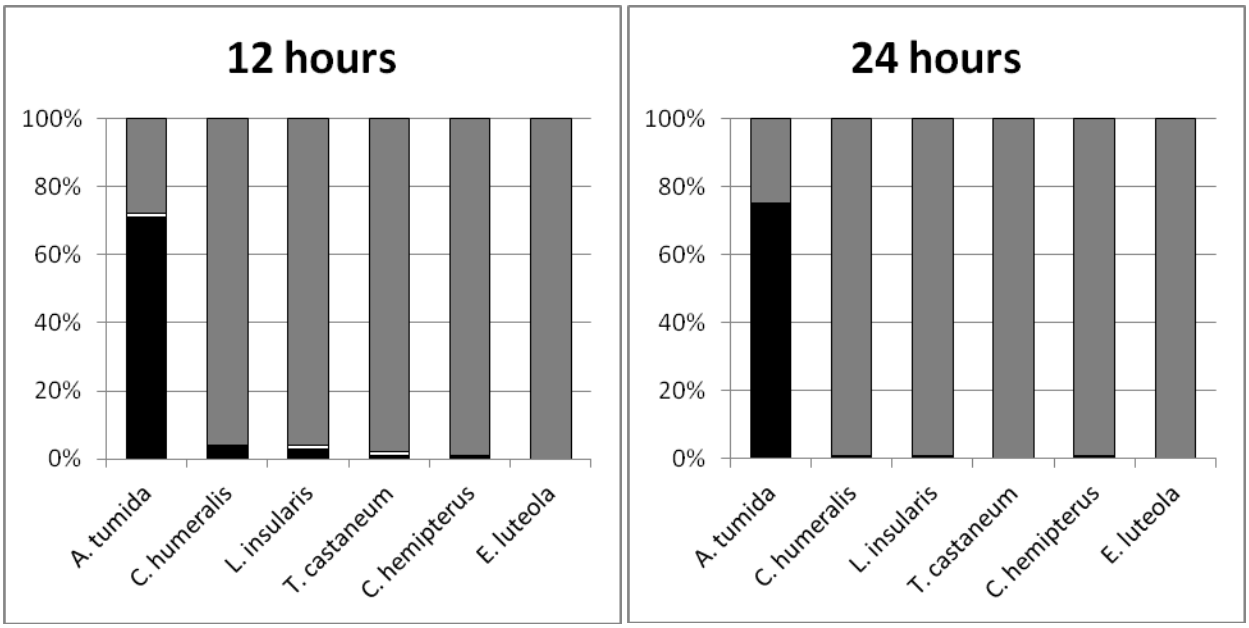

Figure 6-2. Continued.

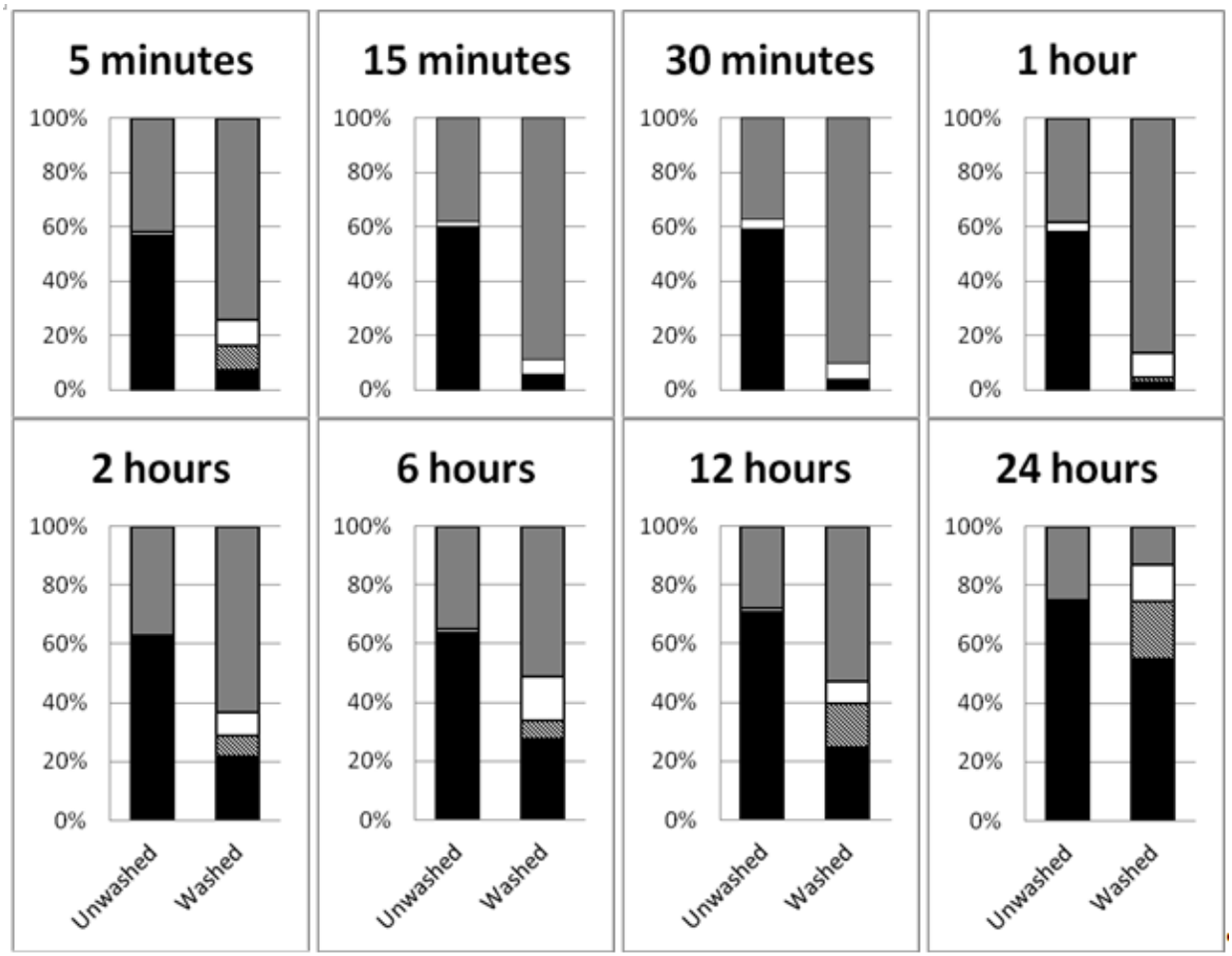

Figure 6-3. Percentage distribution of *A. tumida* in colonies where the confinement sites were unwashed and washed. The four potential locations of *A. tumida* within a colony include confinement sites (black), comb (hashed), on the interior walls of the hive (white), or missing (gray).

CHAPTER 7
TEMPERATURE-DEPENDENT CLUSTERING BEHAVIOR OF SHBS IN HONEY BEE COLONIES

Temperate races of honey bees are able to survive cold temperatures by forming thermoregulatory clusters when the ambient temperature falls below 18 ºC (Michener 1974, Stabentheiner et al. 2003). A honey bee cluster is a tight, contiguous mass of bees formed for the purpose of producing and conserving heat. They accomplish this by tightly grouping bees between frames and then having bees occupy empty cells in the combs to form a more solid cluster. The result is a sphere-shaped mass of bees. Through this behavior, the bees are able to maintain a core temperature of 20 – 30 ºC. In contrast, African races do not form winter clusters well, as their native climates often do not necessitate clustering behavior. Consequently, African races of honey bees may perish when experiencing temperatures common in temperate climates (Hepburn and Radloff 1998).

Honey bee clustering behavior also insulates and protects nest parasites present in the colony, such as *Varroa destructor*, which survives by attaching to adults during the winter when few brood are reared (Bowen-Walker et al. 1997, Schäfer et al. 2010b). Similarly, the SHB is able to endure temperate climates by entering the bee cluster when cold temperatures persist (Pettis and Shimanuki 2000, Ellis et al. 2003b, Neumann and Elzen 2004, Schäfer et al. 2010b). If fact, they have been found in individual comb cells with bees, thus illustrating how well they penetrate the cluster (Ellis et al. 2003b). This is surprising since honey bees confine SHBs around the nest periphery most of the year (Neumann et al. 2001, Ellis et al. 2003a, Ellis 2005) and beetles are met with aggression from bees when outside of confinement sites (Schmolke 1974, Elzen et al. 2001). As such, SHB/bee clustering behavior represents a behavioral change for both species during cold temperatures, resulting in an apparently less aggressive relationship between the two. Despite this, little is known about the cluster-entering behavior of SHBs,

especially when the behavior is initiated, when it ceases, what happens during the behavior, and why the beetles, which have native hosts that do not cluster well (Hepburn and Radloff 1998), exhibit the behavior at all.

The purpose of this study was to address the temporal aspects (initiation, cessation) of the cluster-entering behavior in SHBs. To do this, I exposed honey bee observation colonies to different temperatures to determine the temperature at which SHBs (1) leave confinement sites on the nest periphery (Neumann et al. 2001, Ellis 2005) and enter the thermoregulatory cluster maintained by bees and (2) leave bee clusters and return to confinement sites.

Materials and Methods

Beetles

Adult SHBs were captured from experimental honey bee colonies maintained at the University of Florida Bee Biology and Research Unit (BBRU) in Gainesville, Florida (29.63° N, 82.36° W). SHBs were reared in an incubator (25°C; 80% relative humidity; constant darkness) on a diet of honey, pollen, and Brood Builder™ (Dadant and Sons, Inc., Hamilton, IL) in a ratio of 1:1:2 respectively (Ellis et al. 2008, 2010) at the University of Florida Entomology and Nematology Department (29.64° N, 82.36° W).

Observation Hives

Seven observation hives (four for the experimental group, three for the control group) were created from previously established honey bee colonies of mixed European origin located at the University of Florida Plant Science Research and Education Unit in Citra, Florida (29.41° N, 82.17° W). The observation colonies were given three frames containing pollen, capped honey, brood of all ages, worker bees, and an egg-laying queen and housed at the BBRU. The observation hive structure (Figure 7-1) accommodated three, 23.0 × 42.6 cm (L × W) wooden frames. I further modified each observation hive by cutting 8 semicircular grooves (~10 cm^2 total

area) on the periphery of both sides of the hives, totaling 16 grooves per hive (Figure 7-1). The grooves provided locations where invading beetles could escape from bees, thus becoming confinement sites. I drilled a small hole (1 cm diameter) into the side of the colony entrance and used it to introduce beetles into the colony (Figure 7-1). The entrance corridor to the hives could be closed at both ends using Plexiglas doors.

Behavioral Assays

Experiments were performed in mid-February 2010 to ensure winter bees inhabited the colonies (Fluri et al. 1982). Twenty SHBs were introduced into each colony 24 hours before the experiment to allow sufficient time for SHBs to find confinement sites (Chapter 6). Two hours before the experiment, the observation hives were moved from the BBRU to a dark room (24.5 ºC) housing Florida Reach-Ins (Walker et al. 1993) at the Entomology and Nematology Department. At 20:30, the number of SHBs in each of the 16 confinement sites was determined under red-light conditions. Once the number of SHBs in confinement sites was determined at a given temperature, the four colonies were moved to another Florida Reach-In offset at a different temperature. The colonies were left for one hour to allow bees and beetles to acclimate. This procedure was repeated eight more times (nine times total) at five different temperatures, exposing the colonies to thermostatic rooms that were descending in temperature successively, and then ascending in temperature (Table 7-1). The temperature of each room was determined using a digital thermometer (Acu-Rite, Jamestown, NY) placed in each room for an hour prior to data collection. Control colonies were established and remained in a dark room at room temperature (~24.8 ºC, Table 7-2). The control colonies were moved out of the room and back into the room after each beetle count to replicate the movement of treated colonies between the rooms set at different temperatures. Beetle counts for these colonies were conducted at the same time period the treatment colonies were counted to control for time period effects on beetle

presence in confinement sites. The control colonies permitted me to say that any discovered differences in the proportion of beetles in confinement sites were a function of descending/ascending temperature rather than the nightly time period at which the observations occurred.

Statistical Analysis

Because the total number of beetles found in confinement sites varied between colonies initially, the number of beetles in confinement sites in each colony was converted to proportion data relative to the original number of beetles in confinement sites at room temperature. All proportion data (the dependent variable) were transformed with arc-sine √ transformations prior to analyses. For the experimental group data, the data were analyzed using a simple linear regression model (JMP 2008), recognizing descending or ascending temperature as main effects, and the transformed proportions of beetles in confinement sites as the response variables. For the control group data, the transformed proportions were analyzed using a one-way ANOVA (JMP) recognizing observation time period (20:30-04:30) as the main effect. Untransformed means are reported in this chapter.

Results

The proportion of SHBs in confinement sites was positively correlated with temperature when ambient temperatures were descending ($y = 0.08x – 0.57$; $t = 6.67$; $P < 0.01$; Figure 7-2) and ascending ($y = 0.03x – 0.18$; $t = 2.92$; $P < 0.01$; Figure 7-3). Also, temperature accounted for a significant portion of the variance in SHB proportions in confinement sites when temperatures were descending ($R^2 = 0.72$; $F = 45.78$; $df = 1, 18$; $P < 0.01$, Figure 7-2) or ascending ($R^2 = 0.32$; $F = 8.56$; $df = 1, 18$; $P < 0.01$, Figure 7-3). Finally, the slope of the regression line for the proportion of SHBs exiting confinement sites when the temperature was descending was ~2.7× that for when the temperature was ascending (0.08 and 0.03, respectively). The slope describes

the rate at which the SHBs exit the confinement sites and enter the cluster. For the control colonies, the proportion of SHBs in confinement sites at each time period did not differ significantly (Table 7-2).

Discussion

The data suggest that there is a regular pattern followed by SHBs when entering the thermoregulatory cluster of bees as the ambient temperature drops with the reciprocal being true when the ambient temperature rises. However, the difference in the slopes (0.08 and 0.03 for decreasing and increasing temperatures, respectively) suggests that the SHBs exit the confinement sites and cluster with the bees when the temperature decreases more quickly than they reoccupy the confinement sites when the temperature increases. Furthermore, the respective R^2 values (0.72 and 0.32 for decreasing and increasing temperatures, respectively) suggest that SHBs entered the cluster more regularly than they reoccupied the confinement sites.

One possible explanation for the slower return of SHBs to confinement sites is that the beetles were being confined within the cluster of bees. This may not have happened, however, given that age related polyethism (age-based division of labor) in bees does not follow the same pattern when bees cluster (Hepburn and Radloff 1998, Stabentheiner et al. 2010), and confinement behavior is governed by such a division of labor (Ellis et al. 2003c). That said, it is difficult to know exactly what happens within bee clusters since inner clusters cannot be observed directly. SHBs seem to cluster in cells with bees (Ellis et al. 2003b), but other possible interactions between beetles and bees in winter clusters remain unknown.

Another potential explanation is that the cluster of bees may not heat up as rapidly as it cools down. Thus, the bees themselves would remain in the cluster and, likewise, the SHBs would exhibit a slower return to confinement sites as they wait for the cluster temperature to increase. Furthermore, the discrepancy between the R^2 values may be the result of variation

between colonies in the bees' ability to warm up following exposure to low ambient temperatures, so the SHBs within the clusters did not leave and reoccupy confinement sites in a pattern that was consistent between the four colonies.

The data from control colonies suggest that the proportion of SHBs confined does not vary throughout the night. This is consistent with previous results, which suggest that honey bees are capable of keeping a moderate number of SHBs in confinement sites "indefinitely" (Ellis et al. 2003a). The present study was performed at night, when SHBs are more active within the hive (Ellis et al. 2003a). Previous investigators have found that there are more bee "guards" keeping SHBs confined at night, which also may help explain why the number of SHBs in confinement sites remained constant throughout the study and why no SHBs were found on the combs (Ellis et al. 2003a, 2004a, b).

The findings suggest that SHBs have highly-developed adaptation for cold-weather tolerance facilitated through exploitation of their host bees' thermoregulatory capabilities. The behavioral tug-of-war that likely occurs between SHBs and bees in bee clusters should be investigated to understand this adaptation in detail. These behaviors would be studied best through comparisons of the clustering behavior in colonies of European and African races of honey bees. Through such studies, I may be able to elucidate another adaptation that enables the SHB to integrate into honey bee colonies.

Table 7-1. Proportion of beetles confined at each temperature in experimental colonies (colonies exposed to changing temperatures). Data are mean ± SE (*n*) proportion of beetles in confinement sites of each colony.

Time	Temperature (ºC)	Proportion Beetles
20:30	24.5	0.829 ± 0.075 (4)
21:30	21.6	0.938 ± 0.063 (4)
22:30	16.1	0.231 ± 0.149 (4)
23:30	9.1	0.094 ± 0.094 (4)
00:30	6.6	0 ± 0 (4)
01:30	9.4	0.108 ± 0.079 (4)
02:30	15.9	0.088 ± 0.030 (4)
03:30	21.7	0.219 ± 0.129 (4)
04:30	24.3	0.538 ± 0.197 (4)

Table 7-2. Proportion of beetles confined at each temperature in control colonies (colonies remaining at room temperature throughout study). Data are mean ± SE (*n*) proportion of beetles in confinement sites of each colony.

Time↓	Temperature (ºC)	Proportion Beetles
20:30	24.6	0.808 ± 0.048 (3)
21:30	24.8	0.891 ± 0.003 (3)
22:30	24.7	0.891 ± 0.064 (3)
23:30	24.9	0.832 ± 0.039 (3)
00:30	24.9	0.856 ± 0.032 (3)
01:30	24.9	0.870 ± 0.097 (3)
02:30	24.8	0.780 ± 0.081 (3)
03:30	24.7	0.821 ± 0.055 (3)
04:30	24.7	0.869 ± 0.082 (3)
ANOVA→		F = 0.39; df = 8, 18; P = 0.91

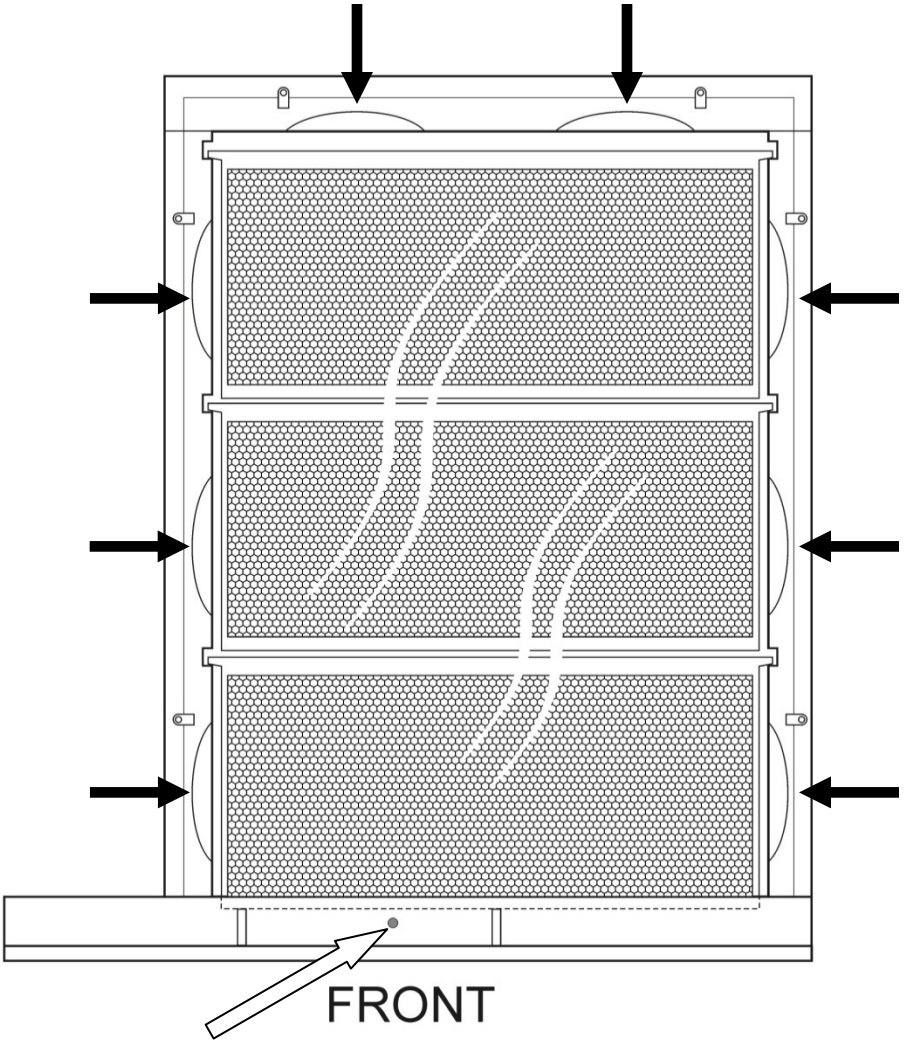

Figure 7-1. Diagram of experimental observation hive. The white arrow indicates the location where beetles were introduced into the observation hive while the black arrows indicate the location of the eight grooves (confinement sites) located on the periphery of the observation hive. The confinement sites were present on both sides, totaling 16 sites.

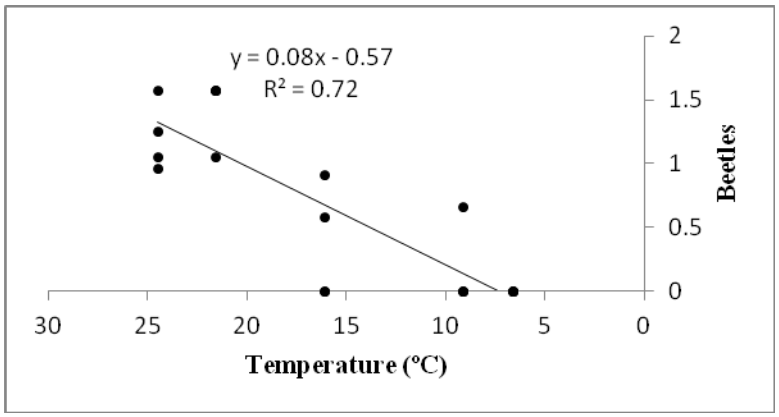

Figure 7-2. The proportion of beetles in confinement sites as the ambient temperature decreased. The line is the best-fit line. The Y-axis beetle proportions are arc-sine √ transformed. For clarity, the X-axis has been reversed to show decreasing temperatures.

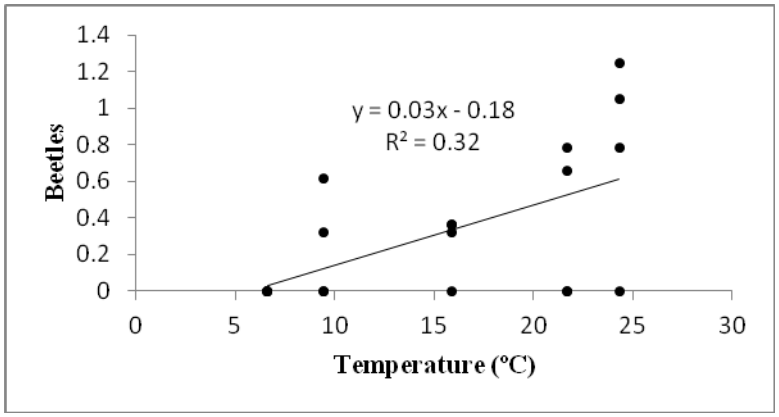

Figure 7-3. The proportion of beetles in confinement sites as the ambient temperature increased. The line is the best-fit line. The Y-axis beetle proportions are arc-sine √ transformed.

CHAPTER 8
DISCUSSION

The panic that accompanied the discovery of the SHB in North America in the late 1990s gradually was followed by a reluctant acceptance of the pest as a way of life and a reason for beekeepers to alter their management techniques. Paranoia that followed the SHB after its introduction largely has waned. In a survey of 571 beekeepers, less than 2% reported SHBs as a source of colony collapse (vanEngelsdorp et al. 2010).

Regardless, the SHB remains a problem in many U.S. apiaries (including Hawaii), and recent evidence suggests that, similar to *V. destructor*, it has the ability to carry and transmit honey bee pathogens (Eyer et al. 2009a, b, Schäfer et al. 2010a). Therefore, the study of SHBs as an applied problem is warranted. Basic research of this pest is fundamental to solving applied problems (see Ellis 2002). For example, the discovery of hiding and confinement behavior (Lundie 1940, Neumann et al. 2001, Ellis 2005) has guided control strategies, including the design of adult SHB traps and their placement within colonies (Elzen et al. 1999, West 2004, Hood 2006, Cobey 2008, Levot 2008, Neumann and Hoffmann 2008). Also, studies of SHB feeding and attraction to colonies (Lundie 1940, Schmolke 1974, Ellis et al. 2002a, Keller 2002, Hood and Miller 2003, Suazo et al. 2003, Torto et al. 2005, 2007b, Arbogast et al. 2009, 2010). The discovery of the SHB-vectored yeast *Kodamaea ohmeri* (Torto et al. 2007a), has also led to the development of lures for attracting and capturing SHBs (Arbogast et al. 2007, Torto et al. 2007c, Nolan and Hood 2008).

The results of the research presented herein indicate that the SHB is unique among nitidulids in its adaptive leg morphology (Chapter 3), treatment by honey bee hosts (Chapter 4), attraction to bee colony odors (Chapter 2), and hiding and subsequent confinement by honey bees (Chapter 6). Furthermore, the SHB exhibits adaptive behaviors which allow it to survive

low temperatures (Chapter 7) and, potentially, avoid recognition by hosts (Chapter 5), though the latter requires further investigation.

Re-examination of the Low Number of Honey Bee Nest Symbionts

One of the motivations for this research was the perceived lack of symbionts in nests of social bees compared to that in ant and termite nests (Wilson 1971, Kistner 1982). However, an analysis of the diversity of arthropods found in bee nests (Chapter 1) provides evidence to the contrary, that social bee nests do, in fact, host many symbionts. Furthermore, the number of symbiont species found in some bee nests may be comparable to that found in ant and termite nests (Kistner 1979). However, the degree of specialization (morphological, behavioral, and chemical) is relatively low among bee nest symbionts (Wilson 1971) (Tables 1-1, 1-2). A discussion of why honey bees have few nest invaders is warranted if one is to understand how SHBs, and melittophiles in general, have been able to overcome obstacles to inhabiting honey bee nests.

One hypothesis offered to explain the scarcity of symbionts in honey bee nests is that honey bees often nest in trees (Wilson 1971), though many nest in the ground as well (Seeley 1985). Therefore, a potential pest first would have to become adapted to arboreal life to become adapted to the nest environment. Many relatives of the SHB are associated with fungal mats and sap flows at trees (Cease and Juzwik 2001), so the association of SHBs with honey bee nests may have developed through regular contact.

Another explanation offered for the lack of honey bee symbionts is that the colonies are kept relatively free of debris through the cleaning behaviors of the nest inhabitants (Wilson 1971, Kistner 1982). Cavity-nesting honey bees regularly remove debris and waste from their colonies (Seeley 1982), and these habits minimize the opportunity for scavengers to invade. This is done because the food-stuffs (pollen and nectar) on which bees feed is concentrated, thus leaving little

refuse and resources for scavengers that may become pests eventually. Scavenging arthropods compose the second largest group of symbionts found within ant and termite nests, nests that often are full of debris (Kistner 1982).

Finally, honey bee nests have limited entrances, which reduce the number of potential nest invaders. Furthermore, the nest entrances are guarded perpetually by a guild of guard bees that are physiologically specialized for the task (Breed et al. 2004). Ants and termites also excel at nest defense, but they have multiple entrances to their nests, which may represent multiple opportunities for nest invasion. Of the hypotheses offered to explain why honey bees have fewer symbionts, the efficient defense of limited entrances is, perhaps, the most probable.

SHBs are able to overcome hive defenses in a variety of ways (Chapter 1). I hypothesized that SHBs are able to penetrate and persist within honey bee colonies as symbionts as a result of morphological, behavioral, and chemical adaptations. It is clear that they have morphological adaptations (Chapter 3) that assist them when entering the nest. They can withstand attacks by defensive guard bees at the entrance by retracting their appendages beneath their body (Neumann et al. 2001, Neumann and Elzen 2004), a behavior that is accommodated by specialized morphological adaptations (Chapter 3). Specifically, SHBs have grooved femora that likely accommodate the flattened tibiae to allow for more complete retraction, though this has not been tested behaviorally. Also, SHBs have various behavioral adaptations (Chapters 2, 4, 6, 7) that are unique among related species that were used. Once within the colony, the SHBs find cracks and crevices around the colony in which to hide from the bees, with 60% of the beetles hiding within 15 minutes (Neumann and Elzen 2004, Chapter 6). Thus, although SHBs are treated defensively by guard honey bees (Chapter 4), the majority are able to enter the nest rather quickly (Chapter 6).

What is not clear is whether they use chemical means to integrate into colonies. In Chapter 6 I found that SHBs find the confinement sites more quickly if they have not been washed, suggesting that there is an attractive chemical or chemical blend present at these sites, potentially released by SHBs that previously occupied the sites. Furthermore, in Chapter 5 I found that SHBs reared on a presumably more nutritious diet of honey, pollen, and protein supplement produce a different cuticular chemical profile than that produced by SHBs reared on the less nutritious sugar-water diet. That more lipids are present on the cuticle of SHBs reared on an essentially lipid-free diet is intriguing, and may suggest an ability to "blend in" after feeding on hive products (particularly stored pollen). However, the differences in cuticular profiles do not appear relevant at the honey bee nest entrance. Thus, neither study (Chapters 5, 6) provides conclusive evidence for chemical use in SHBs. That said, were SHBs to lack either the morphological or behavioral traits that they possess, they likely would not be able to thrive within honey bee nests as they do.

Similarly, the death's head hawkmoth, *Acherontia atropos*, has a suite of adaptations that allows it to enter and feed on nectar and honey within honey bee colonies. However, this moth utilizes all three methods of integration. Morphologically they have a thickened cuticle that allows it to endure honey bee stings (Moritz et al. 1991). Behaviorally, the moth emits a squeaking sound which is thought to reduce the defensive behavior of its hosts (Moritz et al. 1991). Finally, the moth endogenously produces a cuticular chemical profile that allows it to blend into the colony (Moritz et al. 1991). The moth requires each method of integration, as the persistent odor allows it to blend in and, if noticed, it can emit the appeasing vibration and, if that fails, it is still protected by a thickened cuticle.

The Future of SHB Research

Aggregation pheromones, which are ubiquitous among Nitidulidae (Bartelt et al. 1991, 1992, 1994, 1995, 2004, Dowd and Bartelt 1993, Williams et al. 1993, Nardi et al. 1996, Cosse and Bartelt 2000), have yet to be found in SHBs, although results reported in Chapter 6 suggest that they may exist. Torto et al. (2007a) report that no such pheromones have been detected after several bioassays. Therefore, presenting the SHBs with an appropriate context to express such pheromones may be needed to discover the compound. Aggregation pheromones have been utilized extensively to trap various insect pests for monitoring and control (Phillips and Throne 2010), and elucidating such a pheromone for SHBs would be exceedingly useful in SHB management practices. Regardless, the data presented in Chapter 6 suggest that such a pheromone may exist and this is further evidence that SHBs use chemical cues to integrate into honey bee colonies. Furthermore, testing the effects of the potential pheromone on heterospecific beetles would be worthwhile.

The use of other chemical cues also may exist in SHBs. For example, although the implications of the data from Chapter 5 remain unclear, the cuticular profiles of SHBs may be beneficially altered as a consequence of their diet or surroundings. This chemical profile plasticity might be relevant to survivability within the honey bee nest and may shed light on the confinement behavior of honey bees and the phenomenon of captive SHBs being fed by honey bees (Ellis et al. 2002b). Furthermore, the evolution of confinement behavior itself remains a mystery (Chapter 6). Future chemical studies will help answer many of these remaining questions.

Recent evidence indicates that SHBs are capable of mechanically transmitting honey bee pathogens (Eyer et al. 2009a, b, Schäfer et al. 2010a). Following the consumption of infected honey bee brood, SHBs were found to be infected with sacbrood virus (Eyer et al. 2009b),

deformed wing virus (Eyer et al. 2009a), and *Paenibacillus larvae* (Schäfer et al. 2010a). The latter is a bacterium that causes American foulbrood in honey bees. Furthermore, the study by Schäfer et al. (2010a) suggests that such infections can be transmitted to healthy honey bee colonies. Nitidulids, including SHBs, are known to carry different fungi and some have the potential to transmit diseases between secondary organisms (Cease and Juzwik 2001, Torto et al. 2005, Benda et al. 2008). The potential for SHBs to carry fungal and viral pathogens is especially important in light of recent evidence which suggests that there is a correlation between the fungal microsporidian genus *Nosema*, an invertebrate iridescent virus, and the recent phenomenon colony collapse disorder (CCD) (Bromenshenk et al. 2010). Thus, if SHBs are able to transmit these two pathogens, and these pathogens are involved in CCD, then the beetles may be contributing to these collapses.

Relevant to all issues regarding SHB problems in apiaries is a means of measuring dispersal patterns and population dynamics. In Chapter 6 I investigated the intracolonial distributions of various beetle species, including SHBs. However, SHB infestations remain largely enigmatic in that they are very difficult to predict and can vary and change within an apiary almost daily. Mathematical modeling and, perhaps, epidemiological techniques may aid in uncovering intercolonial SHB distributions.

Also, the research presented in this dissertation, as well as that of others, focuses on adult morphology, behavior, and chemistry. It would be worthwhile to repeat many of the experiments using immature stages. Little is known about interactions between honey bees and larvae beyond the fact that some honey bee races can remove them from colonies (Neumann and Härtel 2004, Spiewok and Neumann 2006a). Might the SHB larvae use similar techniques as the adults to get fed by the bees (Ellis et al. 2002b)? Do they possess any morphological adaptations which

accommodate their persistence within the colony as the adults do (Chapter 3)? Lundie (1940) suggests that the protuberances act to prevent the larvae's drowning in the honey. However, this is based entirely on anecdotal evidence and has yet to be demonstrated. Furthermore, though they can be removed effectively by African honey bee races, do the SHB larvae have any behavioral methods of avoiding honey bee aggression like the hiding behavior in the adults (Chapter 6)? Also, are there any other stages that can overwinter within or around honey bee colonies besides the adults (Chapter 7)? Finally, do SHB larvae produce chemicals to attract other larvae, as is suggested in adults in Chapter 6, and is evidenced in larvae by the common larval masses on foodstuffs; or does their cuticular profile change based on their diet, perhaps to their own advantage (Chapter 5)? Such investigations would provide further insight into the relationship between SHBs and their honey bee hosts.

A final project on SHBs would compare the survivability, longevity, and reproductive capacity of several beetle species (as per Chapters 2, 3, 4, 6) on various diets of honey bee and non-honey bee foodstuffs. Though I initiated this project multiple times, the developing larvae were overtaken repeatedly by the mold mite *Tyrophagus putrescentiae* (Acari: Acaridae), which has shown the ability to disrupt the SHB life cycle (Ellis et al. 2010). It would be interesting to investigate whether these other (non-SHB) beetle species, which include species that have been found in honey bee nests and beetles that have not, are able to survive and reproduce on the honey bee products.

SHBs provide an excellent opportunity for studies on the development of inquilinism, as they are highly integrated into bee nests but do not complete their entire life cycle within the nest. Furthermore, they are not obligate symbionts and can survive in nests of alternative hosts (Ambrose et al. 2000, Stanghellini et al. 2000, Spiewok and Neumann 2006b, Hoffmann et al.

2008, Greco et al. 2010) as well as on fruits (Ellis et al. 2002a, Keller 2002, Buchholz et al. 2008, Arbogast et al. 2009, 2010). As such, SHBs can be considered a potential step toward obligate symbiosis within honey bee nests, and studies of a species with such plasticity can yield interesting results with ecological and evolutionary ramifications.

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BIOGRAPHIAL SKETCH

Edward Blake Atkinson was born in Ocala, Florida to a musician mother and a biologist father. He received a bachelor's degree from the Florida State University in 2005 with a double major in physics and philosophy and a minor in mathematics. Shortly after graduating, Eddie married Crystal Taylor who also is an entomology graduate student at the University of Florida. Soon after enrolling in graduate school, they had a daughter, Ella (now 3) and a son, Eli (now 1). Eddie plans on pursuing research on honey bee pests and inquiline integration.