

The Ecology and Control of Small Hive Beetles (*Aethina tumida* Murray)

Volume I

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Chapter 7: Ellis J.D., Hepburn H.R., Elzen P.J. 2004. Confinement of small hive beetles (*Aethina tumida* Murray) by Cape honeybees (*Apis mellifera capensis* Esch.). *Apidologie*, in press.

Chapter 8: Ellis J.D., Hepburn H.R., Ellis A.M., Elzen P.J. 2003. Prison construction and guarding behaviour by European honeybees is dependent on inmate small hive beetle density. *Naturwissenschaften*, 90: 382-384.

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Abstract

The small hive beetle (*Aethina tumida* Murray) is an endemic scavenger in colonies of honey bee (*Apis mellifera* L.) subspecies inhabiting sub-Saharan Africa. The beetle only occasionally damages host colonies in its native range and such damage is usually restricted to weakened/diseased colonies or is associated with after absconding events due to behavioral resistance mechanisms of its host.

The beetle has recently been introduced into North America and Australia where populations of managed subspecies of European honey bees have proven highly susceptible to beetle depredation. Beetles are able to reproduce in large numbers in European colonies and their larvae weaken colonies by eating honey, pollen, and bee brood. Further, adult and larval defecation is thought to promote the fermentation of honey and large populations of beetles can cause European colonies to abscond, both resulting in additional colony damage. The economic losses attributed to the beetle since its introduction into the United States have been estimated in millions of US dollars.

Although beetles feed on foodstuffs found within colonies, experiments *in vitro* show that they can also complete entire life cycles on fruit. Regardless, they reproduce best on diets of honey, pollen, and bee brood. After feeding, beetle larvae exit the colony and burrow into the ground where they pupate. Neither soil type nor density affects a beetle's ability to successfully pupate. Instead, successful pupation appears to be closely tied to soil moisture.

African subspecies of honey bees employ a complicated scheme of confinement (aggressive behavior toward and guarding of beetles) to limit beetle reproduction in a colony. Despite being confined away from food, adult beetles are able to solicit food and feed from the mouths of their honey bee guards. Remarkably, beetle-naïve European honey bees also confine beetles and this behavior is quantitatively similar to that in African bees.

If confinement efforts fail, beetles access the combs where they feed and reproduce. Two modes of beetle oviposition in sealed bee brood have been identified. In the first mode, beetles bite holes in the cappings of cells and oviposit on the pupa contained within. In the second mode, beetles enter empty cells, bite a hole in the wall of the cell, and oviposit on the brood in the adjacent cell. Despite this, African bees detect and remove all of the infected brood (hygienic behavior). Similarly, European bees can

detect and remove brood that has been oviposited on by beetles. Enhancing the removal rate of infected brood in European colonies through selective breeding may achieve genetic control of beetles.

Additional avenues of control were tested for efficacy against beetles. Reducing colony entrances slowed beetle ingress but the efficacy of this method probably depends on other factors. Further, the mortality of beetle pupae was higher when contacting species of the fungus *Aspergillus* than when not, making biological control an option. Regardless, no control tested to date proved efficacious at the level needed by beekeepers so an integrated approach to controlling beetles remains preferred.

The amalgamation of the data presented in this dissertation contributed to a discussion on the beetle's ecological niche, ability to impact honey bee colonies in ways never considered, and the ability to predict the beetle's spread and impact globally.

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“It is surprising that the insect to be described in this paper, although it is so common and can be so troublesome to the beekeeper, has not been mentioned previously in our apicultural literature.”

- A.E. Lundie, 1940

Chapter 1: An Introduction to the Study of Small Hive Beetles (*Aethina tumida* Murray)

Small hive beetles (*Aethina tumida* Murray) are native to sub-Saharan Africa where they are scavengers in colonies of African subspecies of honey bees (*Apis mellifera* L.). As is often the case in science, small hive beetles had remained relatively unstudied since they were first described over 140 years ago owing to their presumed lack of economic value/importance. However, for reasons that will be outlined extensively in this dissertation, small hive beetles have recently garnered much international coverage since they escaped their endemic range 5-10 years ago. Along with their spread around the world has come a landslide of studies outlining their potential impact on honey bee colonies outside of Africa.

Most investigations concerning small hive beetles have contributed, in part, to an overall understanding of the beetles' biology and natural history. However, there remain voids in our knowledge of this honey bee pest and it is for this reason that I explore the ecology of the small hive beetle. Through the original research reported in Chapters 2 - 13, I illuminate various aspects of the beetle's biology, behavior, and control. As an introduction to these investigations, I outline what currently is known about small hive beetles and introduce the original research published herein.

History

In 1940, A.E. Lundie (then a Research Apiculturist for the South African Department of Agriculture and Forestry) published information concerning a beetle that he observed to be a pest in honey bee colonies of South Africa. He suggested that his subject be called the 'small hive beetle' to distinguish it from another much larger beetle

(Scarabaeidae: *Hyplostoma fuliginus*) that was also commonly associated with bee hives in South Africa. The small hive beetle had remained unexamined until Lundie's studies on its biology, behavior, and control.

The small hive beetle (hereafter referred to as 'beetle' unless it is necessary to distinguish small hive beetles from other beetles) was first named and described in 1867 by Andrew Murray who was working in London (Murray 1867). Two specimens were sent to Murray from Old Calabar (in present day Nigeria) on the West Coast of Africa; but at that time, no reference was made to the beetle being associated with honey bee colonies (Lundie 1940). Since then, Grouvelle (1899) worked on the beetle's taxonomy but very little else was written concerning it after Murray's 1867 paper. Subsequently, the first record of the beetle's presence in South Africa was from a 1920 specimen collected in Durban. Because these beetles were occasional colony pests and only anecdotal knowledge on them existed, Lundie decided to investigate further and he began studying the beetles in 1931 (Lundie 1940). The manifestation of these studies was a 1940 paper by Lundie that dealt with various aspects of the beetle's ecology and biology including its distribution, life history, longevity, diet, number of generations, parasitism, and control. Through his work, Lundie demonstrated that the beetle was a colony nuisance that only occasionally damaged (or destroyed) host colonies of African subspecies of honey bees (Lundie, 1940).

M.D. Schmolke, in a 1974 thesis he submitted for a Certificate in Field Ecology at the University of Rhodesia (Zimbabwe), revisited the beetle with the intention of continuing where Lundie's studies had terminated. Schmolke's efforts furthered our knowledge on beetle biology with investigations into beetle distribution in infested hives, life cycle, sexual maturity and number of eggs, diet effects on oviposition, wandering period of larvae, photo-taxis of larvae and adults, sexual dimorphism, sex ratios, size of beetles, size of larvae, behavior reactions, predation on honey bee eggs, larvae and pupation, and various control measures. Together, Lundie and Schmolke's papers provided pivotal starting points from which all, current-day beetle work would be spawned.

Classification, Taxonomy, and General Description

Small hive beetles are members of the Coleopteran family Nitidulidae which are commonly referred to as 'sap beetles' since many are primarily saprophagous and mycetophagous (Habeck 2002). Some nitidulids live in flowers, although most live in decaying fruits, fermenting plant juices, and in fungi (Borror and White 1970; Scholtz and

Holm 1985; Habeck 2002; Picker et al. 2002). The primitive feeding habit of this family is believed to be the association with decaying organic matter, wood, and wood fungi with more derived nitidulid species being associated with flowers and pollen (Blackmer and Phelan 1995). Examples of diversity within the feeding habits of Nitidulidae are *Nitidula* sp. and *Omosita* sp., which breed in carrion, and members of *Carpophilus*, which can be major pests in stored products (Habeck 2002).

There are also members of this family that live in close association with various social insect groups and in some instances these relationships are symbiotic. Members of the genus *Epuraea* can live in *Bombus* nests (Parsons 1943) while *Amphotis* is closely associated with *Formicidae* (the relationship between *Amphotis* and *Formicidae* will be considered in Chapter 14)(Hölldobler and Wilson 1990; Habeck 2002). The larvae of *Brachypeplus auritus* Murray feed on wax and honey of wild *Trigona* colonies in Australia (Lundie 1940; Habeck 2002). Because of their wide range of diets, nitidulids are considered important scavengers of the insect world.

Murray's (1864) original list of essential characteristics of Nitidulidae includes "Ventral segments free, five in number, the first visible both at the middle and sides, some of the dorsal segments membranous. Antennae more or less clavate, but not geniculate. Tarsi five-jointed, in general dilated; fourth article the smallest, usually very minute. Anterior coxae transverse, not prominent; anterior cotyloid cavities transverse, oblique, more or less open, and tapering towards the outer side." Recent work on Nitidulidae taxonomy adds grooved metacoxae and antennal club with three antennomeres as characteristics that distinguish nitidulids from other coleopterans (Habeck 2002). The antennae of nitidulids, which have 11 antennomeres, are inserted between the eyes and base of the mandibles (Habeck 2002). The elytra are shortened, often exposing the last abdominal segments. The pronotum is shield-like and, in the case of small hive beetles, has two lateral lobes projecting toward the posterior end of the insect (Figure 1.1). Nitidulids, including small hive beetles, may be covered in a fine pubescence (Blatchley 1910 cited in Lundie 1940).

The genus *Aethina*, *sensu stricto*, which contains about 30 species, has its origins purportedly in Africa and extending through the continent to Asia, Australia, and the Orient (Andrew Cline, personal communication; Kirejtshuk and Lawrence 1999). It is, however, mostly Indo-Malayan (Kirejtshuk and Lawrence 1999) in its current distribution. Cline states that one of the most defining features for the genus, *sensu stricto*, is the presence of numerous pits along the basal margin of the pygidium. However, there may be

some discrepancy within the genus with members being misclassified (Michael Thomas, personal communication). The most authoritative paper concerning the *Aethina* complex remains the one by Kirejtshuk and Lawrence (1999) and these authors state that Lundie's 1940 paper is the most thorough investigation into the biology of the genus *Aethina*. Most members of this genus are mycetophagous and anthophagous (Kirejtshuk and Lawrence 1999).

Newly eclosed small hive beetles are light brown in color (Lundie 1940), becoming progressively darker (almost black) as sclerotization occurs. These color changes may occur in the pupal cell before the adult ecloses (Lundie 1940). Adult female (5.7 ± 0.02 mm) beetles are generally longer than males (5.5 ± 0.01 mm) (Mackay unpublished data cited in Schmolke 1974; Ellis et al. 2002) but both are nearly identical in width (~ 3.2 mm)(Ellis et al. 2002). Adult females (14.2 ± 0.2 mg) are also heavier than males (12.3 ± 0.2 mg)(Ellis et al. 2002) and occur in greater proportions of the population (Schmolke 1974; Neumann et al. 2001a; Ellis et al. 2002). Despite average general biometry, naturally occurring small hive beetles can vary greatly in size (Schmolke 1974), possibly depending on diet, climate, etc.

Hive beetle eggs are 1.4×0.26 mm ($l \times w$), arcuate, and pearly white in appearance (Schmolke 1974). Newly emerged larvae of the beetle have relatively large heads and numerous protuberances covering their bodies (Figure 1.2)(Lundie 1940). Larval growth rate varies depending on diet but Lundie (1940) and Schmolke (1974) state that the majority mature in 10-14 days. Upon full maturation, larvae will have reached a length and width of 9.5 mm and 1.6 mm respectively (Schmolke 1974). Early-stage pupae of the beetle are pearly white, having characteristic projections on the thorax and abdomen (Lundie 1940). Upon changing to adults, sclerotization darkens the pupae until final eclosion as an adult.

General Biology and Life History

Small hive beetles are endemic scavengers in honey bee colonies of sub-Saharan Africa (Lundie 1940; Schmolke 1974; Hepburn and Radloff 1998). Reports of their natural occurrence indicate beetle endemism in South Africa (Walter 1939a, b; Lundie 1940, 1952a, b; May 1969; Buys 1975), Uganda (Roberts 1971), Nigeria (Mustaers 1991), Zimbabwe (Schmolke 1974), Botswana (Phokedi 1985), Ethiopia, Kenya, Namibia, Eritrea

(Mostafa and Williams 2002), Angola (Rosário Nunes and Tordo 1960), Congo Republic (Castagné 1983), Zambia (Clauss 1992), Tanzania (Smith 1960; Ntenga 1970; Ntenga and Mugongo 1991), Central African Republic (Lepissier 1968), Senegal (N'diaye 1974), Guinea-Bissau (Svensson 1984), Ghana (Gorenz 1964; Adjare 1990), and the Democratic Republic of Congo (Aurelien 1950; Dubois and Collart 1950)(also cf. Hepburn and Radloff 1998)(Figure 1.3). However, beetle home range probably includes all of sub-Saharan Africa (Hepburn and Radloff 1998), certainly encompassing the natural occurrence of honey bees on the African continent.

In their native range, the beetles are not considered major economic pests to indigenous subspecies of honey bees although they may damage or even totally destroy the occasional weakened colony (Lundie 1940; Schmolke 1974; Anderson et al. 1973). Economically, they are often considered less important than the cosmopolitan greater (*Galleria mellonella*) and lesser (*Achroia grisella*) wax moths although they probably serve the same function (to clean up dead or weakened honey bee colonies) as do their lepidopteran counterparts (Lundie 1940; Hepburn and Radloff 1998). Most beetle damage stems from the feeding habits of adults and larvae which eat honey, pollen, and, preferentially, bee brood (Lundie 1940; Schmolke 1974; Elzen et al. 2000). However, as a secondary effect of adult and larval feeding, stored honey in a colony is rendered useless as it quickly fouls and ferments due to significant beetle populations, likely owing to beetle defecation (Lundie 1940; Schmolke 1974; Elzen et al. 1999; Hood 2000).

General beetle biology is fairly well understood. Upon eclosion from the ground, adult beetles search for honey bee colonies, probably identifying the host colony by a suite of olfactory cues (Elzen et al. 1999). Studies have shown that beetles fly before or just after dusk (Schmolke 1974; Elzen et al. 2000) and that odors from various hive products (honey, pollen) and adult bees are very attractive to flying beetles (Elzen et al. 1999). It is also possible that beetles are attracted to substances that beekeepers use in colonies. It has been demonstrated that beetle populations can be significantly higher in colonies containing patties made of vegetable shortening and sugar (which is a substrate often used to facilitate the delivery of antibiotics)(Elzen et al. 2002). Initial indications suggest that males are earlier fliers than females, or that they respond to fresh food sources more readily than do females (Elzen et al. 2000).

Upon entering the host colony, beetles seek out cracks and crevices where they hide from bee aggression (Schmolke 1974; Hepburn and Radloff 1998; Neumann et al. 2001b). Remarkably, at least some subspecies of African honey bees (in particular, the

Cape honey bee of South Africa, *A.m. capensis* Esch.) station guards around the cracks where beetles hide. The ‘prison’ guards keep the beetles confined to the cracks and out of the brood combs where there is an ample supply of honey, pollen, and brood which promote beetle reproduction (Hepburn and Radloff 1998; Neumann et al. 2001b). The confinement of beetles in prisons of propolis, resinous material collected from trees, has also been reported, perhaps implicating the use of propolis by bees in managing beetle outbreaks (Hepburn and Radloff 1998; Neumann et al. 2001b).

Mating behavior of small hive beetles (including whether female beetles mate one or multiple times) is not known but adult beetles do not appear to be sexually mature until about 1 week after eclosion. Regardless, if allowed to reproduce, female beetles will oviposit directly onto food sources such as pollen or brood combs (Lundie 1940; Schmolke 1974). Alternatively, female beetles may deposit irregular masses of eggs in crevices or cavities away from the bees (Lundie 1940) as female ovipositors are long and flexible, being perfectly designed to lay eggs in tiny, concealed places (Schmolke 1974). Schmolke (1974) speculated that a female beetle may lay 1,000 eggs in her lifetime although recent data suggests that the number of eggs produced in one female’s lifetime might be upwards of 2,000 (Somerville 2003). Lundie (1940) collected data on the incubation period of 1,299 eggs. The majority of these eggs had hatched by the 3rd day following oviposition; however, some eggs were still viable and hatched after 5 days. Humidity appears to be a crucial factor influencing hatching rates, as beetle eggs are prone to desiccation if exposed to circulating air and relative humidity below 50% (Schmolke 1974; Pettis unpublished data cited in Somerville 2003).

Hatching larvae immediately begin feeding on whatever food source is available including honey, pollen, and bee brood (Lundie 1940; Schmolke 1974; Elzen et al. 1999; Hood 2000) although they have demonstrated a preference for bee brood (Elzen et al. 2000). Lundie (1940) and Schmolke (1974) demonstrated that the maturation time for larvae is generally 10-14 days, although some were shown to feed for 29 days. Once the larvae have finished feeding, a ‘wandering’ phase is initiated where larvae leave the food source and migrate out of the colony to find suitable soil in which to pupate (Schmolke 1974). Apparently, larvae in this stage are remarkably resilient to climatic conditions and may even wander great distances to find suitable soil (Schmolke 1974).

Despite the fact that larvae may migrate some distance from the hive in an effort to find ideal soil, Pettis and Shimanuki (2000) showed that most beetle larvae, pupae and newly eclosed adults are found within 90 cm of the hive. Nearly 80% of the larvae burrow

down into the soil less than 10 cm from the soil surface but not generally more than 20 cm (Pettis and Shimanuki 2000). It has been suggested that soil type affects various aspects of beetle pupation biology (Lundie 1940; Schmolke 1974) because of larval vulnerability when burrowing into the soil. Lundie (1940) suggested that investigations would probably show the absence of beetles in certain geographical areas due to the physical or chemical nature of the soil. Schmolke (1974) partially tested this assertion and found that soil moisture, but not soil type, was correlated with pupation success possibly indicating that larvae need moist soils in order to pupate successfully.

Once larvae cease burrowing, they construct a smooth-walled, earthen cell in which they pupate (Lundie 1940). Lundie (1940) demonstrated that the period of time spent in the ground pupating can vary greatly depending on factors such as soil temperature, etc. However, he maintained that the majority of adults eclose after being in the soil 3-4 weeks. Upon adult eclosion, the entire life cycle begins again. The turnover rate from egg to adult can be as little as 4-6 weeks; consequently, there may be as many as 6 generations in a 12-month period under moderate US and South African climatic conditions (Somerville 2003).

Occurrence Outside of Sub-Saharan Africa

In June of 1998, unidentified specimens of adult and larval beetles were sent to M.C. Thomas of the Florida Department of Agriculture for identification after it was discovered that these beetles were destroying colonies of European-derived honey bees in east-central Florida, United States (Elzen et al. 1999; Hood 2000). The specimens were positively identified as small hive beetles. Although this was the initial identification of the pest outside of sub-Saharan Africa, the beetles were later positively identified from earlier specimens collected in Charleston, South Carolina in November of 1996 (Hood 2000). Subsequently, the beetles were found in Georgia and North Carolina in 1998 and anecdotal evidence suggests that the beetle may have been present in Georgia as early as 1996 (Evans et al. 2003).

A record of their movement through the US indicates that small hive beetles were found in 4 states in 1998 (Florida, South Carolina, Georgia, North Carolina), 9 additional ones in 1999 (New Jersey, Maine, Pennsylvania, Minnesota, Iowa, Wisconsin, Massachusetts, Ohio, Michigan), 6 additional in 2000 (Louisiana, New York, North Dakota, Tennessee, Indiana, Vermont), 6 additional in 2001 (Maryland, Virginia,

Delaware, Illinois, Missouri, Mississippi), 3 additional in 2002 (Arkansas, Alabama, Kentucky), and 1 additional in 2003 (West Virginia)(Figure 1.4; Patti Elzen, personal communication).

How the beetle arrived in the US has been the subject of much speculation. The US restricts importation of honey bees from other countries so presumably the beetles did not enter the US with package bees or queens. Hood (2000) suggests that beetles may have crossed the Atlantic Ocean on cargo ships as Charleston, South Carolina (one of the earliest places identified as having beetles) is home to a large, international port.

Mitochondrial DNA (mtDNA) analyses of beetles in the US and South Africa indicate that there are two distinct haplotypes of beetles in the US and at least 13 in South Africa (Evans et al. 2000). The two US haplotypes matched those found in South Africa although the data did not allow a precise estimate of the point of origin. Both haplotypes were found across and within several geographic regions, possibly owing to a single introduction. However, a broad survey across the beetle's new range revealed significant heterogeneity in haplotype frequencies, which could have resulted from multiple introductions (Evans et al. 2000). In the end, the mechanism of beetle introduction into the US remains unclear.

In a more recent effort, findings by Evans and colleagues (2003) suggest that there is limited beetle movement between apiaries but that there may have been different invasion histories of the two haplotypes, with the beetles currently intermixing. In Hood's 2000 review, the beetle had only been discovered in 12 states. Now in 2003, the beetle has been positively identified in 29 states (Mostafa and Williams 2002; P. Elzen personal communication), indicating the rapid spread of beetles through the US. The beetles' natural range expansion, and movement of infested managed honey bee colonies, package bees, and empty beekeeping equipment are facilitating their spread (Hood 2000).

Small hive beetles have also been discovered in Manitoba, Canada in 2002 where they arrived with beeswax that was imported from the US (Central Science Laboratory National Bee Unit 2003). Despite the beetle's presence in Canada, it has, as of yet, failed to establish and cause serious damage to European bees in that country. The beetle was also discovered in Egypt (Figure 1.3) in June 2000 (Mostafa and Williams 2002) and this is the first record of the beetle being found north of the Sahara although it was probably transported there by 'unnatural' causes such as beekeeper-assisted migration of colonies. In October of 2002, the small hive beetle was formally identified in New South Wales, Australia and later in Queensland (Somerville 2003)(Figure 1.5); however, it is not known

how the beetle arrived in Australia. Spreading faster around the world than the beetle itself are fears that globally, European honey bees are in jeopardy (Waite and Brown 2003).

Impact in Introduced Regions

The biological effects of small hive beetles on honey bee colonies of European-descent in the new world are not altogether different from those on colonies in their endemic range. Beetle damage in European colonies follows the characteristic 1) beetle invasion into colonies, 2) population build-up of beetles, 3) reproduction of beetles, 4) significant damage to brood, pollen, and honey stores by scores of feeding larvae, 5) mass exodus of larvae from the hive, 6) pupation in the soil, and 7) eclosion as adults and subsequent re-infestation of colonies (Sanford 1998a, b; Elzen et al. 1999, 2000; Hood 2000).

Lundie (1940) and Schmolke (1974) report these same effects of beetles in colonies of African subspecies of honey bees. There does, however, appear to be a fundamental difference between beetle effects on African and European colonies. Beetle damage in Africa is almost solely restricted to weakened or diseased colonies (Lundie 1940; Schmolke 1974; Hepburn and Radloff 1998) while in its introduced range, beetles may damage weak and strong colonies alike (Elzen et al. 1999; Hood 2000). In fact beetle ability to destroy entire apiaries in the US is well documented (Hood 2000). In its inaugural year in Florida, the beetle caused an estimated \$ US 3 million damage to the beekeeping industry (P.J. Elzen, personal communication). It is not uncommon to hear of thousands of colonies lost to commercial beekeepers, who then discard infested equipment (Somerville 2003).

Beetle damage to living colonies is not the only loss experienced by beekeepers. Adult and larval beetles can be a significant problem in the honey house (Lundie 1940; Schmolke 1974; Eischen et al. 1999a; Hood 2000). As a result, beekeepers realize the necessity of extracting honey quickly and moving the equipment out of honey houses to discourage ensuing build-up of beetle larvae (Hood 2000). Further, stored supers of honey or supers containing pollen residues are prime targets for beetle reproduction and subsequent damage.

The queen and package bee industry is also negatively affected in areas where beetles occur. The potential for beetles traveling in queen cages and packages has sparked such concern that scientists have tried to devise beetle controls for traveling packages

(Baxter et al. 1999). As a result, initial movement of bees and queens from areas having beetles to areas ‘free’ of beetles dropped drastically. Beekeepers did not want to buy bees and queens from areas having beetles, and rightly so since it has been shown that the initial introduction of beetles into Manitoba occurred on shipped beeswax (Central Science Laboratory National Bee Unit 2000) indicating that beetles can travel on hive ‘products’ (possibly including queens and package bees). Australia is already feeling the impact of fears associated with beetles spreading in queen cages; Canada has shut its border to the importation of queens from Australia, which was a major export commodity for Australian beekeepers (T. Weatherhead, personal communication).

There are still greater fears associated with the spread of small hive beetles in introduced regions. Studies have shown that beetles can complete entire life cycles in bumble bee colonies of North America (Stanghellini et al. 2000; Ambrose et al. 2000). Although this work was done *in vitro* under controlled conditions, the findings generated many concerns regarding the impact of beetles on non-*Apis* native or beneficial insects. For example, *Trigona* (a native, stingless bee in Africa, Asia, and Australia) stores honey and pollen (much like species of *Apis*) possibly making it an ideal candidate for non-specific beetle invasion. Further, Asia is rich in *Apis*-biodiversity which may be susceptible to damage caused by beetles. Therefore, the potential impact of beetles on global bee-biodiversity alone is a possibility that can only be paralleled by the spread of *Varroa destructor* Anderson and Truemann (a parasitic, hemolymph-feeding mite which has caused world-wide damage to various subspecies of honey bees).

The impact of beetles in introduced regions may also manifest itself in the commercial fruit production industry. Eischen et al. (1999b) studied alternate feeding habits on various fruits in the absence of bee hive products. They found beetles to be most attracted to cut or whole cantaloupe (= ‘spanspek’ in southern African English) and mature larvae were seen on some of the other tested fruits (Eischen et al. 1999b). In fact, entire beetle life cycles were completed on the tested fruit, raising the possibility that in the absence of bee hives, beetles may be sustained on fruits. Despite this, beetles have never been identified on fruits in the wild, although Eischen and co-workers clearly showed that the possibility remains (Eischen et al. 1999b).

Introduction to the Research Presented in this Dissertation

Because the study of small hive beetles is in its relative infancy, a review of the relevant literature will show that information on beetles is incomplete and sometimes inconsistent. The fault does not lie with entomologists because they have had to begin anew when the beetle escaped its native range. Even Lundie and Schmolke's original and thorough studies left fundamental questions unanswered.

Elementary information regarding beetle biology is understood; however there is a great deal that is unknown about the newest pest facing honey bees. A more thorough exploration of beetle biology will allow one to 1) understand the beetles' natural reliance on honey bee colonies and need for tightly regulated environmental conditions, 2) predict its effects on non-African honey bees and non-target species, 3) predict its spread outside of its native range, and 4) possibly develop control measures for the pest.

Further, virtually nothing is known about intra-colonial beetle and honey bee interactions. As already discussed, Hepburn and Radloff (1998), Neumann et al. (2001b), and Solbrig (2001) noted that subspecies of African honey bees will 'encapsulate' beetles in prison-like structures made of propolis. The authors suggest that the confinement behavior may be an important resistance mechanism of African honey bees toward small hive beetles. Apart from these studies, very little else is known about what beetles do when they enter colonies and how bees deal with them. It is, therefore, essential that confinement of beetles be studied in greater detail to determine if the behavior is 1) present in European bees, 2) essential to the relative immunity of African bees to beetles, 3) an initial defense of European and African bees against invading beetles, or 4) a more general defense by honey bees against small colony intruders. Further, studying small hive beetle behavior in honey bee colonies will 5) illuminate the apparent symbiotic relationship both insects share and 6) place this relationship in context with those of other arthropods that inhabit various social insect colonies.

Obviously, understanding beetle biology and behavior inside of bee hives should ultimately lead one to develop efficacious controls for the menace. This avenue of research has not gone unexplored. Lundie (1940) and Schmolke (1974) both investigated a number of possible control measures for the beetle, ranging from the use of chemicals to the use of larval trapping devices. Chemical controls are becoming less popular today as fears concerning their impacts on humans escalate. Because of this, investigations into the efficacy of various 1) cultural, 2) behavioral, and 3) biological controls on beetle populations are not only needed, but are essential if one is to find a suitable control

candidate or possibly employ integrated pest management (IPM) schemes to effectively control beetles and/or eliminate beetle-associated damage.

The bulk of this thesis is divided into three main sections each dealing with a different avenue of small hive beetle research. In the three sections, I report original research that answers fundamental/focused questions regarding: the biology of small hive beetles (Section I: Chapters 2 - 5), intra-colonial interactions between beetles and honey bees (Section II: Chapters 6 - 10), and beetle control schemes (Section III: Chapters 11 - 13). The discoveries reported in each chapter contribute to the General Discussion (Chapter 14), which constitutes a holistic approach to answering the major topics of the thesis outlined as numbered points above.

Section I: Small hive beetle biology

Lundie (1940) and Schmolke (1974) are the original pioneers of small hive beetle research and, as already noted, each focused most of their efforts on studying beetle biology. Most scientists suggest that beetles only affect honey bees of European-decent but do little to African ones in their native range. Further, there has been a general consensus among those working with small hive beetles that only the larval stage presents a direct threat to honey bee colony health and in certain circumstances, European colonies can host thousands of adult hive beetles without suffering visible side effects (Wenning, 2001); however, no quantitative study has tested any of these assertions. In **Chapter 2**, I report the results of an intercontinental, quantitative study of the productivity of artificially beetle-infested or non-infested Cape and European honey bee colonies in an attempt to identify the actual impacts of adult beetles on honey bees in their native and introduced ranges. While conducting the study reported in Chapter 2, I collected observations on beetle behavior during European honey bee clustering and absconding events and on beetle oviposition. These behavioral observations are reported in **Chapter 3**.

Studies on the longevity of small hive beetle adults are few and conflicting and it is unclear how longevity is related to different food regimes and reproductive success, which is the major factor affecting the economic impact of beetles. Indeed, beetles are provided a range of diets in their native habitat (brood, pollen, honey), the reproductive effects of which are not yet known. Further, Eischen et al. (1999b) stated that small hive beetles can feed and reproduce on fruits, raising the possibility that beetle impacts may not be limited to honey bee colonies. In **Chapter 4**, I report on the longevity and reproductive success of newly emerged adult small hive beetles assigned different, natural diets. The pupation

success of larvae reared on the same diets as their parents and sex ratios of the resulting adults were also analyzed. The data shed light on the reproductive success and life history of small hive beetles telling us if small hive beetles are obligate or facultative scavengers of honey bee colonies; the possibility of their survival outside of honey bee colonies; and their longevity as adults (which may be crucial for beetle reproduction).

After feeding on various foodstuffs found in honey bee colonies, beetle larvae exit the colonies and pupate in surrounding soils (Lundie 1940; Schmolke 1974; Pettis and Shimanuki 2000). How different soil types affect various aspects of beetle pupation biology was considered long ago (Lundie 1940; Schmolke 1974) because of larval vulnerability when burrowing into the soil. Further, Lundie (1940) reasoned that investigations would probably show the absence of beetles in certain geographical areas due to the physical or chemical nature of the soil. Schmolke (1974) partially tested this assertion and found that soil moisture, but not soil type, was correlated with pupation success but his experiments did not involve large sample sizes or adequately replicated trials. Because of this ambiguity, I tested the effects of six different soils, two moisture extremes, and two soil densities on beetle eclosion. I also determined the effects of soil type and beetle sex on the time spent pupating. These findings are reported in **Chapter 5**.

Section II: Interactions between small hive beetles and honey bees

Neumann et al. (2001b) demonstrated the confinement of beetles by Cape bees lasts 1-4 days as bees have sophisticated guarding strategies, including a high degree of aggressiveness toward beetles, for limiting the escape of beetles during confinement (Neumann et al. 2001b; Solbrig 2001). As a result, beetle access to honey, pollen, and bee brood in the combs, where beetle reproduction potentials are high, is restricted.

Incarcerated beetles lack access to the combs because worker bees continuously guard the entrances of the confinement sites ('prisons') and prevent many attempted escapes by the beetles. Nonetheless, despite no access to food in the combs, imprisoned beetles may endure two months or longer and the beetle's survival is not due to metabolic reserves because starved beetles die within two weeks (Neumann et al. 2001b). While documenting bee/beetle interactions I observed what appeared to be trophallactic encounters between guard bees and imprisoned beetles. The beetles characteristically approach guard bees and extend their heads towards and make antennal contact with guard bees (mimicking normal honey bee begging behavior). In **Chapter 6** I report the results of a simple experiment designed to determine whether long-term survival of incarcerated

beetles derives from a form of behavioral mimicry which induces honey bees to feed them by trophallaxis.

While documenting intra-colonial interactions of European bees and beetles, I discovered that although beetle-naïve, subspecies of European honey bees also confine and guard beetles. Nonetheless, European bees remain susceptible to depredation caused by beetles while their African counterparts rarely do. In **Chapter 7**, I quantify beetle and Cape and European honey bee behaviors that are associated with beetle confinement to determine if there are any differences in the behavioral repertoires of African and European subspecies of honey bees that could explain their highly different susceptibilities to beetle infestations. Further, I describe time differences in the behavior and record intra-colonial distribution of small hive beetles in Cape and European colonies in order to determine the efficacy of beetle confinement by both honey bees (the efficacy is gauged by how well the bees limit beetle access to the combs and/or reproduction).

To take the study of beetle confinement a step further, I report the effects of increasing beetle density on prison construction and guarding behavior of both Cape and European honey bees in **Chapter 8**. I decided to explore this avenue because ‘infested’ African colonies rarely host large populations of beetles while infested European colonies often do. Therefore, the overall success or failure of beetle confinement by Cape and European honey bees may be dependent on intra-colonial beetle density. The data deepen our understanding on confinement of beetles by African bees, allow for comparisons to be made between confinement schemes of African and European honey bees, and ultimately place the efficacy of these behaviors as resistance mechanisms to beetles in context. In **Chapter 9** I determine the age of the guard bees and the duration of beetle guarding for each honey bee subspecies.

The goal of beetle confinement by honey bees is to limit beetle access to the combs. If female beetles reach the brood combs, they puncture the waxy capping of brood cells and lay eggs on and around the honey bee pupa. Nonetheless, honey bees show hygienic responses to other pests and diseases and remove infected brood (Rothenbuhler 1964a; cf. Boecking and Spivak 1999). I therefore tested for hygienic behavior of Cape honey bees toward beetle eggs oviposited in bee brood. The results from this study are presented in **Chapter 10**. While doing this, I also discovered a second mode of beetle oviposition in bee brood; this is discussed in Chapter 10 as well.

Section III: Controlling small hive beetles

Since the introduction of small hive beetles into the US, little progress towards developing beetle control methods has been made. In-hive applications of coumaphos-impregnated plastic strips (Check-Mite®) can be used to treat for beetles, but control is not consistent (Elzen et al. 1999; Hood 2000; Wenning 2001). Further, coumaphos does not provide extended control because the strips are not registered to remain in colonies continuously. Treating soil around infested colonies with permethrin (GardStar® 40% EC) is recommended (Hood 2000; Pettis and Shimanuki 2000) because beetles pupate in soil (Lundie 1940; Schmolke 1974). However, this treatment is not always effective (Hood 2000; Wenning 2001), killing few beetles unless application is correctly timed (Pettis and Shimanuki 2000). The need for new, efficacious beetle controls is apparent; therefore, in Section III I test original cultural (Chapter 11), genetic (Chapter 12), and biological (Chapter 13) control schemes against small hive beetles.

Beekeepers in Georgia, US suggested that colony invasion by adult beetles may be reduced by sealing and replacing the regular hive entrance with a section of polyvinyl chloride (PVC) pipe 10.2-cm long and 1.9-cm inside diameter (ID), inserted 7.6-10.2 cm above the bottom board. In **Chapter 11**, I report the results of a 2-part experiment testing the efficacy of screened bottom boards (used for control of *Varroa destructor* Anderson and Truemann in honey bee colonies: Pettis and Shimanuki 1999; Ostiguy et al. 2000; Ellis et al. 2001) and PVC pipes of two different diameters on beetle control.

The findings outlined in Chapter 10 suggest that hygienic behavior is an important component of Cape (and possibly other African subspecies) bee resistance to beetles. If true, then a possible lack (or negligible amount) of hygienic behavior toward beetle eggs by European bees in the US and Australia could be a substantial reason they are susceptible to beetle depredation. In **Chapter 12**, I tested for hygienic behavior of Cape and European honey bees toward beetle eggs oviposited in bee brood in order to highlight differences between the two bee subspecies and possibly further illuminate why Cape bees are ‘resistant’ to beetles while European bees are often not. I also looked for colony differences in removal rates of infested brood within each bee race. If such differences exist, European colonies that are ‘more’ hygienic may be used in selection programs for increased hygienic tendencies toward beetle eggs as is often done in breeding for varroa mite resistance (Harbo and Harris 1999).

The last control avenue I explore is biological in nature. Many of the biological studies that I report in Section I require the rearing of beetles in the laboratory. In some

instances, mortality of beetle pupae in numerous soil containers (where they pupate) was quite high and the pupae appeared to be dying due to a fungal infection (an observation also made by Lundie 1940). Because of this, I exposed healthy larvae to diseased larvae via 1) contact and 2) ingestion of dead larvae emulsion. The effects of the exposure on beetle pupae mortality are discussed in **Chapter 13**.

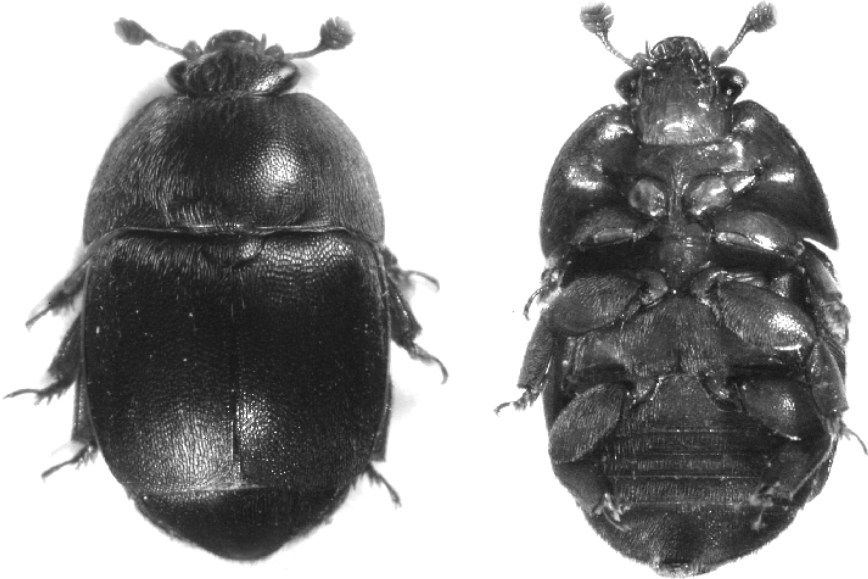


Figure 1.1. An adult small hive beetle.



Figure 1.2. Small hive beetle larvae.

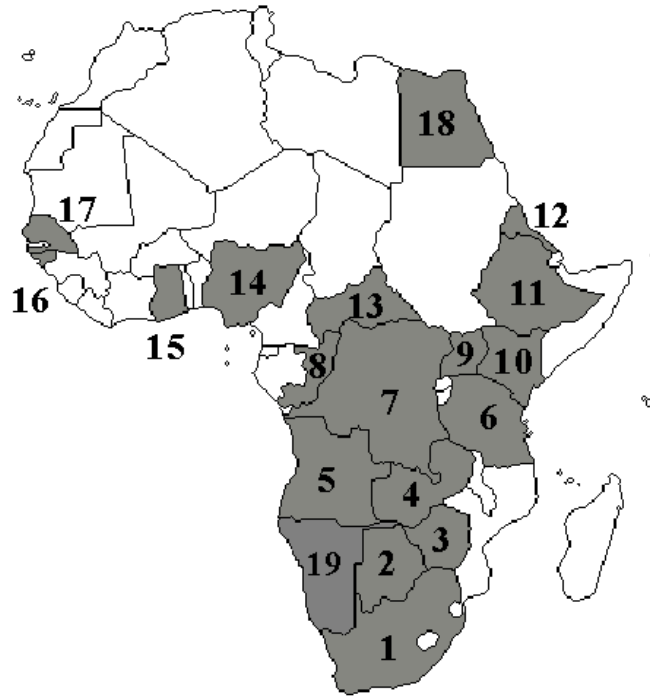


Figure 1.3. Known distribution of the small hive beetle in Africa as of October 2003. Countries where small hive beetles have been recorded include: 1) South Africa 2) Botswana, 3) Zimbabwe, 4) Zambia, 5) Angola, 6) Tanzania, 7) Democratic Republic of Congo, 8) Congo Republic, 9) Uganda, 10) Kenya, 11) Ethiopia, 12) Eritrea, 13) Central African Republic, 14) Nigeria, 15) Ghana, 16) Guinea Bissau, 17) Senegal, 18) Egypt, and 19) Namibia. Map used with permission of P. Neumann and was modified from Neumann and Elzen 2003.

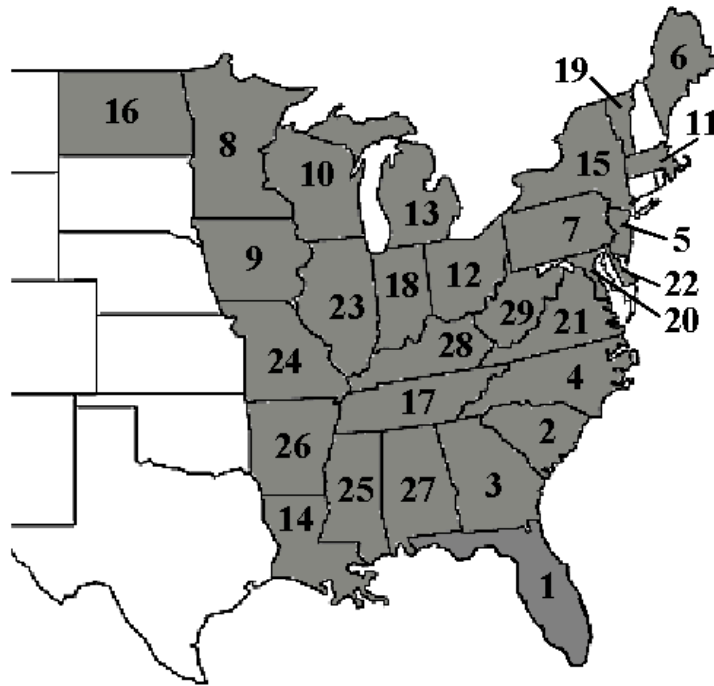


Figure 1.4. Known distribution of the small hive beetle in the United States as of October 2003. States where small hive beetles are present and the year of their discovery (in brackets) include: 1) Florida (1998), 2) South Carolina (1998), 3) Georgia (1998), 4) North Carolina (1998), 5) New Jersey (1999), 6) Maine (1999), 7) Pennsylvania (1999), 8) Minnesota (1999), 9) Iowa (1999), 10) Wisconsin (1999), 11) Massachusetts (1999), 12) Ohio (1999), 13) Michigan (1999), 14) Louisiana (2000), 15) New York (2000), 16) North Dakota (2000), 17) Tennessee (2000), 18) Indiana (2000), 19) Vermont (2000), 20) Maryland (2001), 21) Virginia (2001), 22) Delaware (2001), 23) Illinois (2001), 24) Missouri (2001), 25) Mississippi (2001), 26) Arkansas (2002), 27) Alabama (2002), 28) Kentucky (2002), 29) West Virginia (2003) and Manitoba (2003 not shown). Map used with permission of P. Neumann and was modified from Neumann and Elzen 2003.

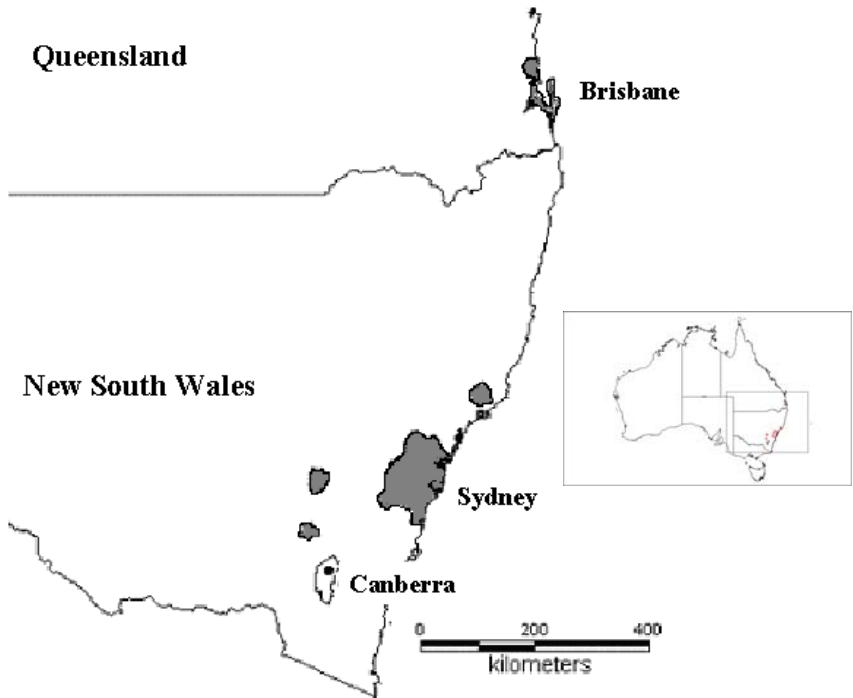
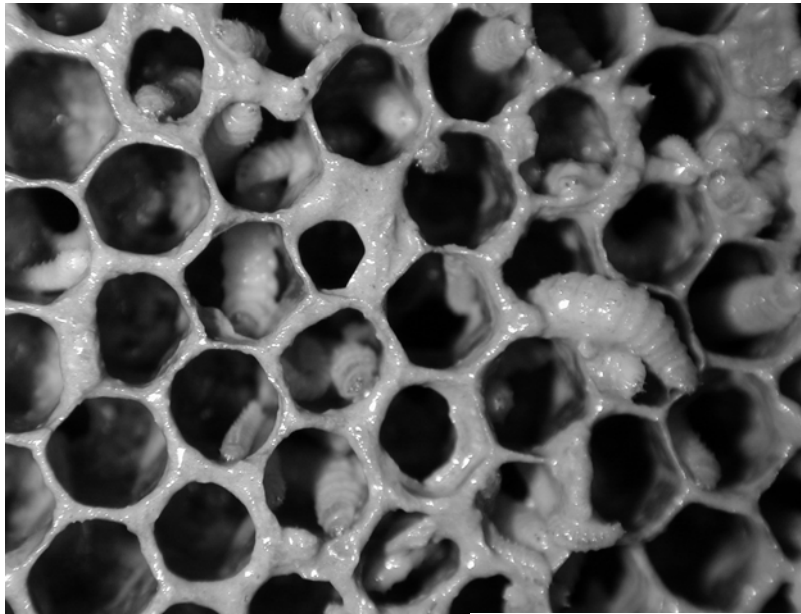


Figure 1.5. Known distribution of the small hive beetle in Australia as of October 2003. Map used with permission of P. Neumann and was modified from Neumann and Elzen 2003.

Section I: Small Hive Beetle Biology



Photography by Gerald Kastberger

Chapter 2: The Effects of Adult Small Hive Beetles on Nests and Flight Activity of Cape and European Honey Bees

Abstract - This study identifies differences in the effects of small hive beetles on flight activity and nests of European-derived honey bees in the United States and Cape honey bees in South Africa. Treatments consisted of control colonies (<5 beetles/colony) and experimental colonies receiving beetles (treatment: 100 beetles/day for 15 days). The number of days to absconding did not differ significantly between treatment or bee race but absconding was greater between the two treatments in European colonies than in Cape ones. Cape bees used significantly more propolis than European bees. Honey stores were significantly greater in Cape honey bee colonies than in European ones. Bee weight did not differ significantly between treatments or bee race. Treatment did not significantly affect bee populations, brood area, or average flight activity in Cape colonies but did significantly lower all of these variables in European colonies. The effects of treatment in European colonies are symptomatic of absconding preparation. Treatment significantly lowered the amount of pollen stores in Cape colonies, but this effect was not found in European colonies. The number of beetles in control colonies was significantly higher in European colonies than Cape ones while the percentage of beetles remaining in non-absconding, treated colonies was higher in Cape colonies than European ones. These data indicate that adult small hive beetles are sufficient to cause significant harmful effects on honey bees of European, but not Cape, origin.

Introduction

Successful reproduction of the small hive beetle in its native range is often restricted to weak host colonies, due to behavioral resistance mechanisms of their honey bee hosts (Elzen et al. 2001; Neumann et al. 2001b), or is associated with after absconding events (Hepburn et al. 1999). Absconding is frequent in African honey bee subspecies and can be triggered by parasite infestations (Hepburn and Radloff 1998). Indeed, severe small hive beetle infestations may cause such absconding (Hepburn and Radloff 1998).

In sharp contrast, colonies of European-derived honey bee subspecies are highly susceptible to small hive beetle depredation (Elzen et al. 1999; Hood 2000; Wenning 2001). This damage stems from the feeding habits of both adult and larval beetles (Hood 2000). It has been reported that only the larval stage presents a direct threat to colony health and European colonies can host thousands of adult hive beetles without suffering visible side effects (Wenning 2001); however, this has not yet been confirmed in quantitative analyses.

Such quantitatively different responses of Cape (and presumably most other African subspecies) and European host colonies towards adult small hive beetles are very likely to be reflected in colony productivity. Since European honey bees are highly susceptible to beetles, a reduction in colony productivity is more likely to be expressed in European host colonies than in Cape ones. Although the impact of hive beetles on European host colonies is striking (Hood 2000), this effect has not yet been measured quantitatively.

Here I report the results of an intercontinental quantitative study of the productivity of artificially infested or non-infested Cape (*A.m. capensis*) and European honey bee (*A. mellifera* mixed races) colonies. The variables measured included number of days to absconding, total propolis, honey stores, bee weight, sealed brood, number of adult bees, pollen stores, flight activity and the number of small hive beetles remaining in treated colonies of Cape honey bees in South Africa and European honey bees of mixed origin in the United States.

Materials and Methods

Cape honey bees

Experiments were conducted at Rhodes University (Grahamstown, South Africa) in late summer/early fall (April 2001). Twenty propolis-free nucleus colonies (about 20 l in volume) of Cape honey bees (an African honey bee subspecies that is geographically distributed in the region of study) were established with 3 frames of workers, 1 frame of honey, 2 frames of brood, and a laying queen. Ten treated colonies were artificially infested with 100 adult small hive beetles on a daily basis between 17:00 - 21:00 h for 15 consecutive days. The small hive beetles used were reared in the laboratory according to standard procedures (Neumann et al. 2001a). By the end of the experiment, 1,500 beetles (100 beetles/colony for 15 d) had been introduced into all of the treated colonies. This level of beetle infestation is high for African honey bee colonies, but is common in infested European ones. Ten control colonies (<5 beetles/colony) were otherwise treated identically to the treated colonies. All nucs were placed in the same apiary, blocked together by treatment.

The number of returning bees was counted for all colonies twice daily, 1 minute each count, between 11:00 – 11:40 and 15:00 – 15:40 h because of data indicating peak foraging times for honey bees at 11:00 and 15:00 in southern Africa (Hepburn and Magnuson 1988). Overall flight activity was determined by averaging the number of incoming bees per minute for both times.

Each colony was monitored three times daily (11:00, 15:00, 20:00) to identify its date of absconding, immediately after which, the colony was dismantled to determine number of adult small hive beetles present; sealed brood area (cm²), honey area (cm²), and pollen area (cm²) (using a calibrated plastic grid); and total weight of propolis (g) in the colony.

On the evening of day 16 all remaining colonies were closed up, gassed with CO₂, frozen at -10°C, and then analyzed. For each colony, data were collected for the amount of sealed brood, honey, and pollen (cm²), number of adult small hive beetles, total weight of bees (g), weight of a sub-sample of bees (g) and number of bees in the sample (used to derive the number of bees in the colony), and total weight of propolis (g).

Honey bees of mixed European origin

A slightly modified procedure was conducted on honey bees of mixed European origin (unknown history) in Warren County, Georgia, USA in late summer/early fall

(August - September 2001). Adult beetles were reared from larvae collected in the field. The larvae were supplemented on a diet of pollen, honey, and bee brood (Neumann et al. 2001a; Chapter 4) until they reached the wandering phase (Lundie 1940), after which they were transferred to soil chambers for pupation and emersion as adults. Each treated European colony cumulatively received 1,400 beetles (100 beetles/day for 14 d).

European colonies that did not abscond in the experimental period were collected early morning on the 17th day of the experiment, cooled at 7°C for 1 d, and then frozen for an analysis identical to that done on non-absconding Cape bee colonies.

Data analysis

The effects of treatment [small hive beetles added or not added (control)] on absconding day, total propolis content, honey area, bee weight, number of bees, sealed brood and pollen area, and average flight activity were tested with a randomized design analysis of variance, blocked on location (United States or South Africa) and accepting differences as statistically significant at the $\alpha \leq 0.05$ level. When the treatment \times location interaction was significant, analyses were run separately by location. For the variables absconding day, total propolis content, brood and pollen area, analyses included absconding colonies. Absconding colonies were excluded from analyses of honey area, bee weight, and number of bees because these parameters were either unavailable or confounded in empty hives. The effects of time and increasing beetle numbers on average daily bee flight activity were tested with regression analyses testing for linear, quadratic, and cubic effects. The final number of small hive beetles in non-absconding control colonies and the percentage of beetles remaining in non-absconding treated colonies were analyzed for location effects with ANOVA. Beetle numbers in both absconding and non-absconding treated colonies were analyzed separately by location because the absconding \times location interaction was significant. All reported data are given as means \pm standard errors; *n*. Analyses were conducted using SAS (1992) and Statistica (2001).

Results

Absconding

There were no treatment effects ($F = 1.6$; $df = 1, 13$; $P = 0.2220$), location effects ($F = 2.8$; $df = 1, 13$; $P = 0.1201$), or location \times treatment effects ($F = 2.6$; $df = 1, 13$; $P =$

0.1308) for absconding day. Treated colonies did not abscond earlier than control colonies (Table 2.1). In South Africa, 44% of control and 60% of treated colonies absconded while in the United States, 10% of the control and 60% of treated colonies absconded.

Prior to absconding, treated European colonies aborted much of their brood. This was evident by the piles of mutilated brood on the ground outside of each colony. Further, worker bees were seen carrying brood out of the colony and discarding it on the ground. Upon post-absconding analysis of these colonies, no uncapped brood remained.

Propolis

There were no treatment effects ($F = 2.2$; $df = 1, 32$; $P = 0.1447$) or location \times treatment interactions ($F = 1.4$; $df = 1, 32$; $P = 0.2461$) for the amount of propolis in colonies. Treated colonies did not have more propolis than control colonies (Table 2.1). There were location effects for the total propolis content ($F = 30.1$; $df = 1, 32$; $P < 0.0001$). Cape honey bee colonies had significantly more propolis than did European honey bee colonies (Table 2.1).

Honey area

There were treatment ($F = 7.5$; $df = 1, 18$; $P = 0.0136$) and location ($F = 100.4$; $df = 1, 18$; $P < 0.0001$) effects for honey area. Control colonies had significantly more stored honey than treated colonies while Cape honey bees had significantly greater stores of honey than did European honey bees (Table 2.1). There were no location \times treatment interactions found for honey area ($F = 1.4$; $df = 1, 18$; $P = 0.2455$).

Bee weight

There were no treatment effects ($F = 1.4$; $df = 1, 18$; $P = 0.2495$), location effects ($F = 0$; $df = 1, 18$; $P = 0.9746$), or location \times treatment interactions ($F = 1.5$; $df = 1, 18$; $P = 0.2361$) for bee weight. There were no differences in Cape and European colonies with respect to weight (Table 2.1). Bee weight was not significantly different across all tested control and treated colonies (Table 2.1).

Brood area

There was a significant location \times treatment interaction for sealed brood area ($F = 9.6$; $df = 1, 35$; $P = 0.0039$) so analyses were run separately by location. In Cape colonies, treatment did not significantly affect the amount of sealed brood ($F = 0$; $df = 1, 17$; $P =$

0.9712) but it did in European colonies ($F = 12.7$; $df = 1, 18$; $P = 0.0022$). In European colonies there was significantly less brood in treated colonies than in control ones (Table 2.2).

Number of bees

There was a significant location \times treatment interaction for number of bees ($F = 7.3$; $df = 1, 18$; $P = 0.0144$) so analyses were run separately by location. In Cape colonies, treatment did not affect the number of bees in colonies ($F = 3.2$; $df = 1, 7$; $P = 0.1174$) while it did in the European colonies ($F = 5.2$; $df = 1, 11$; $P = 0.0432$). European treated colonies had significantly fewer adult bees than did control colonies (Table 2.2).

Pollen area

There was a significant location \times treatment interaction for pollen area ($F = 5.3$; $df = 1, 35$; $P = 0.0276$) so analyses were run separately by location. Treatment affected pollen area in Cape colonies ($F = 5.8$; $df = 1, 17$; $P = 0.0278$) whereas it did not in European bee colonies ($F = 1.0$; $df = 1, 18$; $P = 0.3398$). Cape treated colonies had significantly less pollen than did control colonies (Table 2.2).

Flight activity

There was a significant location effect for average flight activity ($F = 13.3$; $df = 1, 474$; $P = 0.0003$). European colonies (13.5 ± 0.5 ; 262) had significantly more activity than Cape colonies (10.2 ± 0.6 ; 216). There was also a significant location \times treatment interaction for average flight activity ($F = 6.4$; $df = 1, 474$; $P = 0.0120$). In Cape colonies, treatment did not affect average flight activity ($F = 1.4$; $df = 1, 214$; $P = 0.2387$). Cape honey bee treated colonies had similar flight activity as control colonies (Table 2.2). In contrast, treatment significantly affected average flight activity ($F = 25.8$; $df = 1, 260$; $P < 0.0001$) in European colonies. The number of incoming bees was significantly greater in control colonies than in treated colonies (Table 2.2).

Regression analyses of flight activity trends over time reveal pronounced differences between locations (Figure 2.1). In Cape colonies, average flight rates increased linearly over time in both treated and control colonies. Thus, flight activity appeared unaffected by increases in beetle numbers and the sampling period was universally and increasingly favorable for foraging. However, in European colonies there were measurable differences in trends between treated and control colonies. A cubic regression model in

which rates fell, then rose, then fell again over time explained flight activity in treated colonies. A quadratic model in which rates rose then fell explained flight activity in control colonies. Rates were generally lower in treated colonies. Moreover, the increasing rates of flight by control colonies early in the sampling period contrast strongly with the decreasing rates by treated colonies at the time when conditions were apparently favorable for foraging. In spite of a mid-period surge by treated colonies, rates began decreasing more rapidly in treated colonies by the end of the sampling period when foraging conditions appeared to be deteriorating universally and when rainy weather was prevalent.

Beetle counts

There were significant differences between locations for the number of small hive beetles present in control colonies at the end of the experiment ($F = 14.0$; $df = 1, 12$; $P = 0.0028$) and for the percentage of beetles remaining in non-absconding treated colonies ($F = 18.0$; $df = 1, 6$; $P = 0.0054$). There were significantly more beetles present in European control colonies (12.9 ± 1.3 ; 9) than in Cape control colonies (5.6 ± 1.3 ; 5). A significantly higher percentage of beetles remained in Cape bee non-absconding treated colonies (87.8 ± 0.7 ; 4) than did in European bee non-absconding treated colonies (42.1 ± 10.7 ; 4). Indeed, that percentage was over twice as high for Cape bee colonies.

There were location effects ($F = 13.2$; $df = 1, 16$; $P = 0.0022$) and location \times absconding effects ($F = 22.7$; $df = 1, 16$; $P = 0.0002$) for the number of beetles remaining in absconding and non-absconding treated colonies. Cape treated colonies (pooled absconding and non-absconding) had more beetles present (713.5 ± 165.0 ; 10) on colony analyses than did European treated colonies (481.3 ± 83.3 ; 10). Because the interaction term was significant, the number of beetles remaining in absconding and non-absconding treated colonies was analyzed by location. For Cape honey bees, there was a significant difference between the number of beetles remaining in non-absconding and absconding treated colonies ($F = 656.8$; $df = 1, 8$; $P < 0.0001$). Non-absconding treated colonies had significantly more beetles remaining (1316.3 ± 11.2 ; 4) than did absconding ones (311.7 ± 30.5 ; 6). For European colonies, there was no difference ($F = 1.1$; $df = 1, 8$; $P = 0.3174$) between the number of beetles remaining in absconding treated colonies (409.3 ± 95.8 ; 6) and the number of beetles in non-absconding treated colonies (589.3 ± 150.3 ; 4).

Discussion

Abscending

An analysis of absconding is of particular interest because most African honey bee subspecies readily abscond in response to nest predation and many other forms of disturbance (Hepburn and Radloff 1998) while by contrast, temperate races of *A. mellifera* very seldom abscond (Simpson 1959; Martin 1963; Winston 1992; cf. Lipiński 2001). In this study, control and treated colonies alike in both locations absconded; but there were no effects of treatment or location on the latency to abscond (Table 2.1). For Cape bees, 44% of the controls absconded and 60% of treated colonies absconded. Because a large percentage of both Cape treatment and control colonies absconded, other factors (colony disturbance, nectar dearth, etc.) probably caused them to abscond and not merely the presence of large numbers of adult small hive beetles.

Because 60% of European treated colonies absconded and only 10% of control colonies, I infer that, unlike Cape bees, European colonies absconded in response to the presence of large numbers of adult beetles in the hives. European colonies exhibited “prepared absconding” because these colonies had no uncapped young brood (based on post-abscond analyses), few workers emerged after the colony absconded, and honey stores were reduced. Other authors (Woyke 1976; Winston et al. 1979; Koeniger and Vorwohl 1979; Koeniger and Koeniger 1980; Punchihewa 1990; Nakamura 1993; Mutsaers 1991, 1993, 1994; cf. Lipiński 2001) record these symptoms as behavior typical of colonies preparing to abscond.

Moreover, European treated colonies (including the non-absconding colonies) uncapped and discarded all or most of their capped pre-pupae and pupae, as evidenced by the piles of mutilated pupae on the ground in front of treatment colonies. Further, bees were observed pulling pupae from the combs. By the end of the experiment, there was no open brood observed in any non-absconding European treated colony. These observations are similar to those of Woyke (1989) who showed that colonies of *A. m. adansonii* ate all of their uncapped larvae and most of their sealed brood before absconding. This suggests that the remaining 4 treated colonies were going to abscond soon and this is a likely explanation for the beetle effects seen on adult bees, brood, and flight activity in these colonies. None of this behavior was observed in the control European colonies. Therefore, the data clearly indicate that European colonies do respond to large adult small hive beetle infestations by having high, prepared absconding rates.

Propolis

We found that European honey bees used almost 4 times less propolis than Cape honey bees (Table 2.1) which is consistent with the findings of others (Bro. Adam 1983; Ruttner 1988; Dietz 1992; Hepburn and Radloff 1998) though this difference could be due to environmental effects. Because confinement of adult small hive beetles in propolis prisons appears to be a resistance mechanism of African honey bees (Hepburn and Radloff 1998, Neumann et al. 2001b), this could be a reason European colonies are highly susceptible to beetle infestations while Cape honey bees are more resistant (Tribe 2000). Because Cape bees use more propolis than European bees, more propolis is available in Cape colonies for use in beetle confinement systems. Although imprisoning behavior is also present in European honey bees (Ellis 2002; Section II) the data suggest that it may not be as efficient as African honey bee imprisoning behavior, possibly due to the lesser use of propolis by European bees.

Honey area

Treatment clearly reduced the amount of honey stores in bee colonies (Table 2.1). Because flight activity was not reduced, this difference could be due to the feeding habits of adult beetles (Lundie 1940; Schmolke 1974; Chapter 4), or general colony stress conditions due to beetle presence. European treated colonies had no honey stores at the end of the study, possibly reflecting preparation for absconding (Winston et al. 1979; Koeniger and Vorwohl 1979; Koeniger and Koeniger 1980; Punchihewa 1990).

Brood area

It has been reported that small hive beetles feed on honey bee eggs and brood (Lundie 1940; Schmolke 1974; Elzen et al. 1999; Chapter 4) and indeed, that they do so preferentially (Elzen et al. 2000). These data support the finding of significant differences in sealed brood areas between treated and control European honey bee colonies. Despite beetles feeding on bee brood, the major factor contributing to a decline in brood area between treatment and control European honey bee colonies was most likely due to the observed absconding preparation behavior, namely brood abortion and cannibalism. On the other hand, Cape honey bees did not experience the same decline in brood area when infested with hive beetles (Table 2.2), also suggesting a superior ability to cope with beetle infestations.

Adult bees and bee weight

The data show that the presence of adult small hive beetles lowers the number of adult bees present in European honey bee colonies, but not in Cape honey bee colonies, although beetle infestations did not compromise bee weight. However, threshold values have not yet been determined. Contrary to what has been reported by others (Wenning 2001), this shows that beetle larvae are not the only stage of the small hive beetle's life cycle that damages honey bee colonies. European treated colonies also had significantly less brood than control colonies and this is probably related to the differences in adult bee populations between treatments in European colonies.

Pollen area

The only impact small hive beetle infestations had on infested Cape honey bee colonies was a reduction in pollen stores. It is possible (but was not shown) that beetles in these colonies were feeding on pollen stores. Although beetles preferentially feed on bee brood (Elzen et al. 2000), it is evident that Cape bees are efficient at guarding their brood because there was no significant loss of brood area in beetle infested Cape colonies. In these circumstances the beetles would have had to feed on alternative food sources, such as pollen stores. It is well established that beetles feed on pollen (Lundie 1940; Schmolke 1974; Elzen et al. 2000; Hood 2000; Neumann et al. 2001a) and that they reproduce most successfully on a diet of pollen alone (Chapter 4). In European colonies the beetles caused a significant reduction in brood area (probably by feeding and ovipositing on it and because of prepared absconding behavior by the bees) and there were no differences in the pollen stores between the treatments. The data suggest that beetles are restricted to pollen in Cape bee colonies, but gain access to brood in European ones, which likely triggers explosive reproduction by beetles.

Flight activity

The fact that European bees had greater flight activity than Cape bees is probably due to nectar flow differences in each country for the time of year the experiments were conducted. The pertinent information lies in the interactions found between location × treatment. The data show that treatment significantly lowered average flight activity in European bee colonies but not in Cape ones. Small hive beetle presence in European colonies was sufficient to lower flight activity. Although the causes for this are unknown, it may be that small hive beetles cause general disruption in European colonies (Hood

2000; Wenning 2001) and flight behavior is thus compromised. The difference appears related to prepared absconding behavior of the treated European colonies; a behavior that likely limited the number of available foraging workers. Further, in Chapter 9 I show that European honey bees guarding small hive beetle prisons belong to the same age cohort as foraging bees. Therefore, an increasing population of small hive beetles could cause more foraging-age bees to begin guarding beetle prisons thus explaining the overall decrease in flight activity between European treatment and control colonies seen in this study. Increasing beetle densities affected flight activity only in treated European colonies.

Beetle counts

All colonies in both locations were created from colonies having small populations of hive beetles. All colonies started with < 5 beetles per colony (visual estimates). Therefore, the number of beetles found in Cape control colonies could be considered ‘background noise’, being close to the original population of beetles present in the colony at the beginning of the experiment. A total of 2565 small hive beetles were unaccounted for in Cape colonies by the end of the experiment. These beetles were put into the hives, but not re-collected. At the same time the data show that these beetles were not migrating into control colonies. Even though European control colonies had significantly more beetles than did Cape control colonies, they too were not heavily infested with “stray” beetles (unaccounted beetles totaling 4487 individuals in the U.S.).

Why beetles tended to migrate from European non-absconding treated colonies and not from Cape non-absconding treated colonies is unclear. This could be indicative of a superior ability of Cape bees to imprison and guard beetles more efficiently than European bees (Hepburn and Radloff 1998; Neumann et al. 2001b; Ellis 2002). Regardless, over half of the beetles introduced into European colonies were not in the hives at the end of the experiment. These beetles may have been host seeking, even though they were not going to control colonies.

Small hive beetle populations in both European absconding and non-absconding treated colonies were the same. This occurred regardless of the number of beetles introduced into the colonies (which totaled 1400 beetles/colony for treated colonies that did not abscond and an average of 617 beetles/colony for treated colonies that absconded). This implies a “carrying capacity” for small hive beetles in European bee colonies. It could also imply a threshold, that when met, European colony health is compromised and, even in extreme situations, absconding preparation begins.

At the same time, the carrying capacity for beetles in Cape colonies is either much higher, or non-existent. I base this on the data which shows that most of the beetles put into Cape colonies stayed in those colonies. Because this large number of beetles in Cape colonies never significantly affected measured colony parameters, with the exception of reduced pollen stores (Table 2.2), Cape bees must have either superior imprisoning techniques (Hepburn and Radloff 1998; Neumann et al. 2001b), or other behavioral mechanisms (Elzen et al. 2001) that make them better able to handle large infestations of small hive beetles.

Table 2.1. Analyses of absconding day, honey area (cm²), total propolis (g), and bee weight (mg) for Cape and European host colonies.

absconding day				honey area			
	treatment	control	row total		treatment	control	row total
Cape	7.3 ± 2.0 (6)	6.3 ± 2.6 (4)	6.9 ± 1.5 (10)a	Cape	663.8 ± 82.7 (4)	960.0 ± 124.9 (5)	828.3 ± 90.5 (9)a
European	7.5 ± 2.2 (6)	17.0 ± 0 (1)	8.9 ± 2.3 (7)a	European	0 ± 0 (4)	115.6 ± 38.9 (9)	80.0 ± 30.6 (13)b
column total	7.4 ± 1.4 (12)a	8.4 ± 2.9 (5)a		column total	331.9 ± 131.1 (8)a	417.1 ± 122.1 (14)b	
total propolis (g)				bee weight (mg)			
	treatment	control	row total		treatment	control	row total
Cape	15.5 ± 3.1 (8)	10.8 ± 2.3 (8)	13.1 ± 2.0 (16)a	Cape	91.4 ± 4.4 (4)	91.3 ± 4.1 (5)	91.4 ± 2.8 (9)a
European	3.7 ± 0.6 (10)	3.2 ± 0.4 (10)	3.5 ± 0.3 (20)b	European	87.6 ± 3.1 (4)	95.3 ± 1.7 (9)	92.9 ± 1.8 (13)a
column total	9.0 ± 2.0 (18)a	6.6 ± 1.4 (18)a		column total	89.5 ± 2.6 (8)a	93.9 ± 1.8 (14)a	

Values are mean ± standard error with sample size (*n*) in parentheses. Row total and column total means followed by the same letter are not different at the $\alpha \leq 0.05$ level. For the variables absconding day and total propolis, analyses were run including absconding colonies. For the variables honey area and bee weight, analyses were run without including absconding colonies.

Table 2.2. Location × treatment interactions for amount of sealed brood (cm²), number of adult bees, stored pollen area (cm²), and average flight activity (number of bees returning per minute) in Cape and European host colonies.

	Cape colonies	
	treatment	control
sealed brood area	201.9 ± 78.8 (10)a	205.6 ± 58.3 (9)a
number of adult bees	6552.8 ± 675.5 (4)a	4823.4 ± 675.4 (5)a
stored pollen area	27.7 ± 11.2 (10)a	116.9 ± 37.1 (9)b
average flight activity	9.6 ± 0.7 (102)a	10.9 ± 0.8 (114)a
	European colonies	
	treatment	control
sealed brood area	54.1 ± 18.0 (10)a	739.7 ± 191.6 (10)b
number of adult bees	3246.8 ± 234.3 (4)a	6321.0 ± 869.9 (9)b
stored pollen area	67.5 ± 42.4 (10)a	25.1 ± 8.6 (10)a
average flight activity	10.4 ± 0.6 (103)a	15.5 ± 0.7 (159)b

Values are mean ± standard error with sample size (*n*) in parentheses. Analyses were run separately by location for these variables. For number of adult bees, analyses were run without including absconding colonies; for sealed brood and stored pollen area analyses included absconding colonies. Row means followed by the same letter are not different at the $\alpha \leq 0.05$ level.

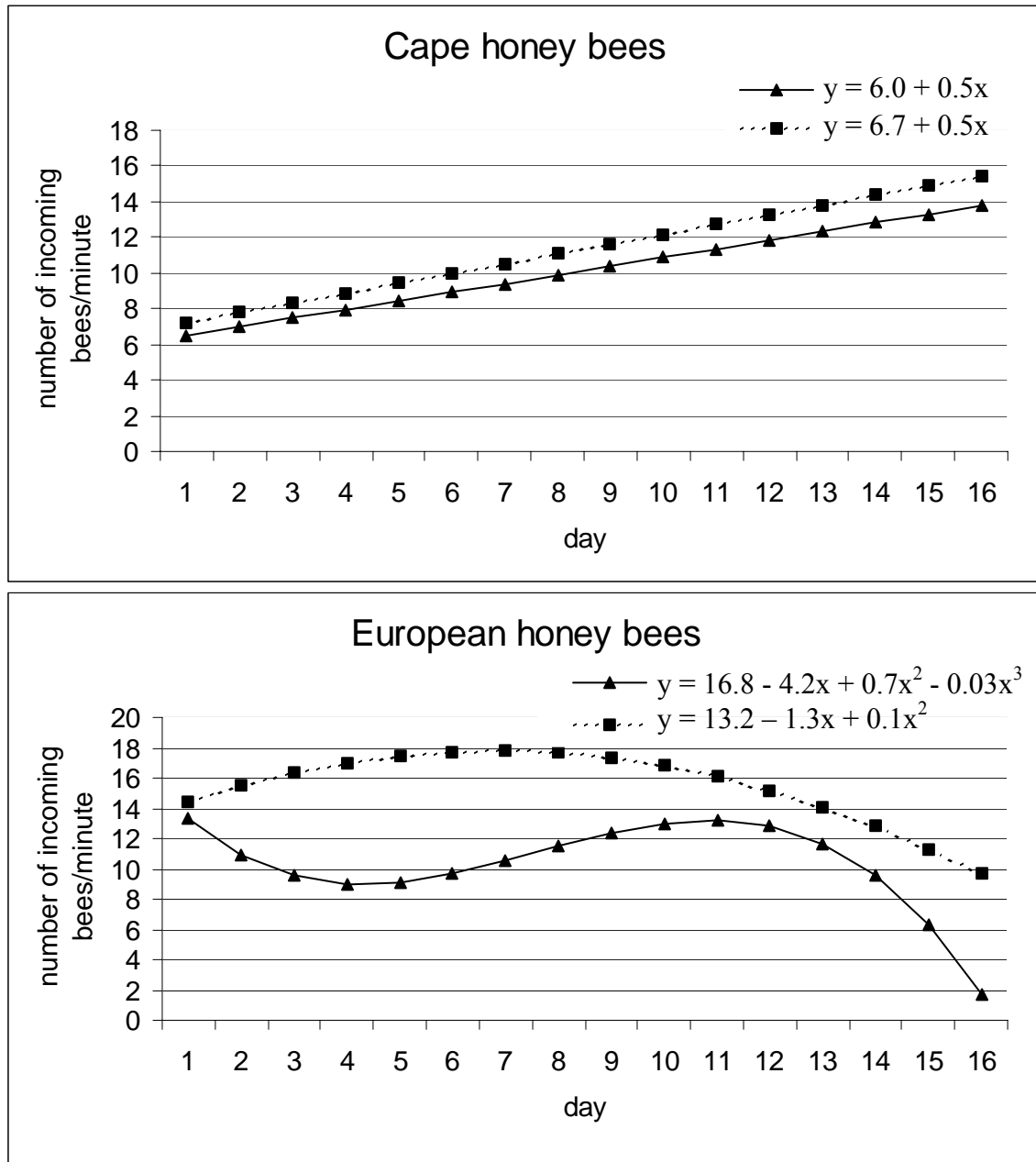


Figure 2.1. Predicted daily average number of returning workers for control and treated (beetles added) colonies of Cape and European-derived honey bees. Data were measured by averaging the number of incoming bees per minute at hours 11:00 and 15:00. Each day corresponds to an increase of 100 beetles/colony. Control colonies are represented by squares and dashed lines while treated colonies are triangles with solid lines.

Chapter 3: Small Hive Beetle Oviposition and Behavior during Honey Bee Clustering and Absconding Events

Abstract – The results of observations on small hive beetle behavior during European honey bee clustering and absconding events and on beetle oviposition are reported. Six, 3-frame nucleus colonies were formed from standard Langstroth-style hives. One hundred small hive beetle adults were introduced into each of the colonies at dusk for 14 consecutive days. Two of the colonies absconded and beetles were observed in the resulting clusters. In the 4 non-absconding colonies, 253 - 905 beetles were found. In every case, most of beetles were found inside the bee cluster. Upon examining frames from the non-absconding colonies, many puncture marks in the brood cell cappings were observed. When the cappings to these cells were pulled back or removed, >10 beetle eggs per cell were often observed. Approximately one-third of the remaining capped brood in all non-absconding colonies was affected in this way. The data suggest that female beetles bite holes in the brood cappings and insert their long, flexible ovipositors to lay eggs on the immature bees.

Introduction

While there is some data on the biology of small hive beetles (Lundie 1940; Schmolke 1974), studies into the behavior of this pest are limited. Knowing various aspects of the beetle's behavior might illuminate possible control methods for them. In conducting the study reported in Chapter 2, I collected observations on beetle behavior during European honey bee clustering and absconding events and on beetle oviposition. These behavioral observations are reported here.

Materials and Methods

Experiments on honey bees of mixed-European origin were conducted in Warren County Georgia, USA during August-September 2002. Six, 3-frame nucleus colonies were formed from standard Langstroth-style hives; each colony had bees on all three combs, 1 comb with honey, 2 combs with brood, and a laying queen. One hundred small hive beetle adults were introduced into each of the colonies at dusk for 14 consecutive days. Two colonies absconded and beetle behavior was monitored during the absconding events. On the 17th day of the experiment, the remaining 4 colonies were put into a cool storage room (7°C) for 1 d (which elicited honey bee clustering behavior), after which they were frozen for two weeks. The colonies were thawed and the honey bee clusters examined for the presence of beetles. The bees were then removed from the comb in order to examine female beetle oviposition.

Results and Discussion

The first of the two colonies that absconded did so on day 8. During absconding, 5-10 beetles were seen leaving the colony with the bees. The queen and the swarm settled on the ground about 15 m from the hive. The following day, an empty hive box was placed beside the swarm. A single small hive beetle entered the box with the cluster of bees indicating that the beetle had been present in the cluster. It is possible, however, that the beetle entered the cluster while the bees were on the ground. With the second colony, beetles were also seen leaving with the absconding bees; however this swarm was not captured and the presence of beetles in its cluster could not be confirmed.

A range of 253 - 905 beetles were found in the 4 non-absconding colonies. In every case, most of beetles were found inside the bee cluster. Of the few beetles found outside the cluster (always <50), most (>75%) were within 5 cm of the cluster perimeter. Clustering bees often enter cells head first in order to form a more contiguous cluster. All such bees were pulled from their cells to facilitate individual cell examination. I found >50 cells per colony containing beetles (some having >5 beetles per cell). It is likely that the bees were clustering naturally and perhaps the beetles infiltrated the cluster to keep warm. The findings support earlier observations (Eischen 1999; Pettis and Shimanuki 2000) of small hive beetles in bee clusters.

Data were also collected on beetle oviposition. Lundie (1940) and Schmolke (1974) have shown that female beetles often oviposit on pollen reserves and in cracks of hives. However, there were no pollen reserves in any of the non-absconding colonies. Upon examination, I observed many puncture marks in the brood cell cappings (Figure 3.1a). When the cappings to these cells were pulled back or removed, I observed small hive beetle eggs (Figure 3.1b). In most instances, there were >10 beetle eggs per cell; eggs were laid directly on or around the honey bee pupae. Approximately one-third of the remaining capped brood in all non-absconding colonies was affected in this way. Beetles may be able to bite holes in the brood cappings and insert their long, flexible ovipositors (Schmolke 1974) to lay eggs on the immature bees. Further, I noted that all 4 colonies had begun aborting brood by the end of the experiment, as indicated by the presence of cannibalized larvae and pupae outside the hive entrances. Bees may have detected and removed the beetle-infested brood.

Analyses of four beetle-infested colonies (1500 beetles each) of Cape honey bees in South Africa, established identically (food stores, number of bees, etc.) to the European colonies, showed no punctured brood cappings in any colony. However, punctured cappings appeared in the brood cells two days after beetle females were given brood comb ($4 \times 4 \text{ cm}^2$) in the laboratory (free from adult bees). The data possibly suggest that in their native range beetles do oviposit in brood combs but only in the absence of adult honey bees, which contrasts with the results I found for European honey bees in the United States. Therefore, there may exist fundamental differences between European and Cape honey bee behaviors toward small hive beetles that help explain the comparative tolerance exhibited by Cape bees. Further, a reproductive threshold for beetles in European honey

bee colonies may exist, above which beetle females are free-running in colonies and able to oviposit in unprotected brood combs.

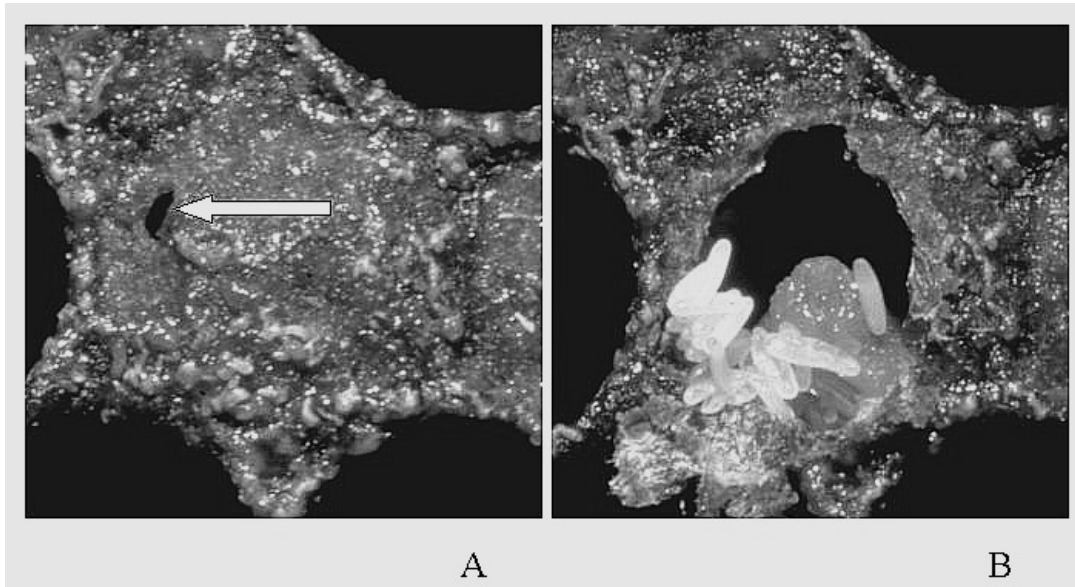


Figure 3.1. Evidence for small hive beetle ovipositing behavior. Photo A shows a brood cell capping with an arrow pointing to a small puncture. After the capping of this cell was removed (photo B), one can see more than 15 beetle eggs oviposited on or around the honey bee prepupa. Photography by Keith Delaplane.

Chapter 4: Longevity and Reproductive Success of Small Hive Beetles Fed Different Natural Diets

Abstract - The longevity and reproductive success of newly emerged, unfed adult small hive beetles assigned different diets (control = unfed; honey-pollen; honey; pollen; empty brood comb; bee brood; fresh kei apples; and rotten kei apples) were determined. Longevity of honey-fed beetle adults (average maximum: 167 d) was significantly higher than on other diets. Beetles fed empty brood comb lived significantly longer (average maximum: 49.8 d) than unfed beetles (average maximum: 9.6 d). Beetle offspring were produced on honey-pollen, pollen, bee brood, fresh kei apples, and rotten kei apples but not on honey alone, empty brood comb, or in control treatments. The highest reproductive success occurred in pollen fed adults (1773.8 ± 294.4 larvae per 3 mating pairs of adults). The data also show that beetles can reproduce on fruits alone, indicating that they are facultative scavengers in honey bee colonies. The pupation success and sex ratio of beetle offspring were also analyzed. Larvae fed pollen, honey-pollen, or brood had significantly higher pupation success rates of 0.64, 0.73, and 0.65 respectively than on the other diets. Sex ratios of emerging adults fed diets of pollen or brood as larvae were significantly skewed towards females. Because beetle longevity and overall reproductive success was highest on foodstuffs located in honey bee colonies, beetles are efficient at causing large-scale damage to colonies of honey bees resulting in economic injury for the beekeeper. Practical considerations for the control of beetles are briefly discussed.

Introduction

The economic impact of small hive beetles appears to depend both on beetle longevity and ability to mass reproduce on the foodstuffs located in honey bee colonies. Studies on the longevity of beetle adults are few and conflicting. Lundie (1940) reported that beetle adults fed honey and pollen can live 180 - 188 d but given only water and beeswax, adults lived a maximum of 19 d (Schmolke 1974). Schmolke (1974) added that adult beetles deprived of food and water died within 2 d while Pettis and Shimanuki (2000) reported that adult beetles can live 5 d when entirely deprived of food and water. In another study (Flügge 2001), newly emerged adults deprived of food and water lived 7 d. Thus, it is unclear how long beetles can live and how this is related to different food regimes and reproductive success, which is the major factor affecting the economic impact of beetles.

Unlike other species of Nitidulidae that mainly feed and reproduce on rotten fruit (Borror et al. 1989), small hive beetle adults and larvae have been reported to feed on honey bee nest contents, including pollen, honey, (Lundie 1940; Schmolke 1974; Neumann et al. 2001a) and, preferentially, honey bee brood (Elzen et al. 2000). It is when beetle adults and larvae are feeding on these foodstuffs that colony health begins to decline. Eischen et al. (1999b) stated that beetles can feed and reproduce on fruits, indicating that the beetles may only be facultative scavengers in honey bee colonies. However, the relative reproductive success of beetle adults on different diets afforded by a honey bee nest or by fruits has not yet been quantitatively investigated.

Successful reproduction of the beetle in its native range is often restricted to weak colonies or associated with after absconding events (Hepburn et al. 1999) because of behavioral resistance mechanisms of their honey bee hosts (Elzen et al. 2001; Neumann et al. 2001b). In these cases, a variety of food stores, brood combs and freshly emerged bees, are often left behind by the absconding swarms (Hepburn and Radloff 1998). Thus, beetles are provided a range of diets in their native habitat, the reproductive effects of which are not yet known.

Sex ratios of adult beetle populations in the wild show no statistically significant sex bias although females most always outnumber males (Schmolke 1974; Ellis et al. 2002). However, Neumann et al. (2001a) showed that sex ratios could significantly favor female offspring in vitro supporting a similar observation made by G.F. Mackay (unpublished cited in Schmolke 1974). It is possible that, in instances where populations of

beetles exhibit biased sex ratios, beetle larval diet affects emerging adult sex ratios. That possibility is also investigated here.

In this study, I report the longevity and reproductive success of newly emerged adult beetles assigned different diets. The pupation success of larvae reared on the same diets as their parents and sex ratios of the resulting adults were also analyzed. These data will shed light on the reproductive success and life history of beetles telling us if they are obligate or facultative scavengers of honey bee colonies; the possibility of their survival outside of honey bee colonies; and their longevity as adults, which may be crucial for beetle reproduction. All of these factors contribute to beetles' success in causing honey bee colony collapse.

Materials and Methods

Experiments were conducted at Rhodes University in Grahamstown, South Africa from February – October 2001. Beetles were obtained from infested colonies at Grahamstown and Port Elizabeth, South Africa and were reared in the laboratory according to standard methods, being fed a mixture of bee brood, honey and pollen combs, and water *ad lib* (Neumann et al. 2001a), and sexed according to standard protocols (Schmolke 1974).

Three recently emerged unfed adult males and females were put in pairs in plastic containers (11 × 11 × 9 cm) and provided with water *ad lib*. This was replicated five times for each of the following eight treatments: 1. control (no food); 2. empty brood comb (6 × 6 cm); 3. comb with honey (6 × 6 cm); 4. comb with pollen (6 × 6 cm); 5. comb with both honey and pollen in roughly equal volumes (6 × 6 cm); 6. brood comb containing live brood of all stages (6 × 6 cm); 7. rotten kei apples (*Dovyalis caffra*; $n = 4$); and 8. fresh kei apples ($n = 4$). All foodstuffs were frozen before use to kill any beetle eggs. As the supply of food in the feeding chambers was exhausted, an amount of food equivalent to the original amount was added to the containers. This was repeated as needed for the duration of the experiment. The feeding containers (with adult beetles and individual diets) were kept from light and at room temperature throughout the experiment.

To determine longevity, the number of live adults in each container was counted weekly until all adults were dead. Because I was interested in determining average maximum longevity (giving one an idea how long one can expect the longest-lived adults

to survive), I used data on the last date beetle adults were recorded alive in each container. When larvae feeding in the same containers as their parents reached the wandering phase prior to pupation (which normally occurs in the ground outside of honey bee colonies, Lundie 1940), they were transferred into containers with slightly moist soil (Neumann et al. 2001a) and were kept from light and at room temperature. Due to the high number of hatching larvae on the pollen diet, larvae reaching the wandering phase were put into several soil chambers to eliminate a possible larval density effect on pupation success (Neumann et al. 2001a). Adults emerging from the pupation chambers were sexed (Schmolke 1974). Because adult beetles often congregated under the soil surface in the pupation containers the contents of the containers were sifted in order to collect the mature adult beetles.

Data analysis

The number of larval and adult beetle offspring produced, ratios of emerging adults per larvae, and longevity of the parental adults in the food containers were compared between the treatments using ANOVAs and Newman-Keuls *post hoc* comparisons. The sex ratios of emerging adults were evaluated using χ^2 -tests. All calculations were performed using the software package Statistica[®] (Statistica 2001).

Results

A total of 13, 926 larvae were transferred into pupation containers across all diets and 8532 male and female adult beetles emerged in the pupation containers.

Diet effects on longevity

Diet affected the longevity of parental adults ($F = 45.2$; $df = 7, 32$; $P < 0.0001$). Adults fed brood or nothing (control) lived significantly shorter than adults fed all other diets (Table 4.1). Adults fed empty brood comb, fresh kei apples, and rotten kei apples had statistically similar longevities (Table 4.1). Honey-fed adults lived significantly longer than adults fed all other diets (Table 4.1) with the longest-lived adults surviving for 176 d. The longevity of pollen-fed adults was significantly different from those of all other diets and was second only to honey-fed adults (Table 4.1). Finally, longevities in honey-pollen

fed adults, fresh kei apple fed adults, and rotten kei apple fed adults were statistically similar (Table 4.1).

Diet effects on reproductive success

There were diet effects on the number of wandering larvae available to put into soil chambers ($F = 97.3$; $df = 7, 32$; $P < 0.0001$). No larvae were found in the control, empty brood comb, or honey diet containers (Table 4.1). The numbers of larvae produced from adults fed pollen, brood, or honey-pollen were significantly different from one another and from all other treatments (Table 4.1) with the pollen diet having the highest reproductive success followed by brood and honey-pollen respectively (Table 4.1). Adults feeding on both fresh kei apples and rotten kei apples did not produce significantly more larvae per 3 pairs of adults than any of the adults fed diets on which no larvae were produced (Table 4.1). After the 81st experimental day, an estimated number of > 5000 unidentified common pollen mites infested only the pollen diet containers. Upon mite infestation, the adult beetles feeding on the pollen diets stopped reproducing.

Diet effects on pupation success

Diet affected the number of adult beetles emerging from the soil pupation chambers ($F = 93.3$; $df = 7, 32$; $P < 0.0001$). Because no larvae were found in the control, empty brood comb, or honey containers (Table 4.1) no adults emerged from these diets. The numbers of emerged adults from larvae fed pollen, brood, or honey-pollen were significantly different from one another and from all other treatments (Table 4.1) with the pollen diet yielding more adult beetles followed by the diets brood and honey-pollen respectively. Adults emerging from larvae feeding on both fresh kei apples and rotten kei apples were not significantly more numerous than on those diets where no adults emerged (Table 4.1).

There were also diet effects on the average ratios of adults per larvae ($F = 5.0$; $df = 4, 18$; $P = 0.0072$). Adults per larvae ratios are the number of emerged adults from the number of larvae initially placed into their respective soil containers, or the proportion of larvae that pupated successfully. The adults per larvae ratios for the pollen, honey-pollen, and brood diets did not significantly differ from one another and they were higher for these diets than for all other diets. Additionally, the adults per larvae ratios for brood and fresh kei apples did not significantly differ from one another; neither did the adults per larvae ratios for the fresh kei apple and rotten kei apple diets (Table 4.1).

Diet effects on sex ratio

In all diets except for rotten apples, the sex ratios of adult beetle offspring were skewed towards females (Table 4.2). For the diets pollen ($\chi^2 = 21.8$; $df = 4$; $P = 0.0002$) and brood ($\chi^2 = 19.1$; $df = 4$; $P = 0.0008$), the number of emerging adult female beetles was significantly higher than the expected value of 50% of emerging adults being females. The number of emerging females did not statistically deviate from the expected value of 50% in honey-pollen ($\chi^2 = 6.9$; $df = 4$; $P = 0.1416$), fresh kei apple ($\chi^2 = 2.9$; $df = 4$; $P = 0.5725$), and rotten kei apple ($\chi^2 = 0.4$; $df = 4$; $P = 0.9817$) diets (Table 4.2).

Discussion

Diet effects on longevity

The data of Table 4.1 and that of Lundie (1940) show that adult beetles feeding on honey alone can live for over 5 months. Dadd (1985) states that carbohydrate (especially sugar) utilization is very important in insect longevity, which is consistent with the findings that honey-fed adults live the longest. So, it is possible that beetles can live in honey houses for at least 5 months and reproduce once locating acceptable food sources. Therefore, beekeepers should strive to maintain clean honey houses. Pollen fed adults were also long-lived (Table 4.1) indicating a need for beekeepers to properly store any frames or equipment that contains pollen.

Beetle adults feeding on honey-pollen, fresh kei apple, and rotten kei apple diets all lived less than those feeding on honey and pollen diets (Table 4.1), despite the fact that the former are also high in carbohydrates. However, I still show that adult beetles can live on diets of fruit alone for over 2 months, thus identifying a potential pathway for beetles from their native range in Africa to the USA and elsewhere via fruit transports on cargo ships.

Beetles living on empty brood comb survived for an average maximum of 49.8 d (Table 4.1), indicating nutritional foodstuffs in empty brood comb (Shimanuki et al. 1992), but not enough to support reproduction. Therefore, adult beetles are able to live on old comb for over a month, further strengthening recommendations (Hood 2000) to properly store beekeeping equipment.

Unfed adult beetles in this experiment had longevities similar to those found by others (Schmolke 1974; Pettis and Shimanuki 2000; Flügge 2001). Surprisingly, longevity in adult beetles feeding on brood diets did not differ significantly from those on control

diets. This is likely due to the rancid environment created in those containers. A possible improvement in design would have been to separate the parental adult beetles from the feeding larvae across all treatments.

Because of the findings, I recommend to beekeepers suffering from beetle infestations of their hives to properly store all equipment (especially combs) and to be assiduous in cleaning up rotten fruit piles and piles of discarded hive equipment.

Diet effects on reproductive success

The data (Tables 4.1 and 4.2) and that of others (Lundie 1940; Schmolke 1974) show that beetles can successfully reproduce on diets of brood, pollen, and mixtures of honey and pollen, all of which contain the proteins and carbohydrates essential for the maturation of larvae and adult reproduction (Dadd 1985). On average, pollen contains 24% protein (Buchmann 1986) and 27% carbohydrates (Schmidt and Buchmann 1992). These factors probably contributed to the high reproductive success on pollen diets, which raises the question why the honey-pollen diets were less efficacious.

A possible explanation may be that beetle feces causes honey to ferment (Lundie 1940; Schmolke 1974) creating an unhealthy environment in the chambers. I observed that fermented honey filled the bottoms of the plastic containers, possibly jeopardizing oviposition and larval health. The presence of unidentified pollen mites in the pollen chambers after day 81 likely inhibited further oviposition by female beetles, since no more larvae appeared after the initial mite infestations. Despite this, the number of larvae maturing on pollen was still significantly higher than on all other diets. It is a common practice among beekeepers to use in-hive pollen traps to collect pollen from foraging bees. The pollen is collected in an area of the trap that is separated (therefore unprotected from beetle invasion) from the bee colony. The data suggest that pollen traps should not be left in colonies for extended periods because of the beetles' ability to successfully reproduce in pollen, especially pollen that is unguarded.

Bee brood is another source of nutrients, and an analysis of *A. m. scutellata* sealed bee brood showed them to contain 20 - 35% protein, 50 - 62% carbohydrate and ash, and 10 - 18% lipid (Hepburn et al. 1979). Thus, the question emerges about the less than optimal reproductive success of beetles on bee brood. Beetle adults and larvae feeding on decomposing honey bee brood caused a rancid environment, which probably led to reduced oviposition and longevity on this diet.

Schmolke (1974) showed that female beetles do not oviposit on diets of honey, an observation in accord with the fact that beetle larvae never appeared in any honey container in this study. Although adult beetles were able to survive for great lengths on empty brood comb (see diet effects on longevity), no larvae occurred in any empty brood comb containers likely indicating the lack of any volume of foodstuffs in the comb to allow for beetle reproduction.

Adult beetles were able to reproduce on fresh and rotten kei apples which is consistent with other findings (Eischen et al. 1999b). Because larvae were not produced to the extent found in any other diet, it is likely that kei apples and other fruits (Eischen et al. 1999b) barely meet the minimum requirements needed for reproduction and larval growth. As in the brood and honey-pollen diets, the environment in the fruit containers became quite rancid shortly after the presence of larvae. This too could have had an effect on the number of larvae produced. Although the beetles were not able to reproduce in great numbers on fruit alone, the data suggest the possibility that beetles may reproduce on fruit in the wild in instances where no bee colonies are present.

Diet effects on pupation success

Larval diet also plays a critical role in their pupation success (Slansky and Scriber 1985). Because of this, adults per larvae ratio is the most critical value in determining the effects of diet on pupation success and not simply the number of adults emerging from the containers. The numbers of adults emerging from the soil chambers paralleled numbers of larvae reaching the wandering phase.

The three diets (pollen, honey-pollen, and brood) with the highest adults per larvae ratios also yielded the highest number of larvae. Because these adults per larvae ratios did not significantly differ, it is inferred that all three diets are equally efficacious for pupal fitness. The adults per larvae ratios from brood and fresh kei apples did not differ significantly, although more larvae were found in the brood containers (Table 4.1). Fewer larvae were produced in the fresh kei apple diets (Table 4.1) providing abundant food for the small number of larvae. The sheer abundance of larvae produced on brood (Table 4.1) gives a great reproductive benefit to adult beetles feeding on brood as opposed to feeding on kei apples.

The poor adults per larvae ratios in both apple diets likely reflect a non-optimum nutrition accumulation by larvae in those diets (Slansky and Scriber 1985; Dade 1985). Although beetles can successfully reproduce on fruits alone they are not optimal diets for

beetles as shown by others (Eischen et al. 1999b). Nonetheless the results show that beetles are only facultative scavengers because they can reproduce on a diet of fruit alone. Because beetles can feed and reproduce on fruits, it is possible that fruit transporters in the United States could spread beetles to un-infested areas.

Diet effects on sex ratio

There were significantly more females than males in the brood and pollen diets (Table 4.2), the same for which there were significantly more larvae than in other diets (Table 4.1). Laugé (1985) states that density and crowding of larvae can act indirectly on sex ratio because of food competition and selective mortality that usually benefits female offspring. In all diets except for rotten apples, there were more female offspring than male, which is consistent with other findings (G.F. MacKay unpublished, cited in Schmolke 1974; Schmolke 1974; Neumann et al. 2001a; Ellis et al. 2002).

Female insects tend to be heavier than males (Slansky and Scriber 1985) indicating a general nutrient accumulation needed for their role as egg-layers. Female beetles do indeed tend to be bigger and heavier than males (Schmolke 1974; Ellis et al. 2002). Slansky and Scriber (1985) state that this generally results from increased food consumption by female larvae. In cases of crowding, female larvae might be able to out-compete male larvae when feeding, leading to the selective mortality of male larvae. Although never shown to be significant, Ellis et al. (2002) reported beetle populations with numerically higher female ratios found in natural populations of beetles.

Table 4.1. Treatment means and mean separations for number of wandering larvae produced per 3 mating pairs of adult small hive beetles put into soil chambers; number of adult beetles emerging from soil chambers; average ratio of emerging adults per larvae per diet; and longevity of parental adults.

diet	no. larvae	no. adults	adults per larvae	longevity (d)
control	0a	0a		9.6 ± 4.0a
empty brood comb	0a	0a		49.8 ± 10.2b
honey comb	0a	0a		167.2 ± 8.7c
pollen comb	1,773.8 ± 294.4 (8869)b	1,096.4 ± 236.4 (5482)b	0.64 ± 0.19a	123.4 ± 17.5d
honey-pollen comb	337.0 ± 134.3 (1685)c	230.6 ± 53.3 (1153)c	0.73 ± 0.19a	81.0 ± 15.7e
brood comb	597.4 ± 217.5 (2987)d	353.6 ± 55.5 (1768)d	0.65 ± 0.23a,b	9.0 ± 0a
fresh kei apples	50.6 ± 55.7 (253)a	15.2 ± 16.5 (76)a	0.32 ± 0.13b,c	63.6 ± 30.4b,e
rotten kei apples	26.4 ± 23.7 (132)a	10.6 ± 14.0 (53)a	0.24 ± 0.25c	58.6 ± 30.0b,e

Values are means ± standard deviations; $n = 5$ replicate containers for all values; numbers given in brackets are total individuals produced per diet. Column means followed by the same letter are not different at the $\alpha \leq 0.05$ level. Mean separations were determined by Newman-Keuls *post hoc* comparisons.

Table 4.2. Sex data on small hive beetles emerging from pupation chambers having been reared on different diets as larvae.

diet	adult females	adult males	sex ratio female to male	<i>P</i> values for no. adult females
pollen	587.2 ± 137.6 (2936)	493.4 ± 112.9 (2467)	1.19 ± 0.06	0.0002
honey-pollen comb	122.0 ± 23.6 (610)	102.4 ± 31.5 (512)	1.23 ± 0.18	0.1416
brood comb	188.6 ± 34.7 (943)	142.4 ± 28.4 (712)	1.34 ± 0.17	0.0008
fresh apples	9.6 ± 10.8 (48)	5.6 ± 5.7 (28)	1.62 ± 0.40	0.5725
rotten apples	5 ± 7 (25)	5.6 ± 7.0 (28)	0.86 ± 0.25	0.9817

Values are mean ± standard deviation; $n = 5$ replicate containers for all values; numbers given in parentheses, where applicable, are total number of individuals emerging per diet. Variables are number of emerging adult females per diet replication; number of emerging adult males per diet replication; average sex ratio of adult females/adult males per diet; and *P* values for number adult females, as determined by χ^2 -tests.

Chapter 5: The Effects of Soil Type, Moisture, and Density on the Pupation Success of Small Hive Beetles

Abstract - The effects of six different soil types, two moisture extremes ('wet' and 'dry'), and two soil densities ('packed' and 'tilled') on the pupation success of small hive beetles were tested. Further, the effects of soil type and beetle sex on the time spent pupating was determined. A total of 3000 beetle larvae were placed in the moist soil treatments (wet/packed and wet/tilled), of which 2746 eclosed. Additionally, 3000 larvae were placed in the dry soil treatments (dry/packed and dry/tilled), of which none eclosed. Eclosion rates in all soils except one were similar. For every soil, there were significantly more eclosing beetles in the wet treatments than in the dry ones. Eclosion rates of larvae burrowing into moist soils ranged between 92 - 98%. Female beetles pupated slightly faster than male beetles. Soil type affected the length of time beetles spent pupating but average eclosion between soil types occurred within a tight range. The data suggest that biological requirements of beetles may limit/exacerbate their reproductive potentials in various soil environments.

Introduction

Small hive beetle adults and larvae live on various foodstuffs in the honey bee nest including pollen, honey, and bee brood (Lundie 1940; Schmolke 1974; Chapter 4) and the effects of these diets on beetle longevity and reproductive success are well documented (Chapter 4). After feeding, beetle larvae exit the colony ('wandering' phase) and pupate in soil in close proximity to the hive (Lundie 1940; Schmolke 1974; Pettis and Shimanuki 2000).

That different soil types might affect various aspects of beetle pupation biology is of standing interest (Lundie 1940; Schmolke 1974) because of possible larval vulnerability when burrowing into the soil. Further, Lundie (1940) suggested that investigations would probably show the absence of beetles in certain geographical areas due to the physical and/or chemical nature of soils and he even speculated that soil moisture was a critical factor in determining pupation success. Schmolke (1974) partially tested this idea and found that soil moisture, but not soil type, was correlated with pupation success but his experiments did not involve a large sample size or adequately replicated trials.

In this study, I tested the effects of six different soils (soils A - F; Table 5.1), two moisture extremes ('wet' and 'dry'), and two soil densities ('packed' and 'tilled') on the pupation success of small hive beetles (= the number of successfully eclosing beetles). I further determined the effect of soil type and beetle sex on pupation time. The data suggest that biological requirements of beetles may limit/exacerbate their reproductive potentials in various environments.

Materials and Methods

Experiments were conducted at Rhodes University in Grahamstown, South Africa, March - August 2003. Six different soils were collected from agricultural areas in the chicory belt surrounding Alexandria, South Africa and their constituents are reported in Table 5.1 (determined by Central Analytical Laboratories, Somerset West, South Africa). For each soil type, about 950 ml of loose, moistened (moist upon collection) soil were put into each of 20 plastic containers, totaling 120 containers. Four different treatments of 5 replicates per treatment were prepared for each soil (6 soil types \times 4 treatments \times 5 replicates = 120 containers). Treatments consisted of 1) dry/tilled, 2) dry/packed, 3) wet/tilled, and 4) wet/packed soils.

Dry soils were prepared by pouring 1000 ml of distilled water through the soil-filled containers in order to pack the soils naturally (all containers had holes in the bottom to facilitate drainage) and then oven-drying at 85°C for about 6 weeks to constant dry weight. Half of the dry soil containers were reserved for the dry/packed soil treatment (so no further manipulation was necessary) and soils in the other containers were loosened by hand mixing (dry/tilled soil treatment). The wet/tilled and wet/packed treatments were established identically except after being oven-dried, 500 ml of distilled water were poured into each container to remoisten the soils. The soils were then allowed to drain for about 3 weeks (to produce soils with an average of 10% water by weight: determined by weighing a sub-sample of each moist soil and then oven-drying the sample to constant dry weight). Half of the moistened containers contributed to the wet/packed treatment and the wet/tilled treatment was created by hand mixing the remaining moistened soil as for the dry/tilled treatment.

Fifty beetle larvae (reared *in vitro* on diets of bee brood, honey, and pollen) were introduced into each soil container. The soils were kept in a room with an ambient temperature maintained at $24.6 \pm 1.3^\circ\text{C}$ (mean \pm standard deviation). The containers were monitored daily and adult beetles were collected upon eclosion.

In order to determine the effects of soil type and beetle sex on the time spent pupating, about 950 ml of loose soil was put into each of 5 containers for each soil type as before. The soils were moist (about 11% water by weight: moisture content determined as before) when allotted to containers (the soils were moist when collected from the field); they underwent no drying. The soils were packed slightly by tapping the containers on a hard surface. Fifty beetle larvae were placed into each of the soil containers and eclosing beetles were collected and sexed (Schmolke, 1974).

Data analysis

The number of eclosed beetles was analyzed by ANOVA recognizing soil type (soils A - F), moisture (dry or wet), and density (packed or tilled) as main effects. Because every interaction term for this analysis was significant (Table 5.2), I tested the effects of moisture and density on the number of eclosed beetles within each soil type using ANOVAs. Further, the effects of beetle sex (male or female) and soil type on time spent pupating (d) and average number of eclosed beetles were analyzed using ANOVA. All analyses were conducted using Statistica (Statistica 2001). Where necessary, means were compared using Tukey's test and all differences were accepted at $\alpha \leq 0.05$.

Results

Pupation success

A total of 3000 beetle larvae were placed on the moist soil treatments (wet/packed and wet/tilled), of which 2746 eclosed (91.5% pupation rate). Additionally, 3000 larvae were placed on the dry soil treatments (dry/packed and dry/tilled), of which none eclosed.

There were significant moisture \times density, moisture \times soil, density \times soil, and moisture \times density \times soil interactions for the full ANOVA (Table 5.2). Due to the low eclosion rate of beetles in soil D's wet/packed treatment, I removed this soil from the analysis and re-ran a partial ANOVA. After removing this soil from the analysis, there were no significant effects of soil on the number of eclosing beetles ($F = 1.7$; $df = 4, 80$; $P = 0.1697$) and no significant soil \times moisture interactions ($F = 1.7$; $df = 4, 80$; $P = 0.1697$). This indicates that eclosion rates in soil D were different from those in all other soils and that eclosion rates in the other soils did not differ. However, all other variables and interactions significantly affected the number of eclosing beetles ($0.0000 \leq P \leq 0.0372$). Because of this, I tested the effects of moisture and density on the number of eclosed beetles independently for each soil (Table 5.3).

Moisture significantly affected the number of eclosed beetles in all soils (Table 5.3) and in every case, there were significantly more eclosing beetles in the wet treatments than in the dry ones (Table 5.4). No beetles eclosed in any of the dry treatments (Table 5.4). Soil density did not affect the number of eclosed beetles in soils C, E, and F (Tables 5.3 and 5.4).

There were significant moisture \times density interactions in soils A, B, and D (Table 5.3) so density was analyzed by moisture for these soils (Table 5.5). For these soils, there were no differences between the number of eclosed beetles in the dry treatments that were packed as opposed to tilled (Table 5.5). In wet treatments of soils A and B, there were significantly more eclosing beetles in packed conditions than in tilled ones (Table 5.5). The trend was reversed in the wet treatment of soil D with there being significantly more eclosing beetles in tilled conditions than in packed ones (Table 5.5).

All 1500 larvae introduced to the dry/tilled treatment were dead within 9 d of being introduced. None successfully burrowed into the soil. Some larvae (<10%) in the dry/packed treatment survived longer than 3 weeks but all had died by week 4. Larvae

introduced to wet/packed and wet/tilled treatments had begun burrowing within 5 minutes. The only two exceptions were most larvae (>90%) in soil E (which had successfully burrowed into the soil within 2 d) and larvae in soil D. Larvae in soil D experienced a high mortality rate, but only after about 28 d of wandering around in the soil chambers (they were not burrowing into the soil). However, those larvae that survived past day 28 eventually burrowed into the soil from days 32-38, and eclosed from days 43-67. Therefore, the length of time spent pupating in soil D's wet/packed treatment varied greatly and overall mortality was high (Table 5.5).

Length of time spent pupating

A total of 1500 beetle larvae were placed on the six different soil types, of which 1468 eclosed (97.9% pupation rate). There were soil ($F = 35.0$; d.f. = 5, 48; $P < 0.0001$) and sex ($F = 45.5$; d.f. = 1, 48; $P < 0.0001$) effects but no soil \times sex interactions ($F = 0.9$; d.f. = 5, 48; $P = 0.5095$) on the length of time spent pupating. Beetles in soils F, D, and E pupated the fastest (ordered by increasing time) followed by slower pupating beetles in soils A, C, and B (ordered by increasing time, Table 5.6). The difference in time spent pupating between the fastest (soil F) and slowest (soil B) pupating beetles was only 1.4 d. All following values are mean \pm standard error; n . Female beetles (22.9 ± 0.1 d; 30) pupated faster than male beetles (23.3 ± 0.1 d; 30) but only by an average of less than half a day. There were neither soil effects ($F = 0.4$; d.f. = 5, 48; $P = 0.8645$) nor soil \times sex interactions ($F = 2.3$; d.f. = 5, 48; $P = 0.0602$) for the average number of eclosing male and female beetles (Table 5.6). However, sex did affect this variable ($F = 13.6$; d.f. = 1, 48; $P = 0.0006$) with significantly more female beetles (26.3 ± 0.7 d; 30) eclosing than male beetles (22.9 ± 0.7 d; 30).

Discussion

With the exception of soil D, beetles pupated equally well in all of the tested soil types. For whatever reason (presence/absence of certain stimuli), beetle larvae were not burrowing into the wet/packed treatment of soil D until after about 50% of the larvae had died. However, once the surviving larvae did burrow, most successfully pupated. Regardless, soil type per se did not appear to significantly influence overall pupation success. This is contrary to what Lundie (1940) suggested but confirms similar observations made by Schmolke (1974). The data further suggest that soil density (plowed

or tilled) has mixed effects on pupation success. When density does affect pupation, it likely affects successful burrowing and the construction of pupation chambers by the larvae.

The only soil condition that consistently affected the number of eclosing beetles was soil moisture. Both Lundie (1940) and Schmolke (1974) speculated that this would be the case. Lundie (1940) found, while rearing beetles *in vitro*, that pupal mortality was high when there was a free passage of air through the soil chambers (thus drying out the soil). Schmolke (1974) found that when soils were dry, no beetle eclosion occurred (no adults eclosed over the 3 dry soil types)(Schmolke 1974). He also showed that pupation rates of beetles are high in moist (but not soaked) soils (Schmolke 1974).

Why beetles need moist soils in which to pupate is unclear, especially since moist soils can also carry with them a host of potential problems for pupating beetle larvae. Some soil-dwelling parasites (fungi for example: cf. Schmid-Hempel 1998) thrive better under moist conditions. However, the effects of parasites/pathogens such as fungi were likely minimized in this study by drying the soils prior to the experiment.

Most studies concerning the influence of moisture on various insect life-stages have been conducted on insect eggs (Tauber et al. 1986). However, high soil moisture has been shown to play a role in the termination of larval diapause in some insects (Tauber et al. 1986). Rainfall, to an extent, moderates soil moisture and its effects on various insect stages can be direct or indirect (Speight et al. 1999). However, humidity is related to temperature, which was not tested in this study. Pupal stages are often unaffected by humidity over very wide ranges (Gordon 1984) so soil moisture probably would not have an effect on pupating beetles; it may, instead, only affect the larvae's decision and/or ability to burrow. Regardless, it remains unclear whether or not certain insect groups require moisture to induce diapause (as in beetle larvae deciding to burrow into the soil in order to pupate).

Beetle larvae placed onto dry/packed soils would not burrow, probably indicating that the soils were impenetrable (in contrast, larvae could burrow into the wet/packed soils). Moisture serves to make soils more penetrable to burrowing larvae. Further, all larvae placed on dry/tilled soil died within 9 d. These larvae probably desiccated or asphyxiated because of the dry/dusty conditions in the containers. Clearly larvae can live much longer when the soil is moist. Even if some larvae were unable to burrow into the wet/packed treatment of soil D, the data show they could survive some time (over 3 weeks if the soil is dry and packed and over a month if it is moist) and possibly migrate great

distances from the hive in search of suitable soil in which to pupate. Therefore, the increased longevity larvae experience when on moist soils may add to their success at finding a suitable soil in which to pupate.

I further determined that soil type (when lightly packed) did affect the length of time beetles pupate although, average eclosion took place within a tight range (Table 5.2). Had I measured the effects of soil type when packed on the length of time beetles spent pupating, the differences would have likely been exacerbated. The data indicate that it may take longer for beetles to burrow into and make pupal chambers in certain soil types (thus being energetically expensive), because larvae did not always burrow immediately in the more packed soils. Therefore, the actual extension to the time spent pupating may be due in part to it taking 1) larvae longer to burrow and make a suitable chamber in which to pupate and 2) eclosing adults longer to dig out of the soil. Both factors are likely dictated by soil composition.

That female beetles pupate faster than male beetles is not totally unexpected. Female beetles are generally larger and heavier than males (Schmolke 1974; Ellis et al. 2002) possibly reflecting increased food consumption during the larval stage (Slansky and Scriber 1985), which in turn could decrease developmental time. Further, sex ratios of eclosing adults favored females, a finding that has been documented for beetles both *in vitro* and *in vivo* (Schmolke 1974; Neumann et al. 2001a; Ellis et al. 2002; Chapter 4).

The data possibly explain why beetles are not usually problematic to honey bee colonies in their native range of sub-Saharan Africa. Because a large portion of Africa (except equatorial Africa) is semi-arid to arid, negative beetle effects on honey bee colonies in these locations have likely been minimized by lower pupation rates for the beetles. Further, beetles do not naturally occur north of the Sahara. No doubt, the Sahara has proven a formidable barrier to natural beetle dispersal. Even if soil moisture does not limit beetle distribution in the beetle's introduced range (North America and Australia), it likely limits their impact on managed honey bee colonies there. In the end, wherever soils remain moist for much of the time (as in temperate climates where rainfall is moderate), beetle pupation success will likely be high.

Perhaps the most pertinent data presented in this study are the high pupation rates reported for the 4500 larvae burrowing into the various moist soil treatments. This rate ranged between 92 - 98% indicating that in moist soils (regardless of soil type) nearly all larvae burrowing into the soil will eclose as adults. This is especially troublesome for migratory beekeepers who regularly move their hives for pollination services. In such

circumstances, hives are often placed within 10 m of fields managed for fruit/vegetable production where soils are kept tilled and irrigated thus providing beetles ideal conditions in which to pupate.

Table 5.1. The percentage clay, silt, and sand of the 6 test soils.

soil	% clay	% silt	% sand	type
A	10	8	82	loamy sand
B	46	42	11	silty clay
C	11	14	78	sandy loam
D	7	16	77	loamy sand
E	32	39	29	clay loam
F	9	24	67	sandy loam

These soils represent a range of soil types found in agricultural areas of the southwestern area of the Eastern Cape Province, South Africa. Soil composition was determined by Central Analytical Laboratories, Somerset West, South Africa.

Table 5.2. Analysis of variance testing the effects of moisture, density, soil, and associated interactions on the number of eclosed small hive beetles.

source	df	<i>F</i>	<i>P</i>
moisture	1	5804.9	<0.0001
density	1	6.2	0.0142
soil	5	11.0	<0.0001
moisture × density	1	6.2	0.0142
moisture × soil	5	11.0	<0.0001
density × soil	5	16.3	<0.0001
moisture × density × soil	5	16.3	<0.0001

Table 5.3. Analysis of variance testing effects of moisture (m), density (d), and moisture \times density (m \times d) on the number of eclosed small hive beetles within each soil.

soil	source	df	<i>F</i>	<i>P</i>
A	m	1	21032.8	<0.0001
	d	1	11.0	0.0044
	m \times d	1	11.0	0.0044
B	m	1	1035.3	<0.0001
	d	1	8.3	0.0110
	m \times d	1	8.3	0.0110
C	m	1	14433.0	<0.0001
	d	1	0.2	0.6291
	m \times d	1	0.2	0.6291
D	m	1	137.5	<0.0001
	d	1	17.9	0.0006
	m \times d	1	17.9	0.0006
E	m	1	14864.5	<0.0001
	d	1	2.3	0.1470
	m \times d	1	2.3	0.1470
F	m	1	2077.1	<0.0001
	d	1	2.3	0.1466
	m \times d	1	2.3	0.1466

Table 5.4. Moisture and density effects on the number of eclosed adults in each soil type.

soil	moisture		density	
	wet	dry	packed	tiled
A	48.1 ± 0.5a	0b	24.6 ± 8.2	23.5 ± 7.8
B	45.9 ± 1.9a	0b	25.0 ± 8.3	20.9 ± 7.1
C	48.8 ± 0.4a	0b	24.3 ± 8.1a	24.5 ± 8.2a
D	36.0 ± 5.2a	0b	11.5 ± 4.8	24.5 ± 8.2
E	48.0 ± 0.4a	0b	24.3 ± 8.1a	23.7 ± 7.9a
F	47.8 ± 1.1a	0b	23.1 ± 7.8a	24.7 ± 8.2a

Values are mean ± standard error; $n = 10$ for all data. Row totals within moisture (wet or dry) or density (packed or tilled) followed by the same letter are not different at the $\alpha \leq 0.05$ level. There were significant interactions between moisture and density for soils A, B, and D so analyses on density were run separately by moisture for these variables and are reported in Table 5.5.

Table 5.5. Moisture × density data for all soil types.

soil	wet		dry	
	packed	tilled	packed	tilled
A	49.2 ± 0.4a	47.0 ± 0.5b	0a	0a
B	50.0a	41.8 ± 2.9b	0a	0a
C	48.6 ± 0.6	49.0 ± 0.5	0	0
D	23.0 ± 6.1a	49.0 ± 0.4b	0a	0a
E	48.6 ± 0.6	47.4 ± 0.5	0	0
F	46.2 ± 2.1	49.4 ± 0.2	0	0

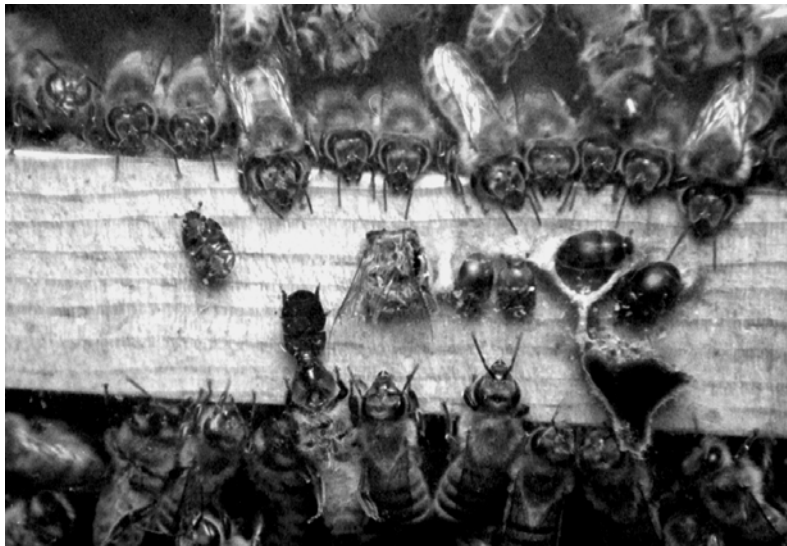
Data are the number of eclosed adults (mean ± standard error; $n = 5$ for all data). There were significant interactions between moisture and density for soils A, B, and D so analyses were run separately by moisture for these variables. For these soils, row totals within moisture type (wet or dry) followed by the same letter are not different at the $\alpha \leq 0.05$ level.

Table 5.6. Effects of soil type on time spent pupating (d) and average number of eclosing small hive beetles (average of males and females eclosing).

soil	time spent pupating (d)	number of adults
A	23.0 ± 0.2a	24.5 ± 0.9
B	23.9 ± 0.1b	24.4 ± 1.2
C	23.4 ± 0.1c	23.4 ± 2.1
D	22.8 ± 0.1a, d	24.9 ± 1.3
E	22.9 ± 0.1a	25.5 ± 1.2
F	22.5 ± 0.1d	24.8 ± 0.9

The time spent pupating is the amount of time (d) between the larvae burrowing into the soil and eclosion. The number of adults is an average of the male and female beetles eclosing. Values are mean ± standard error; $n = 10$ replicates per soil. For time spent pupating, means followed by the same letter are not different at the $\alpha \leq 0.05$ level (compared using Tukey's multiple range test).

Section II: Interactions Between Small Hive Beetles and Honey Bees



Chapter 6: Small Hive Beetles Survive in Honey Bee Prisons by Behavioral Mimicry

Abstract – In this chapter, the results of a simple experiment to determine whether honey bees feed their small hive beetle nest scavengers are reported. Honey bees incarcerate the beetles in cells constructed of propolis (plant resins) and continually guard them. The longevity of incarcerated beetles greatly exceeds their metabolic reserves. I demonstrate that survival of small hive beetles derives from behavioral mimicry by which the beetles induce the bees to feed them trophallactically.

Introduction

As a defense against small hive beetles, African honey bees confine beetles to cracks and crevices (where the beetles naturally hide) throughout the colony (Neumann et al. 2001b, Chapters 7 and 8). Incarcerated beetles lack access to the combs because worker bees continuously guard the entrances of such areas and prevent many attempted escapes of beetles. Nonetheless, despite no access to food in the combs, imprisoned beetles may survive for 2 months or longer (Neumann et al. 2001b). However, their survival is not due to metabolic reserves because starved beetles die within 2 weeks (Chapter 4; Neumann et al. 2001b). So how do beetles survive their tenure as honey bee prisoners deprived of foodstuffs?

While documenting bee-beetle interactions, I observed what appeared to be trophallactic encounters between guard bees and imprisoned beetles (Figure 6.1). The beetles characteristically approach guard bees, extend their heads towards and make antennal contact with guard bees (mimicking normal honey bee trophallaxis). This behavior of the beetles often elicits aggressive reactions from the guard bees, which try to grab the beetles with their mandibles. However, if the beetles are persistent enough, they seem to induce the bees to regurgitate a drop of honey, which the beetles appear to take directly from the mouthparts of the bees. Here I report the results of a simple experiment to determine whether long-term survival of incarcerated beetles derives from a form of behavioral mimicry which induces honey bees to feed them by trophallaxis.

Materials and Methods

To test the bee-beetle trophallaxis hypothesis, I established a three frame observation hive with 100 beetles confined to the upper third of the hive. The lower two-thirds of the hive housed a feeder, a normal comb, and a small but robust colony of Cape honey bees. The colony was partitioned between the upper third and the lower two thirds by metal gauze that prevented mingling of bees and beetles, but did allow antennal and mouthpart contact, hence trophallaxis, between bees and beetles through the gauze mesh. The bees were fed a sugar/water solution containing the vital stain Rose Bengal. The beetles were confined to the upper chamber, and had no direct access to any source of the dyed sugar solution except by being fed trophallactically through the metal gauze by the

bees. Twenty-four hours after their introduction, I collected a sample of 50 beetles and squeezed them to discharge their viscera which were analyzed by UV-spectroscopy.

Results

Twenty out of the 50 beetles sampled showed red-stained emissions that were analyzed spectrophotometrically (Figure 6.2). The spectral analysis indicated that the emissions were indeed stained with Rose Bengal, unequivocally establishing that Cape honey bee workers trophallactically feed the beetles.

Discussion

The spectrograms (Figure 6.2) confirm that beetles use behavioral mimicry to induce trophallaxis from honey bees. The beetles are not always successful in soliciting food and it often takes them more than five attempts to induce trophallaxis. Moreover, after 24 h only 20 out of the 50 beetles sampled contained signs of the Rose Bengal. So, the behavioral mimicry of the beetles, while adaptive, is clearly not fail-safe. Antennation of honey bees by beetles is an easily observed behavior; but this does not exclude the possibility that bee-beetle interactions are also modulated perhaps by chemical mimicry as occurs, for example, in the death's head hawkmoth (Mortiz et al. 1991). Regardless, it is probably tactile stimuli that succeeds in causing bees to regurgitate honey, as is often the case in other relationships between symbionts and their social insect hosts (Wilson 1971).

Usually only minimal tactile stimuli are enough to coerce a social insect into feeding an arthropod intruder (Wilson 1975). Free (1956) observed that more soliciting and offering of trophallactic behavior by nestmate honey bees are directed at one another's heads than at any other part of their bodies and that a freshly served head is sufficient to elicit either the soliciting or offering behavior. Free's study also demonstrated that bee heads lacking antennae were less effective at soliciting than those having antennae. Antennae are so important that when Free inserted imitation wire antennae into the heads lacking antennae, he was able to induce regurgitation. The antennae, therefore, serve as releasers and guides for the bees when they touch each other with their lower mouthparts.

Hölldobler (1967a,b, 1970) also demonstrated the nature of the minimal tactile required. Ants just finishing meals and searching for nestmates with which to share crop contents were most susceptible to regurgitation stimuli. A nestmate only needs to tap the

donor ant's body lightly with its antennae or forelegs, the donor turns, the recipient taps the labium lightly, and the donor regurgitates. This is extremely similar, in nature, to what small hive beetles do and because the beetles, which use their antennae to solicit food, are able to solicit regurgitated food from the bees must mean that they have mastered this form of behavior.

To place the behavioral mimicry of the beetles in a wider context, it must be remembered that there are many other cases where social insects, particularly ants, are tricked by beetles into feeding them. In more adapted instances, such as in some ant-aphid interactions, the relationship is one of virtual aphid husbandry by the ants (Hölldobler and Wilson 1990). In contrast, the behavior of the small hive beetle is simply a case of honey bee exploitation, albeit of a novel kind. Further implications from these findings will be discussed in Chapter 14.



Figure 6.1. Trophallaxis between a worker honey bee and a confined small hive beetle. Notice the bee's antennae touching the beetle's thorax. Photography by Gerald Kastberger and Otmar Winder.

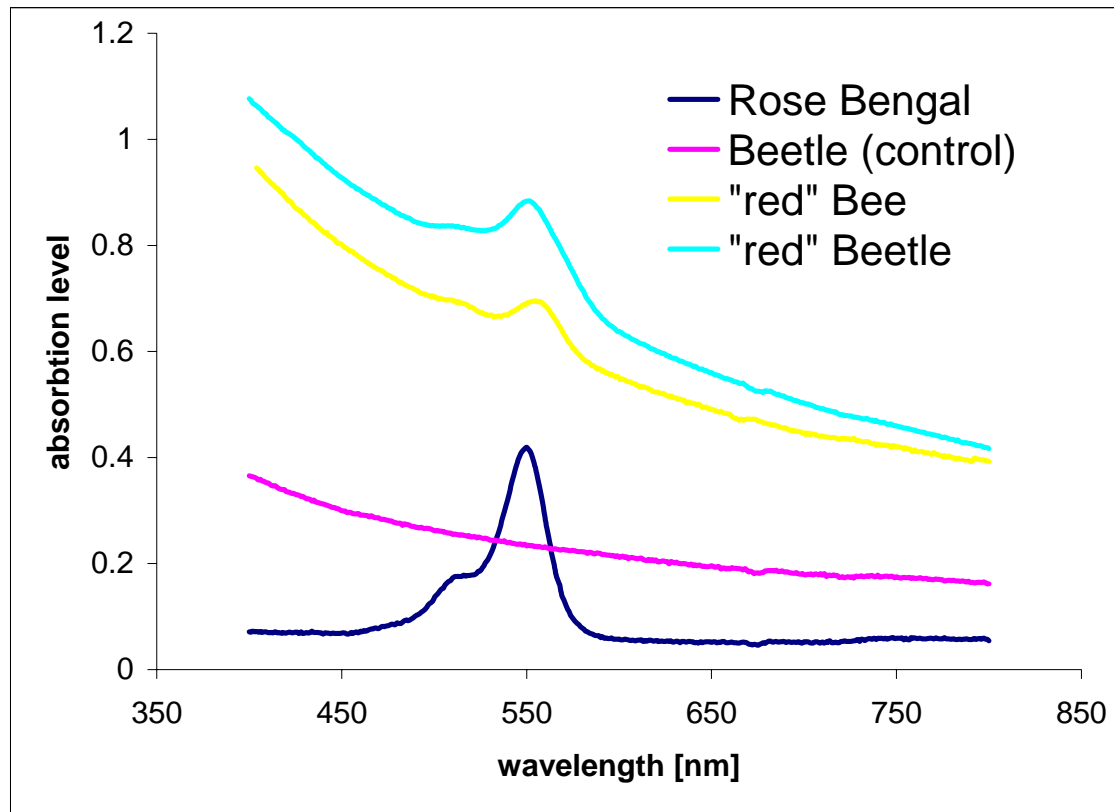


Figure 6.2. UV spectrograph of a Rose Bengal standard, “red bees,” and “red beetles,” and a control beetle. Rose Bengal has a characteristic peak at 550 nm, the red bee at 551 nm, the red beetle at 551nm (the latter two being within error range of the spectrophotometer) and the control beetle no peak at all.

Chapter 7: Confinement of Small Hive Beetles by Cape and European Honey Bees

Abstract - In this study small hive beetle and Cape and European honeybee behaviors that are associated with confinement of small hive beetles are quantified in an effort to understand why Cape bees can withstand large beetle infestations while European bees cannot. Colony and time (morning and evening) differences in these behaviors and intra-colonial beetle distributions are also described. For Cape bees, the almost complete absence of time \times colony interactions indicated that guard bee and beetle behavioral trends were similar for all colonies during morning and evening. There were more beetle guards (worker bees who guard beetle confinement sites) during evening, which was likely an effort to keep increasingly active beetles contained. About one-fifth of the beetles were found at the comb periphery although the colonies suffered no ill effects. Although beetles reached the combs, the bees were able to keep the beetles from accessing brood and pollen stores where they can reproduce. Concerning European bees, there were significant colony differences in a number of confinement behaviors suggesting that successful confinement of beetles by European bees may vary between colonies. In response to increased beetle activity during the evening, there was an increase in the number of prison guard bees during evening. Additionally, European bees successfully kept most (~93%) beetles out of the combs at all times. The data show that beetle confinement behaviors of Cape and European bees do not differ significantly. This suggests that confinement is more likely a general defense against small nest intruders or an initial defense against invading beetles and not the sole reason African subspecies of honeybees are virtually immune to beetle infestations while European bees are not.

Introduction

The existence of sophisticated confinement behaviors in social insects has only recently been described in honeybees (Hepburn and Radloff 1998; Neumann et al. 2001b; Solbrig 2001). African subspecies of honeybees imprison small hive beetles in an effort to minimize harmful beetle reproductive outbreaks. Cape honeybees confine beetles in prisons often made of propolis, a sticky tree resin. The confinement process lasts 1-4 days and bees have sophisticated guarding strategies, including a high degree of aggressiveness toward beetles, for limiting the escape of beetles during confinement (Neumann et al. 2001b; Solbrig 2001). As a result, beetle access to honey, pollen, and bee brood in the combs, where beetle reproduction potentials are high (Chapter 4), is restricted.

In sharp contrast, host colonies of European-derived honeybees in the United States (and more recently in Australia) have proven highly susceptible to infestation by beetles (Elzen et al. 1999; Hood 2000). Although European honeybees also confine beetles (Figure 7.1; Ellis 2002), their confinement efforts do not successfully contain beetles below harmful levels. One explanation for differing susceptibilities of European and African bees to beetle depredation could be that the relative efficacy of beetle confinement and guarding might differ between subspecies (Neumann et al. 2001b; Solbrig 2001). However, studies on confinement behavior of African and European bees have not been conducted in a manner that would allow a direct comparison between confinement schemes of both subspecies of honeybees.

In this study, I quantify the confinement behavior of Cape and European honey bees to determine if there are any significant differences in their behavioral repertoires that could explain their highly different susceptibilities to beetle infestations. The data aid in determining if confinement is 1) essential to the relative immunity of African bees to beetles, 2) an initial defense of European and African bees against invading beetles, or 3) a more general defense by honeybees against small colony intruders. Further, I describe morning and evening differences in these behaviors as honeybees are less active at evening (Kaiser 1988; Moritz and Southwick 1992) and the nocturnal activity peaks of small hive beetles recorded in Africa (Solbrig 2001) may be present in the United States as well. I also discuss hypotheses concerning who the bees guarding the beetle confinement sites are. Finally, I record intra-colonial distribution of small hive beetles in order to determine how effective beetle confinement by Cape and European honeybees is (the efficacy is gauged by how well the bees limit beetle access to the combs).

Materials and Methods

Experiments on Cape honey bees were conducted in Grahamstown, South Africa (January - March 2003) using four observation hives (each containing two frames of brood, one of honey, about 8,000 bees, and a laying queen (all unrelated)). All bees, combs, and queens were from established colonies of Cape honeybees and in a geographic region where beetles commonly occur. A transparent grid, which divided each side of the colony into 160 squares (each square was 5 cm²) was used to define intra-colonial locations that consisted of the top wall (above the uppermost frame), bottom board, front wall, back wall, and rest of the colony (among the combs).

Twenty-five, unsexed beetles were randomly introduced into each hive 2 - 3 d after the hives were established. Random assignments of beetles minimized the chance of sex-specific behaviors biasing the results. Hives were monitored twice daily (at about 08:00 and 20:00 hours) once beetles were confined and guarding behavior by bees was apparent (usually after 24 h). During the 20:00 hour, hives were observed under red-light conditions in order to minimize disturbances.

At each monitoring interval, the observer moved across the top row of the grid, from left to right, and then down one block (or one 5 cm² area) in the grid, followed by another left to right motion. This pattern was followed from top to bottom on both sides of the hive. Neither beetles nor bees were counted twice in any observation because guard bees and beetles do not readily move between locations in the nest. The entire procedure lasted about 30 minutes per hive. Data were collected for 17 subsequent days for 2 colonies, 16 for a third, and 11 for a fourth.

Intra-colonial distribution, behavior, and number of imprisoned beetles, and number and behavior of worker honeybee guards were documented. Observed behaviors of confined beetles included resting (not moving), antennal contact with guard bees (touching prison guard bee antennae with their antennae), trophallactic contact with guard bees (feeding from the mouth of guard bees), and mating (Figure 7.1). Guard bee behavior included biting at (extending head and lunging towards beetles with open mandibles, or attacking beetle), antennal contact with (touching beetle antennae), and trophallactically feeding confined beetles (regurgitating a drop of honey from their mandibles which beetles imbibe), and biting the area around beetle prisons (chewing at prison walls with mandibles)(Figure 7.1). Neumann et al. (2001b), Solbrig (2001), and data in Chapter 6

collectively described these same beetle and guard bee behaviors in African honeybees. All behaviors are reported as the proportion of observed individuals performing a given behavior. This is especially important when reporting beetle behavior, as the total number of introduced beetles ($n = 25$) was not always observed.

Experiments on European honey bees of mixed origin were conducted in Warren County Georgia, USA (August - September 2001) with only slight modifications. Three observation hives were used (opposed to four as with Cape bees) and all hives were observed for 17 consecutive days.

Data analysis

Guard bee and beetle behavioral data were analyzed for Cape and European honey bees with a repeated measure ANOVA design recognizing time (morning and evening) and colony (Cape colony 1, 2, 3, or 4; European colony 1, 2, or 3) as main effects. Where analyzed data were proportions (as with bee and beetle behaviors), the data were transformed before analyses using $\arcsin\sqrt{\text{proportion}}$ to stabilize the variance. Where applicable, means were separated using Tukey's test. Where there were interactions between time and colony for certain variables for each bee subspecies, the variables were analyzed by colony using independent variable t-tests. Beetle intra-colonial distribution was tested for differences between times using Pearson's χ^2 tests. All differences were accepted at the $\alpha \leq 0.05$ level and all analyses were conducted using the software package Statistica (2001).

Results

I propose using 'confinement' as opposed to 'social encapsulation' (previously proposed by Neumann et al. 2001b) because encapsulation implies that trapped beetles are actively encased in prison-like structures (of wax or propolis) especially made for beetles. Actually, I found that beetles are restricted (or confined) to these locations by guard bees but are not completely encapsulated and sealed off; such locations can be voids, crevices, or cracks created by propolis deposits of the kind that beetles seek out, giving the impression that propolis was used especially for beetle confinement. Newly introduced or free-roaming beetles run from bee aggression into cracks and crevices throughout the colony (Schmolke 1974) and it is at these places bees station guards. Such sites can even

include individual cells within the combs. Bees do not actively encapsulate beetles; they only station guards where invading beetles hide.

Cape honey bees

Confinement dynamics

The number of guard bees per beetle was analyzed by colony because of the significant interaction between time and colony (Table 7.1). Although the interaction term was significant, the difference in the number of guard bees per beetle during morning and evening between each colony was a matter of differing magnitudes between each colony (colony 1 morning: 0.74 ± 0.10 , evening: 1.11 ± 0.09 ; colony 2 morning: 0.54 ± 0.06 , evening: 0.75 ± 0.05 ; colony 3 morning: 0.81 ± 0.07 , evening: 1.38 ± 0.12 ; colony 4 morning: 0.69 ± 0.04 , evening: 1.07 ± 0.07 , mean \pm standard error, $n = 11, 17, 17$, and 16 for colonies 1, 2, 3, and 4 respectively). Indeed there were significantly more guard bees per confined beetle during evening than morning in all colonies [$4.8 \leq t \leq 5.6$; $df = 10, 16, 16, 15$ (colonies 1, 2, 3, and 4 respectively); $0.00004 \leq P \leq 0.0002$]. Colony effects (Table 7.1) indicated that colonies 1 and 3 had more guard bees per beetle than colony 2, with the number of guard bees per beetle in colony 4 not being different from those in any other colony (Table 7.2).

There were no time effects or time \times colony interactions for the number of confinement sites (prisons) per colony or the number of beetles per prison although there were colony effects for both (Table 7.1). The number of prisons per colony was significantly higher in colony 4 than in all other colonies (Table 7.2). Colonies 1, 2, and 3 had similar numbers of prisons (Table 7.2). Further, colony 2 had the most beetles per prison, followed by colonies 3, 1, and 4 in decreasing order (Table 7.2).

The number of guard bees per prison varied significantly by time and colony (Table 7.1). There were more guard bees per prison during evening than during morning (Table 7.3). Further, colonies 3 and 2 had the highest number of guard bees per prison followed by colonies 1 and 4 in decreasing order (Table 7.2). There were no time \times colony interactions for this variable (Table 7.1).

Beetle behavior

There were colony and time effects for the proportion of beetles resting and making antennal contact with guard bees (Table 7.1). Beetles rested more in colonies 4 and 2,

followed in colonies 1 and 3 in decreasing order (Table 7.2). There were also more beetles resting during the morning than evening (Table 7.3). Beetles made antennal contact with guard bees more in colony 3 than in 4 (Table 7.2). The proportion of beetles making antennal contact with guard bees in colonies 1 and 2 was not different from those in any other colony. Further, more beetles made antennal contact with guard bees during evening than morning (Table 7.3). There were no time \times colony interactions for either variable (Table 7.1).

There were no colony effects or time \times colony interactions for the proportion of beetles being fed by guard bees (Table 7.1); however, there was a time effect (Table 7.1). Beetles were fed more during evening than morning (Table 7.3). Also, there were no time effects or time \times colony interactions for the proportion of beetles mating although there were colony effects (Table 7.1). Beetles mated more in colony 3 than in colonies 2 and 4 (Table 7.2). The proportion of beetles mating was not different between colony 1 and any other colony (Table 7.2).

Guard bee behavior

There was a colony effect but no time effect or time \times colony interaction for the proportion of guard bees biting at confined beetles (Table 7.1). Higher proportions of guard bees in colony 2 were biting at beetles than in 3 and 4 (Table 7.2). The proportion of guard bees biting at beetles was not different between colony 1 and any other colony (Table 7.2).

There were no colony or time effects or time \times colony interactions for the proportion of guard bees making antennal contact with beetles or biting the area around beetle prisons (Table 7.1). Further, there were no time effects or time \times colony interactions for the proportion of guard bees feeding beetles. There was an overall colony effect for the proportion of guard bees feeding beetles (Table 7.1) although Tukey mean comparison tests indicated that means for no two colonies were different at the $\alpha \leq 0.05$ level (Tukey colony separation values: $0.0714 \leq P \leq 0.9864$).

Intra-colonial beetle distribution

Intra-colonial beetle distributions remained consistent between morning and evening ($\chi^2 = 4.6$; $df = 4$; $P = 0.3256$)(Table 7.4). The highest proportions of beetles were found on the bottom board (~33%) and front wall (~23%) of the colonies, followed by the

combs (~22%), back wall (~18%), and top wall (~4%; percentages are average percentages of beetles found in each location during morning and evening based on the data in Table 7.4). Although 22% of the beetles were found among the combs, most (>90% based on visual estimations) of the beetles reaching the combs were kept out of the brood, honey, and pollen areas by bee aggression and were being guarded by bees in empty cells around the comb periphery.

European honey bees

Confinement dynamics

Because there were significant interactions between time and colony for the number of guard bees per confined beetle and number of beetles per prison, these variables were analyzed by colony (Table 7.5). There were significantly more guard bees per confined beetle during evening than morning for colonies 1 and 2 and the same trend was found for colony 3 (Table 7.6). Furthermore, colony 3 (1.20 ± 0.05 , $n = 17$) had significantly more ($F = 23.6$; $df = 2, 48$; $P < 0.0001$) guard bees per confined beetle than did colonies 1 (0.75 ± 0.05 , $n = 17$) or 2 (0.68 ± 0.06 , $n = 17$).

Trends were different between colonies for the number of beetles per prison during morning and evening with the number decreasing in colony 1 but remaining nearly the same in colonies 2 and 3 (Table 7.6). Further, all 3 colonies had significantly different ($F = 33.9$; $df = 2, 48$; $P < 0.0001$) numbers of beetles per prison (colony 1: 4.16 ± 0.24 ; colony 2: 2.29 ± 0.08 ; colony 3: 3.37 ± 0.15 ; $n = 17$ for all colonies).

Each colony had a significantly different number of beetle prisons (Table 7.7)(colony 1: 4.24 ± 0.25 ; colony 2: 7.18 ± 0.23 ; colony 3: 5.24 ± 0.18 ; $n = 17$ for all colonies), yet the number of prisons did not differ significantly between morning and evening (Tables 7.7 and 7.8). The number of guard bees per prison (Table 7.7)(colony 1: 3.00 ± 0.24 ; colony 2: 1.46 ± 0.12 ; colony 3: 3.93 ± 0.19 ; $n = 17$ for all colonies) did not differ significantly between colonies. However, there were significantly more guard bees per prison during evening than morning (Tables 7.7 and 7.8).

Beetle behavior

There was no significant interaction between time and colony for the proportion of confined beetles resting (Table 7.7). Further, the proportion of confined beetles resting in every colony (colony 1: 0.78 ± 0.02 ; colony 2: 0.73 ± 0.04 ; colony 3: 0.81 ± 0.02 ; $n = 17$

for all colonies) did not significantly differ (Table 7.7). There was, however, a time effect (Table 7.7) with more beetles resting during morning than during evening (Table 7.8).

No significant differences were found with respect to the proportion of confined beetles making antennal contact with guard bees in the colonies (colony 1: 0.14 ± 0.03 ; colony 2: 0.10 ± 0.02 ; colony 3: 0.15 ± 0.03 ; $n = 17$ for all colonies) and during morning and evening (Tables 7.7 and 7.8). Beetles were being fed by guard bees in similar proportions across all colonies (Table 7.7)(colony 1: 0.03 ± 0.01 ; colony 2: 0.06 ± 0.03 ; colony 3: 0.06 ± 0.01 ; $n = 17$ for all colonies). Further, proportionately more confined beetles were being fed at evening than during the morning (Tables 7.7 and 7.8). The same proportion of confined beetles was mating in every colony (colony 1: 0.02 ± 0.01 ; colony 2: 0.01 ± 0.01 ; colony 3: 0.02 ± 0.01 ; $n = 17$ for all colonies) and during morning and evening (Tables 7.7 and 7.8).

Guard bee behavior

There were significant interactions between time and colony for the proportion of guard bees biting at and feeding confined beetles, and biting the area around beetle prisons so these variables were analyzed by colony (Table 7.5). There was a significantly lower proportion of guard bees biting at confined beetles during evening than morning for colony 2 (Table 7.6). Although not significant, the trend was reversed in colonies 1 and 3 (Table 7.6). The presence of colony effects ($F = 3.4$; $df = 2, 48$; $P = 0.0412$) indicated that colony 1 (0.37 ± 0.05 , $n = 17$) had a significantly smaller proportion of guard bees biting at confined beetles than colony 2 (0.56 ± 0.05 ; $n = 17$). With 0.49 ± 0.05 , $n = 17$, the proportion of guard bees biting at confined beetles in colony 3 was not different from that in the other two colonies.

Colony 3 had a significantly higher proportion of guard bees feeding beetles during evening than morning (Table 7.6). Although not significant, the same trend was found in colony 2 but the reverse was found in colony 1 (Table 7.6). The proportion of guard bees feeding confined beetles was similar in all colonies ($F = 0.3$; $df = 2, 48$; $P = 0.7644$)(colony 1: 0.06 ± 0.02 ; colony 2: 0.05 ± 0.01 ; colony 3: 0.04 ± 0.01 ; $n = 17$ for all colonies).

Colonies 1 and 3 had significantly higher proportions of guard bees biting the area around beetle prisons during morning than evening (Table 7.6). This trend was significantly reversed in colony 2 (Table 7.6). Further, no colony differences were found ($F = 1.0$; $df = 2, 48$; $P = 0.3930$) in the proportion of guard bees biting the area around

beetle prisons (colony 1: 0.28 ± 0.05 ; colony 2: 0.19 ± 0.04 ; colony 3: 0.30 ± 0.04 ; $n = 17$ for all colonies).

The same proportion of guard bees were making antennal contact with confined beetles in each colony (colony 1: 0.15 ± 0.03 ; colony 2: 0.15 ± 0.02 ; colony 3: 0.09 ± 0.01 ; $n = 17$ for all colonies) and during morning and evening (Tables 5 and 6).

Intra-colonial beetle distribution

There were time differences for beetle intra-colonial distribution ($\chi^2 = 14.1$; $df = 4$; $P = 0.0070$). Although the proportion of confined beetles remained nearly the same in four of five intra-colonial positions during morning and evening (Table 7.4), there was a migration of beetles from the bottom board to other areas of the hive during evening (Table 7.4). Despite this, there were always more beetles in the nest periphery than among the combs. Indeed, only ~7% of beetles were found among the combs during both morning and evening. Most (~75%) beetles were confined on the colonies' front and back walls (Table 7.4). The remaining beetles were mainly located on the top wall of the hive (~15%) with a few (~5%) being on the bottom board (Table 7.4).

Discussion

The data highlight a number of quantitative differences in confinement efforts between the four Cape and three European colonies tested. However, due to the almost complete absence of time \times colony interactions in the Cape colonies, behavioral trends for Cape honey bees were similar for every colony during morning and evening suggesting that all colonies handled beetles similarly (unlike in European colonies). I found evidence for the existence of circadian rhythms in small hive beetles, as they were more active in the evening rather than morning among both groups of bees. Additionally, both European and Cape bees were able to keep most of the beetles out of the combs. The data, therefore, indicate that overall confinement schemes of Cape and European subspecies of honeybees are not markedly different.

The number of guard bees per beetle for each Cape colony was within the same range reported for guard bees per beetle in European-derived colonies. Further, trends were similar between both European and Cape bees for the number of beetle prisons and

guard bees per beetle, both increasing during evening in European and Cape colonies. Increased guard bee presence during evening may be a response to increased beetle activity during evening in an effort to keep increasingly active beetles confined. If so, that more beetle guards are present in the colony during evening may suggest that some foragers engage in prison guarding, although most foragers remain inactive during evening (Kaiser 1988; Moritz and Southwick 1992) and evidence suggests that foraging and guarding subpopulations are distinct groups of bees (Moore et al. 1987; Breed et al. 1990).

Indeed, who the Cape and European beetle guards are remains unclear, especially since experiments show that guarding behavior, in general, can be further compartmentalized based on indications that genetically and behaviorally different bees perform different subsets of guarding duties (including those workers entrance guarding, 'soldiering,' and perhaps even beetle guarding)(Breed et al. 1990). Therefore, it is possible that bees guarding beetle confinement sites are a distinct subpopulation of 'guard' bees, as described by Breed et al. (1990), not previously considered. However, if increasing guard bee numbers during evening is a result of workers changing from another task to guarding beetles, then it is more likely that young foragers (as opposed to older foragers: Robinson et al. 1992) are reverting to guarding than are nest workers advancing. Guards are less likely to engage in behavior typical of young bees within the nest than in behaviors of old, field bees (Seeley 1985; Trumbo et al. 1997).

The number of beetle prisons per Cape colony was only moderately higher than the range reported for European bees. It is unlikely that the number of prisons per colony affects the success bees have at containing beetle outbreaks. Instead beetle density may be more crucial (see Chapter 8) and the number of beetles per prison (or density per prison) for Cape bees was similar to that reported for European bees.

Beetle activity in Cape (Solbrig 2001) and European colonies increased during evening (indicated by there being fewer beetles resting in the evening than during day). In both locations, the increase in beetle activity corresponded to an increase in their soliciting for food and getting fed in the evening by the bees. These findings make the trophallactic relationship between bees and beetles quite unclear. Beetles are obviously afforded a benefit by the behavior, as they are able to feed while being confined away from foodstuffs in the nest (Chapter 6). However, in both European and Cape colonies, bees feed beetles more when beetle activity increases and this may indirectly benefit the bees as fed beetles may be less likely to escape confinement. Likewise, increased beetle activity during evening may reflect an increased proportion of beetles soliciting for food and thus being

fed (without any control by the bees). Regardless, the trophallactic relationship between bees and beetles is likely exploitive like those of other insects that frequent social insect colonies (Hölldobler and Wilson 1990).

The proportion of guard bees biting at beetles in the three European colonies was similar to those found in the four Cape bee colonies. This suggests that aggressive behaviors by guard bees of both Cape and European origin are very similar quantitatively. However, aggressive similarities between both bee subspecies may only hold true in instances of beetle confinement as worker bees from African colonies are generally more aggressive toward free-roaming beetles than are European bees (Elzen et al. 2001).

In perimeter guarding behavior, guard bees often ‘comb’ the prison perimeter with their front legs while biting the walls of the prison (‘prison wall-working’). What this behavior accomplishes is unclear. The bees may be checking the solidity of the prison walls. Regardless, Cape bees never reached the level of this behavior that European bees did. However, that Cape bee guards spend less time wall working may suggest they spent more time actively guarding at the prison entrances with front legs raised in the air like that done at the colony entrance.

In earlier work on beetle confinement by Cape bees (Solbrig 2001) it was found that most beetles were restricted to areas on the bottom board. Lundie (1940) noted this for beetles in African colonies as well. The data (Table 7.4) and that of Schmolke (1974) do not support this although more beetles were found on the bottom board in Cape colonies than in the European ones (however, intra-colonial beetle location in observation hives may not accurately reflect beetle location in full, Langstroth-style hives). The proportion of beetles restricted to various intra-colonial locations in Cape colonies did not vary with time although it did in European ones. This may indicate that at low intra-colonial populations, beetles move around more freely in European, but not Cape, colonies. Others suggest that strong colonies in general are able to prevent beetles from accessing the comb (Lundie 1952a, b; Swart et al. 2001). Regardless, about one-fifth of the beetles were found at the comb periphery in Cape colonies (as opposed to only ~7% in the European ones) although the colonies suffered no ill effects. Therefore, although beetles reached the combs in Cape colonies, the bees were able to keep the beetles from accessing brood and pollen stores where they can reproduce.

Why confinement is present in European honeybees when they have only recently been exposed to beetles can only be hypothesized. It is possible that confinement is similar to existing guard bee behaviors (guarding at the colony entrance) and that going from

entrance guarding to social confinement is inherent. However, this would not explain why European guard bees display remarkably similar behaviors (trophallaxis, aggression, etc.) to those of African ones. Further, confinement may reflect a more general adaptation towards small intruders that has not previously been considered. These possibilities will be further explored in the General Discussion (Chapter 14).

The findings indicate that beetle confinement by Cape bees is not significantly different from that in European bees except that confinement behavior seems to be more consistent over time within Cape colonies than within European ones. This further suggests that confinement may be a general defense against small nest intruders or the first line of defense against beetle invaders (hypotheses 2 and 3) and not the sole reason Cape bees are virtually immune to beetle infestations while European bees are not (hypothesis 1). The study indicates that additional factors external to confinement efforts (such as soil moisture, colony strength, and bee hygienic behavior towards beetle eggs) are probably responsible for Cape bee immunity and European bee susceptibility to beetles. Regardless, honeybees possess the ability to confine colony intruders and this behavior may be universal, although to varying degrees, among African and European honeybees.

Table 7.1. Analysis of variance testing effects of colony (c), time (t), and time × colony (t × c) on confinement dynamics, confined beetle behavior, and guard bee behavior for Cape bee colonies.

variable	source	df	<i>F</i>	<i>P</i> > <i>F</i>
confinement dynamics				
number of guard bees per beetle	c	3	8.2	0.0001
	t	1	93.9	<0.0001
	t×c	3	4.0	0.0119
number of prisons per colony	c	3	17.4	<0.0001
	t	1	1.8	0.1831
	t×c	3	1.8	0.1538
number of beetles per prison	c	3	44.6	<0.0001
	t	1	1.4	0.2358
	t×c	3	1.0	0.4197
number of guard bees per prison	c	3	6.3	0.0009
	t	1	26.4	<0.0001
	t×c	3	0.9	0.4247
beetle behavior				
resting	c	3	6.5	0.0008
	t	1	11.4	0.0013
	t×c	3	1.5	0.2169
making antennal contact with guard bees	c	3	2.9	0.0413
	t	1	37.0	<0.0001
	t×c	3	0.5	0.7110
getting fed by guard bees	c	3	2.7	0.0543
	t	1	9.8	0.0028
	t×c	3	0.4	0.7816
mating	c	3	4.4	0.0079
	t	1	1.7	0.1915
	t×c	3	1.0	0.4137
guard bee behavior				
biting at beetles	c	3	6.3	0.0009
	t	1	3.7	0.0611
	t×c	3	0.5	0.6908
making antennal contact with beetles	c	3	1.0	0.3795
	t	1	1.4	0.2346
	t×c	3	1.0	0.4018
feeding beetles	c	3	2.8	0.0464
	t	1	1.5	0.2193
	t×c	3	1.1	0.3574
prison wall-working	c	3	2.7	0.0523
	t	1	2.0	0.1663
	t×c	3	1.2	0.3137

Table 7.2. Colony effects for confinement dynamics, and guard bee and confined beetle behavior for Cape bee colonies.

	colony 1	colony 2	colony 3	colony 4
confinement dynamics				
number of guard bees per beetle	0.92 ± 0.08a	0.65 ± 0.04b	1.09 ± 0.09a	0.88 ± 0.05a, b
number of beetle prisons per colony	7.05 ± 0.51a	6.50 ± 0.24a	6.00 ± 0.30a	10.19 ± 0.52b
number of beetles per prison	2.25 ± 0.16a	3.87 ± 0.16b	2.60 ± 0.15a	1.61 ± 0.09c
number of guard bees per beetle prison	1.97 ± 0.19a, b	2.52 ± 0.22a	2.62 ± 0.24a	1.45 ± 0.14b
beetle behavior				
resting	0.67 ± 0.04a	0.75 ± 0.02a, b	0.64 ± 0.03a	0.83 ± 0.02b
making antennal contact with guard bees	0.19 ± 0.03a, b	0.16 ± 0.02a, b	0.23 ± 0.03a	0.11 ± 0.02b
getting fed by guard bees	0.06 ± 0.01a	0.03 ± 0.01a	0.06 ± 0.01a	0.02 ± 0.01a
mating	0.05 ± 0.02a, b	0.01 ± 0.01a	0.05 ± 0.01b	0.01 ± 0.01a
guard bee behavior				
biting at beetles	0.53 ± 0.04a, b	0.66 ± 0.03b	0.50 ± 0.04a	0.46 ± 0.03a
antennal contact with beetles	0.16 ± 0.02a	0.17 ± 0.02a	0.15 ± 0.02a	0.11 ± 0.02a
feeding beetles	0.07 ± 0.02a	0.03 ± 0.01a	0.05 ± 0.01a	0.02 ± 0.01a
biting the area around beetle prisons	0.11 ± 0.03a	0.16 ± 0.02a	0.12 ± 0.02a	0.07 ± 0.02a

For beetle and guard bee behavior, data are the proportion of individuals observed doing the particular behavior. Values are mean ± standard error. For all colony 1, 2, 3, and 4 data, $n = 11, 17, 17,$ and 16 respectively. Row totals followed by the same letter are not different at the $\alpha \leq 0.05$ level. Means were compared using Tukey's test.

Table 7.3. Time effects on confinement dynamics, confined beetle behavior, and guard bee behavior for Cape bee colonies.

	morning	evening
confinement dynamics		
number of guard bees per beetle*	0.69 ± 0.03	1.07 ± 0.05
number of beetle prisons per colony	7.26 ± 0.37a	7.60 ± 0.33a
number of beetles per prison	2.70 ± 0.15a	2.56 ± 0.15a
number of guard bees per beetle prison	1.73 ± 0.11a	2.61 ± 0.18b
beetle behavior		
resting	0.76 ± 0.02a	0.69 ± 0.02b
making antennal contact with guard bees	0.12 ± 0.01a	0.22 ± 0.02b
getting fed by guard bees	0.03 ± 0.01a	0.05 ± 0.01b
mating	0.03 ± 0.01a	0.02 ± 0.01a
guard bee behavior		
biting at beetles	0.58 ± 0.03a	0.50 ± 0.03a
antennal contact with beetles	0.15 ± 0.02a	0.15 ± 0.01a
feeding beetles	0.04 ± 0.01a	0.04 ± 0.01a
biting the area around beetle prisons	0.13 ± 0.02a	0.10 ± 0.01a

For beetle and guard bee behaviors, data are the proportion of individuals observed doing the particular behavior. Values are mean ± standard error; $n = 61$ for all data. Where applicable, row totals followed by the same letter are not different at the $\alpha \leq 0.05$ level. *This variable was analyzed by colony because of a significant time × colony interaction; therefore, Tukey's test is not applicable.

Table 7.4. Proportion of beetles confined to various intra-colonial locations during morning and evening for Cape and European honey bee colonies.

location	Cape bees		European bees	
	morning	evening	morning	evening
top wall of hive	0.03 ± 0.01	0.05 ± 0.01	0.15 ± 0.02	0.15 ± 0.02
bottom board of hive	0.34 ± 0.03	0.32 ± 0.03	0.05 ± 0.02	0.01 ± 0.01
front wall of hive	0.23 ± 0.03	0.23 ± 0.03	0.26 ± 0.03	0.28 ± 0.03
back wall of hive	0.17 ± 0.02	0.19 ± 0.02	0.47 ± 0.03	0.48 ± 0.03
combs	0.23 ± 0.03	0.21 ± 0.03	0.07 ± 0.01	0.08 ± 0.01

Values are mean ± standard error; $n = 61$ for all Cape bee data and $n = 51$ for all European bee data.

Table 7.5. Analysis of variance testing on prison dynamics and guard bee behaviors for which there were time × colony interactions for European bee colonies.

variable	colony	t	df	<i>P</i> < <i>F</i>
prison dynamics				
number of guard bees per beetle	1	5.2	16	0.0001
	2	7.5	16	<0.0001
	3	0.6	16	0.5881
number of beetles per prison	1	1.9	16	0.0704
	2	1.4	16	0.1933
	3	0.1	16	0.9592
guard bee behavior				
biting at beetles	1	1.4	16	0.1741
	2	2.7	16	0.0152
	3	0.2	16	0.8296
feeding beetles	1	1.2	16	0.2519
	2	1.8	16	0.0928
	3	2.9	16	0.0095
biting the area around beetle prisons	1	2.9	16	0.0098
	2	2.1	16	0.0499
	3	2.6	16	0.0178

Because of the interaction, analyses of time effects for these variables were run by colony using independent variable t-tests.

Table 7.6. Time \times colony interactions for prison dynamics and guard bee behavior for European bee colonies.

	colony 1		colony 2		colony 3	
	morning	evening	morning	evening	morning	evening
prison dynamics						
number of guard bees per beetle	0.58 \pm 0.06a	0.93 \pm 0.07b	0.46 \pm 0.06a	0.91 \pm 0.08b	1.17 \pm 0.08a	1.23 \pm 0.07a
number of beetles per prison	4.65 \pm 0.42a	3.66 \pm 0.19b	2.23 \pm 0.10a	2.35 \pm 0.12a	3.38 \pm 0.24a	3.36 \pm 0.19a
guard bee behavior						
biting at beetles	0.31 \pm 0.08a	0.42 \pm 0.06a	0.65 \pm 0.06a	0.47 \pm 0.06b	0.48 \pm 0.07a	0.50 \pm 0.06a
feeding beetles	0.09 \pm 0.03a	0.04 \pm 0.01a	0.03 \pm 0.02a	0.07 \pm 0.02a	0.02 \pm 0.01a	0.07 \pm 0.02b
biting the area around beetle prisons	0.35 \pm 0.08a	0.21 \pm 0.07b	0.17 \pm 0.06a	0.21 \pm 0.06a	0.40 \pm 0.07a	0.20 \pm 0.05b

Analyses were run separately by colony for these variables. For guard bee behavior, data are the proportion of individuals observed doing the particular behavior. Values are mean \pm standard error; $n = 17$ for all data. Row totals within colony followed by the same letter are not different at the $\alpha \leq 0.05$ level.

Table 7.7. Analysis of variance testing effects of colony (c), time (t), and time × colony (t × c) on prison dynamics, beetle behavior, and guard bee behavior for European bee colonies.

variable	source	df	<i>F</i>	<i>P</i> > <i>F</i>
prison dynamics				
number of beetle prisons per colony	c	2	31.7	<0.0001
	t	1	0.2	0.6895
	t×c	2	2.7	0.0769
number of guard bees per beetle prison	c	2	41.0	<0.0001
	t	1	12.1	0.0011
	t×c	2	1.9	0.1542
beetle behavior				
resting	c	2	2.4	0.0999
	t	1	7.8	0.0073
	t×c	2	2.6	0.0832
making antennal contact with guard bees	c	2	1.3	0.2775
	t	1	0.6	0.4434
	t×c	2	0.5	0.6076
getting fed by guard bees	c	2	0.9	0.3967
	t	1	9.7	0.0031
	t×c	2	2.1	0.1364
mating	c	2	0.0	0.9568
	t	1	0.6	0.4273
	t×c	2	1.0	0.3918
guard bee behavior				
antennal contact with beetles	c	2	1.4	0.2608
	t	1	0.8	0.3667
	t×c	2	0.6	0.5594

Table 7.8. Time effects on prison dynamics, beetle behavior, and guard bee behavior for European bee colonies.

	morning	evening
prison dynamics		
number of beetle prisons per colony	5.51 ± 0.27a	5.59 ± 0.23a
number of guard bees per beetle prison	2.47 ± 0.24a	3.13 ± 0.17b
beetle behavior		
resting	0.81 ± 0.02a	0.73 ± 0.03b
making antennal contact with guard bees	0.12 ± 0.03a	0.14 ± 0.01a
getting fed by guard bees	0.02 ± 0.01a	0.08 ± 0.02b
mating	0.01 ± 0.01a	0.02 ± 0.01a
guard bee behavior		
antennal contact with beetles	0.13 ± 0.02a	0.13 ± 0.01a

For beetle and guard bee behaviors, data are the proportion of individuals observed doing the particular behavior. Values are mean ± standard error; $n = 51$ for all data. Row totals followed by the same letter are not different at the $\alpha \leq 0.05$ level.

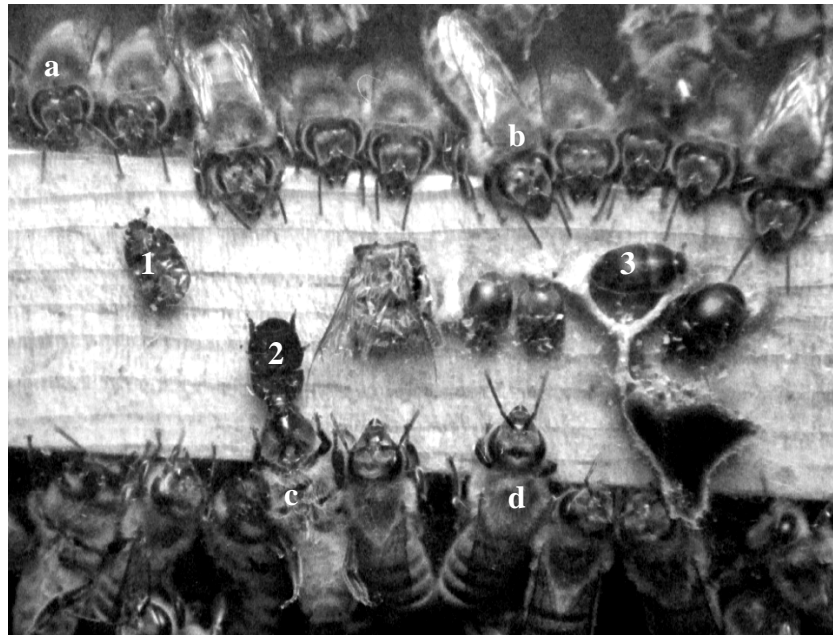


Figure 7.1. Examples of guard bee and confined beetle behavior. Guard bees are labeled “a–d” and confined beetles are labeled “1-3”. Documented guard bee behavior included: a) biting the area around beetle prisons (prison wall-working), b) trying to grab beetles, c) feeding beetles (trophallaxis), and d) guarding beetles (note that the front legs of this bee are raised). Confined beetle behavior included: 1) approaching a guard bee to make antennal contact, 2) getting fed by guard bees, and 3) resting.

Chapter 8: Confinement Behavior of Cape and European Honey Bees in Relation to Population Densities of Small Hive Beetles

Abstract – In this study, the effects of increasing small hive beetle populations on guarding behavior of Cape and European honey bees were quantified. For Cape bees, there were more confinement sites (prisons) at higher (50 beetles per colony) rather than lower (25 beetles per colony) beetle densities. The number of beetles per prison did not change with beetle density. There were more guard bees per beetle during evening than morning. Neither guard bee nor beetle behavior varied with beetle density or over time. Forty-six percent of all beetles were found among the combs at the low beetle density and this increased to 58% at the higher one. In neither instance were beetles causing depredation to host colonies. For European bees, there were more beetle prisons at the higher beetle density; but the number of beetles per prison did not change. Beetles solicited food more actively at the higher density and during evening. Only 5% of all beetles were found among the combs at the low density but this percentage increased 5-fold at the higher one. Within the limits of the experiment, guarding behavior of Cape honey bees is relatively unaffected by increasing beetle density (even if significant proportions of beetles reach the combs) while it is affected in European honey bees.

Introduction

As discussed in Chapter 7, the initial defense used by colonies of African and European honey bees against invading small hive beetles is a confinement scheme where beetle movement is restricted by guard bees who keep the beetles detained in cracks and crevices throughout the colony (Hepburn and Radloff 1998; Neumann et al. 2001b; Chapter 7). In an attempt to explain European bee susceptibility and African bee immunity to depredation caused by beetles, initial studies suggested that confinement schemes by European bees might be less efficacious than those in African ones (Solbrig 2001; Neumann et al. 2001b). However, recent evidence suggests (see Chapter 7) that at low intra-colonial beetle densities, confinement behaviors of African and European honey bees do not differ significantly.

Despite similarities in fundamental confinement behaviors of African and European honey bees, African subspecies of honey bees may handle increasing, intra-colonial beetle populations differently from their European counterparts. Because ‘infested’ African colonies rarely host large populations of beetles while infested European colonies often do, the overall success or failure of beetle confinement by both kinds of honey bees may be dependent on intra-colonial beetle density. Here I report the effects of increasing beetle density on beetle confinement and guarding behavior of Cape and European honey bees. The data allow for comparisons to be made between confinement schemes of Cape and European honey bees and ultimately place the efficacy of these behaviors as resistance mechanisms to beetles in context.

Materials and Methods

Experiments were conducted on three colonies in observation hives [each containing two frames of brood, one of honey, about 8,000 bees, and a laying queen (all unrelated)] in Grahamstown, South Africa (February - March 2003). All bees, combs, and queens were from established colonies of Cape honey bees and in a geographic region where beetles commonly occur. A transparent grid, which divided each side of the colony into 160 squares (5 cm² each square), was used to define intra-colonial locations that consisted of the top wall (above the uppermost frame), bottom board, front wall, back wall, and rest (among the combs) of the colony.

Twenty-five, randomly-collected beetles (to minimize the possibility of sex-specific behaviors biasing the results) were introduced into two of the colonies and fifteen days later, the colonies were monitored twice daily at approximately 08:00 and 20:00 hours (under red-light conditions) for three days. On the fourth day of observations, 25 more beetles were added to both colonies and on days 5-7, the colonies were monitored again. For the third colony, a procedure similar to that described above was conducted, except initial monitoring began 1 day after the introduction of beetles into the colony. At each monitoring interval, the observer moved across the top row of the grid, from left to right, and then down one block (or one 5 cm² area) in the grid, followed by another left to right motion. This pattern was followed from top to bottom on both sides of the hive. Neither beetles nor bees were counted twice in any observation because guard bees and beetles do not readily move between prison areas. The entire procedure lasted approximately 30 minutes per hive.

Intra-colonial distribution, behavior, and number of imprisoned beetles, and number and behavior of worker honey bee guards (guarding at prison entrances) were recorded. Beetle behavior included resting, mating, and antennal or trophallactic contact with guard bees. Guard bee behavior included biting, antennating, and trophallactically feeding beetles, and prison wall-working (all behaviors have been previously described for Cape and European honey bees: cf. Chapter 7; Neumann et al. 2001b; Solbrig 2001).

Experiments on honey bees of mixed-European origin (from Athens, Georgia, USA: at the time of the study, beetles had not yet been discovered in Athens) were conducted in Warren County Georgia, USA (August - September 2001) with only one slight modification. Initial observations on all 3 colonies began 15 days after the introduction of beetles.

Data analysis

For both Cape and European honey bees, guard bee and beetle behaviors and prison dynamic variables were analyzed with a repeated measure ANOVA design recognizing beetle density (25 or 50 beetles) and time (morning or evening) as main effects. Wherever significant time × density interactions occurred, the data were analyzed by beetle density using independent sample t-tests. Because data for guard bee and beetle behaviors were proportions, the data were transformed using $\arcsin\sqrt{\text{proportion}}$ to stabilize the variance. Beetle intra-colonial distribution was analyzed by beetle density using

Pearson's χ^2 tests. Significant differences were accepted at the $\alpha \leq 0.05$ and all analyses were conducted using Statistica (2001).

Results

Cape honey bees

The results from the ANOVA are reported in Table 8.1. There were more beetle prisons at the higher beetle density than at the lower one (Table 8.2) and during evening than morning (Table 8.3). Although the number of prisons increased, the number of beetles per prison did not increase at either beetle density (Table 8.2) or time (Table 8.3). The number of guard bees per beetle increased from morning to evening (Table 8.3) but did not significantly differ over beetle density (Table 8.2). Further, the number of guard bees per prison was not affected by time or beetle density (Tables 8.2 and 8.3).

Beetle activity did not increase at the higher beetle density (Table 8.2) or either time (Table 8.3). Additionally, time and beetle density did not significantly affect the proportion of beetles making antennal contact with guard bees, getting fed by guard bees, or mating. Further, none of the measured behaviors of guard bees (biting at, making antennal contact with, and feeding beetles and prison wall working) were affected by time or beetle density (Tables 8.2 and 8.3).

There was a significant effect of beetle density on intra-colonial beetle distribution ($\chi^2 = 14.9$; $df = 4$; $P = 0.0049$). The proportions of beetles found on the bottom board, front wall, and back wall of the hive all decreased at the higher beetle density leading to a marked increase of beetles among the combs at the higher density (Table 8.4). Despite the high percentage of beetles found among the combs at both beetle densities, most (>90% based on visual estimations) of the beetles reaching the combs were kept out of the brood, honey, and pollen areas by bee aggression and were instead confined to empty cells around the comb periphery.

European honey bees

The results from the ANOVA are reported in Table 8.5. As beetle density increased, so did the number of beetle prisons (Table 8.6); yet the number of beetles per prison did not increase (Table 8.6). Further, the number of guard bees per prison was higher during evening at both the low ($|t| = 3.5$; $df = 8$; $P < 0.01$) and high ($|t| = 7.4$; $df = 8$;

$P < 0.0001$) beetle densities. Similarly, there were more guard bees per imprisoned beetle by evening than morning (Table 8.7).

The beetles were significantly more active at the higher density (Table 8.6) and significantly more beetles made antennal contact with guard bees at the higher density (Table 8.6) and evening (Table 8.7). However, increased proportions of beetles making antennal contact with guard bees did not lead to a corresponding increase in the proportion of beetles being fed at the higher beetle density (Table 8.6) although it did during evening (Table 8.7). No density or time effects for the proportion of beetles mating (Tables 8.6 and 8.7) were found.

Guard bees increased antennal contact with imprisoned beetles at the higher density, and this led to more guard bees feeding beetles (Table 8.6). Guard bees fed beetles more during evening at the lower beetle density ($|t| = 3.7$; $df = 8$; $P = 0.0058$) but not at the higher density ($|t| = 0.7$; $df = 8$; $P = 0.5024$).

The proportion of guard bees biting at the beetles did not significantly increase at the higher beetle density (Table 8.6). Interestingly, prison wall-working by guard bees significantly decreased with increasing beetle density (Table 8.6). More beetles were found among the combs at the higher rather than lower beetle density ($\chi^2 = 118.6$; $df = 4$; $P < 0.0001$) (Table 8.4).

Discussion

Increasing beetle density led to more confinement sites (prisons) in both Cape and European colonies; beetle density per prison did not change. This could mean that there are optimum beetle densities per prison most efficiently guarded by bees or that beetles disperse evenly throughout the colony and are confined wherever they happen to hide. Further, the number of prisons increased during evening in Cape colonies, perhaps indicating a more general increase in beetle dispersal during evening in Cape colonies.

For European colonies, the number of guard bees per prison increased at the higher beetle density and this is probably due to increased beetle activity at the higher beetle density. Conceivably the number of European guard bees will reach a threshold with increasing beetle density; after which beetles become difficult to contain leading to “jail breaks” where beetles escape their prisons and enter the central honey bee nest where the combs are located (which happened at the higher beetle density in this study).

Why the number of guard bees per beetle did not increase with increasing beetle density in Cape colonies as it did in European colonies is unclear; however, it may be due to the absence of increasing beetle activity at the higher density and evening in Cape colonies. Because beetle activity in Cape colonies did not increase, more guards were not needed to keep the beetles confined. The lack of increasing beetle activity at the higher density and evening in Cape colonies may indicate that Cape bees were able to keep beetle activity low. Indeed, beetle activity in Cape colonies was lower than that found in European colonies.

Beetle behavior in Cape bee colonies remained fairly consistent over beetle density and time. Further, guard bee behavior remained relatively unaffected by beetle density or time. This suggests that Cape bees are better able to handle changing beetle density than are European bees, or at least that their confinement behavior is more consistent through changing beetle density than that of European bees. Perhaps Cape bees are more energetically adept as well, spending less energy per beetle per unit time than European bees.

Concerning beetle behavior in European colonies, the increase in the proportion of beetles making antennal contact with guard bees did not lead to a significant increase in the proportion of beetles being fed at either density or time. This could be a reason beetles are problematic in European honey bee colonies. If trophallaxis is used by honey bees to suppress natural beetle feeding habits, then a lack of trophallactic increase by guard bees when beetle density or activity is high could cause incarcerated beetles to leave prisons and move into the combs, possibly triggering beetle reproduction.

In earlier work on beetle confinement by Cape bees (Chapter 7), I found that as much as 23% of beetles in a colony can be found among the combs. In this study, 46% of beetles at the lower density and 58% of beetles at the higher density were found among the combs. These percentages are much higher than those found in the European colonies (5% at the lower density and 25% at the higher one). Although over half of the beetles managed to reach the combs in the Cape colonies, few accessed bee brood, honey, or pollen and this may be due to general bee aggression. Indeed, African bees are significantly more aggressive toward free-roaming beetles than their European counterparts (Elzen et al. 2001).

These findings strongly suggest that confinement of beetles is not the sole mechanism by which Cape bees limit depredation caused by beetles because a large proportion of beetles gained access to the combs where they can reproduce. Although

fundamental confinement behaviors of Cape and European bees are similar, once beetle density in a colony increases, both bee subspecies handle the increase differently. Increasing beetle density did not significantly alter confinement behavior by Cape bees whereas it did in European colonies.

Table 8.1. Analysis of variance testing effects of beetle density (d), time (t), and time \times density (t \times d) on confinement dynamics, beetle behavior, and guard bee behavior in Cape bee colonies.

variable	source	df	<i>F</i>	<i>P</i> > <i>F</i>
confinement dynamics				
number of guard bees per beetle	d	1	0.1	0.7897
	t	1	13.4	0.0021
	t \times d	16	3.9	0.0665
number of prisons per colony	d	1	19.3	0.0005
	t	1	8.9	0.0087
	t \times d	16	0.2	0.6598
number of beetles per prison	d	1	0.3	0.6002
	t	1	0.7	0.4126
	t \times d	16	0.0	0.9834
number of guard bees per prison	d	1	0.9	0.3519
	t	1	4.3	0.0537
	t \times d	16	1.6	0.2175
beetle behavior				
resting	d	1	0.1	0.7342
	t	1	0.4	0.5177
	t \times d	16	1.5	0.2402
making antennal contact with guard bees	d	1	0.2	0.6766
	t	1	2.6	0.1246
	t \times d	16	0.0	0.9356
getting fed by guard bees	d	1	0.0	0.9509
	t	1	0.0	0.9281
	t \times d	16	3.8	0.0692
mating	d	1	0.5	0.4786
	t	1	0.4	0.5548
	t \times d	16	0.5	0.4703
guard bee behavior				
biting at beetles	d	1	0.0	0.8683
	t	1	0.0	0.8272
	t \times d	16	1.5	0.2360
making antennal contact with beetles	d	1	0.3	0.5678
	t	1	0.3	0.5956
	t \times d	16	1.1	0.2999
feeding beetles	d	1	1.1	0.3097
	t	1	0.4	0.5140
	t \times d	16	3.8	0.0683
prison wall-working	d	1	0.0	0.8748
	t	1	0.1	0.7363
	t \times d	16	2.1	0.1661

Table 8.2. Small hive beetle density effects on confinement dynamics, beetle behavior, and guard bee behavior in Cape bee colonies.

	25 beetles	50 beetles
confinement dynamics		
number of guard bees per beetle	0.98 ± 0.10a	1.02 ± 0.09a
number of beetle prisons per colony	7.94 ± 0.63a	14.17 ± 0.98b
number of beetles per prison	2.46 ± 0.19a	2.74 ± 0.34a
number of guard bees per prison	2.25 ± 0.20a	2.60 ± 0.28a
beetle behavior		
resting	0.82 ± 0.03a	0.86 ± 0.02a
making antennal contact with guard bees	0.07 ± 0.02a	0.05 ± 0.01a
getting fed by guard bees	0.02 ± 0.01a	0.01 ± 0.01a
mating	0.02 ± 0.01a	0.01 ± 0.00a
guard bee behavior		
biting at beetles	0.58 ± 0.05a	0.58 ± 0.04a
antennal contact with beetles	0.07 ± 0.02a	0.05 ± 0.01a
feeding beetles	0.02 ± 0.01a	0.02 ± 0.01a
prison wall-working	0.07 ± 0.03a	0.06 ± 0.02a

For beetle and guard bee behavior, data are the proportion of individuals observed doing the particular behavior (mean ± standard error). $n = 9$ for all data. Row totals followed by the same letter are not different at the $\alpha \leq 0.05$ level.

Table 8.3. Time (morning and evening) effects on confinement dynamics, beetle behavior, and guard bee behavior in Cape bee colonies.

	morning	evening
confinement dynamics		
number of guard bees per beetle	0.84 ± 0.06a	1.16 ± 0.10b
number of beetle prisons per colony	9.94 ± 0.94a	12.17 ± 1.21b
number of beetles per prison	2.68 ± 0.26a	2.51 ± 0.29a
number of guard bees per prison	2.11 ± 0.17a	2.74 ± 0.29a
beetle behavior		
resting	0.85 ± 0.02a	0.83 ± 0.03a
making antennal contact with guard bees	0.04 ± 0.01a	0.08 ± 0.02a
getting fed by guard bees	0.02 ± 0.01a	0.01 ± 0.01a
mating	0.02 ± 0.01a	0.01 ± 0.01a
guard bee behavior		
biting at beetles	0.56 ± 0.06a	0.59 ± 0.04a
antennal contact with beetles	0.05 ± 0.02a	0.06 ± 0.01a
feeding beetles	0.02 ± 0.01a	0.02 ± 0.01a
prison wall-working	0.07 ± 0.03a	0.06 ± 0.02a

For beetle and guard bee behavior, data are the proportion of individuals observed doing the particular behavior (mean ± standard error). $n = 18$ for all data. Row totals followed by the same letter are not different at the $\alpha \leq 0.05$ level.

Table 8.4. Proportion of small hive beetles confined in various intra-colonial locations at both beetle densities for Cape and European colonies.

location	Cape colonies		European colonies	
	25 beetles	50 beetles	25 beetles	50 beetles
top wall of hive	0.04 ± 0.02	0.05 ± 0.01	0.22 ± 0.04	0.17 ± 0.04
bottom board of hive	0.24 ± 0.04	0.20 ± 0.04	0 ± 0	0.02 ± 0.02
front wall of hive	0.14 ± 0.04	0.10 ± 0.03	0.32 ± 0.05	0.42 ± 0.05
back wall of hive	0.12 ± 0.02	0.08 ± 0.02	0.41 ± 0.03	0.14 ± 0.02
combs	0.46 ± 0.07	0.58 ± 0.07	0.05 ± 0.02	0.25 ± 0.06

Data are mean ± standard error. $n = 18$ for all data.

Table 8.5. Analysis of variance testing effects of beetle density (d), time (t), and time \times density (t \times d) on confinement dynamics, beetle behavior, and guard bee behavior in European bee colonies.

variable	source	df	<i>F</i>	<i>P</i> > <i>F</i>
confinement dynamics				
number of guard bees per beetle	d	1	3.9	0.0668
	t	1	23.5	0.0002
	t \times d	16	0.2	0.7016
number of prisons per colony	d	1	24.1	0.0002
	t	1	0.4	0.5538
	t \times d	16	0.1	0.7663
number of beetles per prison	d	1	0.6	0.4689
	t	1	0.2	0.6863
	t \times d	16	0.2	0.6788
number of guard bees per prison	d	1	4.4	0.0531
	t	1	56.8	0.0000
	t \times d	16	5.3	0.0349
beetle behavior				
resting	d	1	8.1	0.0116
	t	1	2.3	0.1469
	t \times d	16	0.4	0.5144
making antennal contact with guard bees	d	1	29.6	0.0001
	t	1	9.6	0.0070
	t \times d	16	1.3	0.2783
getting fed by guard bees	d	1	1.3	0.2703
	t	1	4.7	0.0448
	t \times d	16	4.3	0.0552
mating	d	1	1.2	0.2831
	t	1	0.0	0.9910
	t \times d	16	1.4	0.2510
guard bee behavior				
biting at beetles	d	1	3.5	0.0807
	t	1	0.6	0.4539
	t \times d	16	0.2	0.6620
making antennal contact with beetles	d	1	18.7	0.0005
	t	1	1.6	0.2186
	t \times d	16	0.3	0.5649
feeding beetles	d	1	6.7	0.0198
	t	1	5.3	0.0353
	t \times d	16	10.5	0.0051
prison wall-working	d	1	19.5	0.0004
	t	1	1.1	0.3159
	t \times d	16	0.0	0.9153

Table 8.6. Small hive beetle density effects on confinement dynamics, beetle behavior, and guard bee behavior in European colonies.

	25 beetles	50 beetles
confinement dynamics		
number of guard bees per beetle	0.67 ± 0.07a	1.07 ± 0.14a
number of beetle prisons per colony	6.28 ± 0.27a	10.83 ± 0.63b
number of beetles per prison	3.20 ± 0.17a	3.62 ± 0.37a
number of guard bees per prison	2.27 ± 0.31a	3.34 ± 0.26a
beetle behavior		
resting	0.79 ± 0.05a	0.61 ± 0.04b
making antennal contact with guard bees	0.08 ± 0.02a	0.25 ± 0.03b
getting fed by guard bees	0.09 ± 0.05a	0.10 ± 0.02a
mating	0.01 ± 0.01a	0.03 ± 0.02a
guard bee behavior		
biting at beetles	0.68 ± 0.06a	0.86 ± 0.03a
antennal contact with beetles	0.12 ± 0.03a	0.32 ± 0.04b
feeding beetles	0.05 ± 0.01a	0.11 ± 0.02b
prison wall-working	0.32 ± 0.06a	0.04 ± 0.02b

For beetle and guard bee behavior, data are the proportion of individuals observed doing the particular behavior (mean ± standard error). $n = 9$ for all data. Row totals followed by the same letter are not different at the $\alpha \leq 0.05$ level.

Table 8.7. Time (morning and evening) effects on confinement dynamics, beetle behavior, and guard bee behavior in European colonies.

	morning	evening
confinement dynamics		
number of guard bees per beetle	0.73 ± 0.12a	1.02 ± 0.11b
number of beetle prisons per colony	8.67 ± 0.78a	8.44 ± 0.69a
number of beetles per prison	3.37 ± 0.31a	3.45 ± 0.28a
number of guard bees per prison*	2.29 ± 0.30	3.32 ± 0.28
beetle behavior		
resting	0.75 ± 0.05a	0.65 ± 0.05a
making antennal contact with guard bees	0.12 ± 0.03a	0.21 ± 0.03b
getting fed by guard bees	0.05 ± 0.02a	0.14 ± 0.05b
mating	0.02 ± 0.01a	0.02 ± 0.02a
guard bee behavior		
biting at beetles	0.75 ± 0.05a	0.79 ± 0.06a
antennal contact with beetles	0.19 ± 0.04a	0.24 ± 0.04a
feeding beetles*	0.07 ± 0.02	0.09 ± 0.02
prison wall-working	0.22 ± 0.06a	0.14 ± 0.04a

For beetle and guard bee behavior, data are the proportion of individuals observed doing the particular behavior (mean ± standard error). $n = 18$ for all data. Row totals followed by the same letter are not different at the $\alpha \leq 0.05$ level. *For ‘feeding beetles’ and ‘number of guard bees per prison’ there were significant density × time effects so mean separations for time were not applicable.

Chapter 9: Cape and European Honey Bee Guard Age and Duration of Guarding Small Hive Beetles

Abstract - The guard age and duration of European and Cape honey bees guarding small hive beetles were determined using 3-frame observation hives, noting the commencement and termination of beetle guarding by individually labeled honey bees. European honey bees in the United States began guarding beetles significantly earlier (beginning age 18.6 ± 0.5 d; mean \pm standard error), guarded beetles significantly longer (duration 2.4 ± 0.3 d), and stopped guarding beetles at a younger age (ending age 19.9 ± 0.6 d) than Cape honey bees in South Africa (beginning age 20.6 ± 0.4 d; duration 1.4 ± 0.1 d; and ending age 21.0 ± 0.4 d). Although the timing of beetle guarding behavior between the two subspecies was significantly different, it does not explain the differential damage to European and Cape honey bee colonies caused by the beetles.

Introduction

In addition to direct aggressive behavior (biting, stinging) directed at small hive beetles (Elzen et al. 2001), African honey bees confine beetles to cracks and crevices around the hive (Neumann et al. 2001b; Chapters 7 and 8). Similar imprisoning behavior has been documented in European honey bees (Chapters 7 and 8). Both honey bee subspecies station guards around the prison perimeter, keeping the beetles imprisoned (Neumann et al. 2001b; Chapters 7 and 8; Figure 9.1). Despite being imprisoned, beetles are able to remain alive because they are fed by their honey bee captors (Chapter 6).

In this study, I determine the age of European and Cape honey bees that guard beetles and the duration of beetle guarding for each honey bee subspecies. These data show guarding differences between the subspecies, suggesting possible reasons African honey bee subspecies can cope with small hive beetle infestations while European honey bee subspecies cannot. Further, these data aid in describing the recently discovered phenomenon of propolis prisons that are used by honey bees as a defensive tactic against the beetles.

Materials and Methods

The experiments were conducted at Rhodes University in Grahamstown, South Africa (January - April and November - December 2001) and in Warren County, Georgia, USA (August - September 2001). In both locations, three observation hives were used. Each hive contained three frames of bees, two frames of brood, one frame of honey, and a laying queen. Honey bees used in the United States were of mixed European origin, while Cape honey bees were used in South Africa.

Approximately 25 - 40 beetles were added to each hive 2 - 3 d after the observation hives were established. Once small hive beetle imprisoning behavior was apparent in each hive (Neumann et al. 2001b), 150 - 400 newly emerged honey bees, from a mixture of colonies, were individually marked with colored, numbered labels (Opalithplättchen) and added to each colony. No two observation hives were given newly emerged bees from the same colony.

Hives were monitored daily at approximately 09:00, 14:30, and 20:00 h. Location of imprisoned beetles and guarding behavior of marked honey bees (Chapters 7 and 8) were documented noting the commencement and duration of beetle guarding behavior

(Figure 9.1). Data were collected until all marked bees had stopped guarding beetles (ranging from 21 - 28 d).

Data analysis

The beginning age of honey bees guarding beetles, number of days they guarded, and the last day they guarded were analyzed by ANOVA (Statistica 2001). Colonies were nested within location. When colony and location interacted, analyses were run separately by location. Means were compared using Tukey's multiple range tests; differences were accepted at the $\alpha \leq 0.05$ level.

Results

Beginning guard age

European honey bees began guarding beetles two days earlier than did Cape honey bees ($F = 11.0$; $df = 1, 76$; $P = 0.0014$)(Table 9.1). There were colony \times location interactions for beginning guard age ($F = 4.2$; $df = 4, 76$; $P = 0.0039$). In South Africa, workers in one Cape colony (colony 3) began guarding beetles significantly earlier than in the other two colonies ($F = 6.2$; $df = 2, 46$; $P = 0.0040$)(Table 9.2). There were no significant differences with respect to the start of beetle guarding in the European colonies ($F = 2.5$; $df = 2, 30$; $P = 0.0991$)(Table 9.2).

Ending guard age

European honey bees stopped guarding beetles one day earlier than did Cape honey bees ($F = 5.1$; $df = 1, 76$; $P = 0.0266$)(Table 9.1). Colony \times location interactions occurred for this variable ($F = 4.8$; $df = 4, 76$; $P = 0.0016$). Workers in Cape colony 3 stopped guarding beetles earlier than in the other Cape colonies ($F = 9.3$; $df = 2, 46$; $P = 0.0004$)(Table 9.2). There were no significant differences among the European colonies with respect to ending guard age ($F = 2.1$; $df = 2, 30$; $P = 0.1451$)(Table 9.2).

Duration of beetle guarding

European honey bees guarded beetles almost one day longer than did Cape honey bees ($F = 4.3$; $df = 1, 76$; $P = 0.0415$)(Table 9.1). There was no significant colony \times location interaction for this variable ($F = 2.5$; $df = 4, 76$; $P = 0.0509$).

Discussion

European honey bees begin guarding beetles earlier, guard for longer periods of time, and stop guarding at a younger age than Cape honey bees. This European bee behavior may be in reaction to damage beetles cause in European colonies (Elzen et al. 1999, 2000; Hood 2000; Wenning 2001; Chapter 2). Because beetles cause little or no damage in Cape bee colonies (Chapter 2), Cape honey bees could be less inclined to begin guarding beetles and then guard for shorter periods of time once they do begin. This could imply that Cape honey bees are either remarkably efficient at beetle guarding or that there are other factors besides imprisoning techniques that Cape bees use to control small hive beetle infestations (discussed in Chapter 14). This difference between the bee subspecies could also reflect the differences in aggression towards free-running beetles by African and European honey bee subspecies (Elzen et al. 2001). African workers vigorously attack free-running beetles more often than European workers do. Thus beetle guarding in African colonies may not have to be as efficient.

Further, it is possible that age-related division of labor (polyethism) is different between the two honey bee subspecies, with European honey bees advancing in age-specific tasks faster than their African counterparts. However, division of labor in Cape honey bees is poorly studied and therefore no further inferences on this point can be made. Data on guarding behavior do exist for another African subspecies of honey bee (*A.m. scutellata*) and the data suggest that the onset of guarding behavior may occur around 18-20 days of age because of increased synthesis and release of various defensive compounds at this time (Whiffler et al. 1988). Regardless, whether or not one can expect Cape bees to behave in a fashion similar to that of other African subspecies is unknown.

Interestingly, the commencement of hive entrance guarding behavior in European honey bees has been documented at 18 - 19 days of age (Winston 1992). This is consistent with the findings that European bees began guarding beetles at 18.6 days of age (Table 9.1) which may imply that “guarding” behavior is the same for honey bees whether they are doing so at the entrance of a hive or entrance of a beetle prison or that bees from this age cohort perform guarding duties whatever those duties might encompass.

Winston (1992) also noted that guarding behavior in honey bees chronologically overlaps with foraging behavior, indicating that individuals from the same cohort could be doing either of the two tasks. In this study, labeled honey bees in all colonies in both locations were recorded foraging while other labeled bees were guarding beetles.

Therefore, one would expect that if beetle infestations in European honey bee colonies are large, colony foraging activity might be reduced because foraging age bees are guarding beetles instead of foraging. Such reduction in the number of foraging bees for beetle-infested European colonies has been documented (Chapter 2).

African honey bee subspecies south of the Sahara are sympatric with beetles (Lundie 1940; Schmolke 1974; Hepburn and Radloff 1998) and show considerable resistance towards infestations. However, the behavioral mechanisms regulating resistance that have been identified so far [aggressive behavior (Elzen et al. 2001) and prison building (Neumann et al. 2001b)] are also present in European bees (Ellis 2002, Chapters 7 and 8). This strongly suggests that there are only differences in degree, but not in kind, between Cape and European subspecies with respect to resistance behavior. Therefore, one could expect that there is some adaptive advantage to the degree of behavior exhibited by Cape honey bee guards.

Table 9.1. Beginning guard age, ending guard age, and duration of guarding behavior (d) for Cape and European honey bees guarding small hive beetles.

	Cape honey bees	European honey bees
average beginning guard age	20.6 ± 0.4 (49)a	18.6 ± 0.5 (33)b
average ending guard age	21.0 ± 0.4 (49)a	19.9 ± 0.6 (33)b
average duration of guarding behavior	1.4 ± 0.1 (49)a	2.4 ± 0.3 (33)b

Data are mean ± standard error (*n*). The two bee subspecies differed for each parameter at $P \leq 0.05$.

Table 9.2. Location × colony interactions for average beginning guard age and ending guard age (d) of Cape and European honey bees guarding small hive beetles.

Cape honey bees			
	colony 1	colony 2	colony 3
average beginning guard age	22.8 ± 1.5 (6)a	21.0 ± 0.4 (29)a	18.9 ± 0.7 (14)b
average ending guard age	23.2 ± 1.5 (6)a	21.6 ± 0.3 (29)a	19.0 ± 0.7 (14)b
European honey bees			
	colony 1	colony 2	colony 3
average beginning guard age	18.2 ± 1.7 (5)a	17.7 ± 0.7 (17)a	20.1 ± 0.7 (11)a
average ending guard age	18.2 ± 1.7 (5)a	19.5 ± 0.7 (17)a	21.4 ± 0.9 (11)a

Data are mean ± standard error (*n*). Because of the significant interaction, colony analyses were run separately by location for these variables. Row totals followed by the same letter are not different at the $\alpha \leq 0.05$ level. Means were compared Tukey's multiple range tests.



Figure 9.1. Two European honey bees (one labeled “yellow 71”) guarding an imprisoned small hive beetle. Notice the row of propolis, forming a prison wall, at the bottom of the photograph.

Chapter 10: Oviposition by Small Hive Beetles Elicits Hygienic Responses from Cape Honey Bees

Abstract - Two novel behaviors, both adaptations of small hive beetles and Cape honey bees, are described. Beetles puncture the sides of empty cells and oviposit under the pupae in adjoining cells. However, bees detect this ruse and remove infected brood (hygienic behavior), even under such well-disguised conditions. Indeed, bees removed 91% of treatment brood (brood cells with punctured walls caused by beetles) but only 2% of control brood (brood not exposed to beetles). Only 91% of treatment brood actually contained beetle eggs; the data therefore suggest that bees remove only that brood containing beetle eggs and leave uninfected brood alone, even if beetles have accessed (but not oviposited on) the brood. Although this unique oviposition strategy by beetles appears both elusive and adaptive, Cape honey bees are able to detect and remove virtually all of the infected brood.

Introduction

Resistance of African honey bees to small hive beetle depredation is partially due to beetle imprisonment that precludes access to brood, honey, and pollen reserves in the combs (Neumann et al. 2001b) where its reproductive potential is very high (Chapter 4). Although confining beetles was thought to be unique to their natural honey bee hosts in Africa, this behavior also occurs in otherwise beetle-naïve, European-derived honey bees in North America (Chapters 7 and 8), which are often extremely susceptible to beetle depredation (Hood 2000; Chapter 2). Therefore, the confinement of beetles cannot be the sole reason African honey bees are immune to beetles while European bees are not.

If female beetles reach the brood combs, they may puncture the waxy capping of brood cells and lay eggs on and around the honey bee pupa (Figure 10.1a)(Chapter 3). On hatching, beetle larvae feed on the brood and severely damage colonies of European honey bees (Hood 2000). Nonetheless, honey bees generally show hygienic responses to other pests and diseases and remove infected brood (Rothenbuhler 1964a; cf. Boecking and Spivak 1999). I therefore tested for the expression of hygienic behavior by Cape honey bees toward beetle eggs oviposited in bee brood.

Materials and Methods

Experiments were conducted at an apiary near Grahamstown, South Africa in April 2003. Ten hived colonies of Cape honey bees of equal strength and reserves were used. All colonies had existing beetle populations (<50 beetles). For each colony, a frame of capped brood was removed and twenty randomly collected adult beetles were placed on a 10 × 10-cm area on the frame (treatment) in a sheet metal push-in cage (10 × 10 × 2.5 cm; 1 × w × h), the face of which was screen mesh to allow for ventilation. The combs used contained about 50% empty and 50% capped brood cells. A second cage without beetles was pushed into the brood frame as a control. Both caged sections of brood were placed in the center of the bee cluster in each colony.

Twenty-four h later, both cages were removed and the adult beetles from the treatment cage were collected. Beetle oviposition punctures in the capped cells were noted. Previous work showed that beetles puncture brood cell cappings (Figure 10.1a)(Chapter 3); however, in this study, I observed puncture marks well down the sides of capped cells (Figure 10.1b). A transparent sheet of plastic was placed on the comb and all capped brood

with punctures in the cell walls were marked. Similarly, twenty uninfected capped brood cells from the control cage were marked. The treated and control brood were replaced in the center of the bee cluster. After forty-eight h, they were examined and marked cells from which infected or control brood had been subsequently removed by the bees were counted (Table 10.1).

The infection rate of treatment cells containing punctures made by beetles was determined (Table 10.1). For each of seven colonies, twenty adult beetles were confined to one frame of capped brood as before and the frames were replaced in the colonies. Twenty-four h later about thirty cells from each frame having punctures in their walls were opened to determine the presence/absence of beetle eggs. These data were used to determine the infection rate of brood cells containing punctures. The number of beetle eggs per infected cell was also determined.

Data analysis

Differences in the proportion of removed brood were analyzed by independent sample t-tests recognizing brood condition (control brood or treatment brood with oviposition punctures) as the main effect. Because of the analysis of proportions of removed brood, the data were transformed using $\arcsin\sqrt{\text{proportion}}$ to stabilize the variance. Likewise, the proportion of removed treatment brood was tested for differences from the proportion of infected brood (proportion of cells with punctures and containing beetle eggs) using independent sample t-tests and $\arcsin\sqrt{\text{proportion}}$ transformations as before. All differences were accepted at $\alpha \leq 0.05$.

Results

Previously, only one mode of beetle oviposition on brood was known: oviposition directly through cell cappings (mode 1, Figure 10.1a)(Chapter 3). In this study, I also found that beetles enter and puncture the walls of empty cells then oviposit in adjacent cells containing capped brood (mode 2, Figure 10.1b, c). Sometimes, the eggs were laid under the pupa and could only be detected by removing the pupa. In other instances, the punctures were midway down the cell wall and the eggs were laid around the pupa (Figure 10.1b, c). The proportion of treatment cells (having punctures) infected with beetle eggs was 0.905 ± 0.024 (mean \pm std. error, $n = 7$ colonies) (individual colony data are reported

in Table 10.1). Further, 168 infected cells in 7 colonies contained an average of 33.9 ± 1.8 beetle eggs per cell (totaling ~5695 eggs for the 7 colonies or ~814 eggs/colony).

Brood condition (treatment or control) significantly affected the proportion of brood removed by the bees ($|t| = 18.94$; $df = 18$; $P < 0.0001$). The proportion of treatment brood removed by the bees (0.907 ± 0.024 , 10 colonies; mean \pm std. error, n) was higher than the proportion of control cells removed by the bees (0.017 ± 0.011 ; mean \pm std. error, $n = 10$ colonies)(individual colony data are reported in Table 10.1). Indeed, only two colonies removed control cells leading to the mean reported above (the first colony removed 2 and the second removed one); no other colonies removed any of the control brood.

Additionally, there was no significant difference between the proportion of treatment brood removed by the bees and the proportion of treatment brood infected with beetle eggs ($|t| = 0.14$; $df = 15$; $P = 0.8913$). Therefore, it is reasonable to conclude that although bees were only removing 91% of all treatment brood, they were removing all of the brood actually infected with beetle eggs (which was also 91%). I validated this assumption by opening treatment brood cells not removed by the bees and in no case were beetle eggs found.

Discussion

Lundie (1940) and Schmolke (1974) found that beetles oviposit in cracks in the hive around the nest periphery and directly in pollen cells. However, these modes of oviposition appear to contribute little to the overall reproductive potential of the beetles as larvae hatching from eggs in the nest periphery have to crawl to the combs and studies have shown that African subspecies of honey bees very rapidly remove free-roaming beetle larvae from the colony (Neumann and Härtel 2003). Further, at low beetle populations, most beetles are confined to the nest periphery and oviposition has never been observed during beetle confinement (Chapters 7 and 8). Therefore, beetle oviposition directly into bee brood is more likely to result in scores of unnoticed larvae than is beetle oviposition in cracks and crevices around the nest.

In Chapter 3 I described beetles puncturing cell cappings and laying eggs directly on bee pupae in European bee colonies when bees were present (mode 1). Beetles would normally have little chance to oviposit through cell cappings in African colonies as African bees display high aggression toward free running beetles (Elzen et al. 2001). However,

beetles are often found in empty cells among the combs (Chapters 7 and 8) where they retreat to the bottom of the cell exposing only their hard exoskeleton to any bee aggression. Such beetles would then be able to oviposit in adjacent cells containing brood and successfully reproduce (mode 2). These oviposition tactics by the beetles to conceal their eggs appear inevitably foiled because Cape bees removed virtually all infected brood.

Although how bees detect infestation/infection in capped brood is not known (cf. Boecking and Spivak 1999), pathogen-killed brood may be easily recognized and removed by the bees (Rothenbuhler 1964a). However, while pests such as varroa mites (*Varroa destructor* Anderson and Truemann) do not necessarily kill brood, the bees are able to detect and remove the brood nonetheless. There are strong indications that bees cue into the presence of beetle eggs and not the punctures created by the beetles as no brood was removed from punctured cells not containing beetle eggs. Further Neumann and Härtel (2003) have shown that unprotected eggs in a colony are removed within 24 h. If beetle eggs stimulate brood removal by bees, this study does not determine the number of eggs/cell necessary to elicit hygienic responses from the bees because cells in this study contained a large number of beetle eggs. Therefore, there may exist a minimum number of eggs/cell that elicits brood removal.

Despite the fact that Cape bees remove beetle eggs from capped brood (present study) and free-roaming larvae from the colony (Neumann and Härtel 2003), thus minimizing beetle reproduction, beetles maintain a continued presence in Cape bee colonies. This further implies that beetle reproduction in their native range is limited to weakened/diseased colonies (Lundie 1940) or nests left by absconding bees (Hepburn and Radloff 1998) because of behavioral responses of their honey bee hosts.

To place this study in a wider context it must be remembered that Rothenbuhler (1964b) proposed a two-gene model to explain phenotypic variance in hygienic behavior; suggesting that one locus controls the uncapping of brood cells and the second controls removal of the cell contents. However it has recently been suggested (Moritz 1988; Lapidge et al. 2002) that more than 2 loci are responsible for hygienic behavior. This suggests that hygienic behavior is more complex than uncapping and subsequent removal of diseased/infected brood. The data support that hygienic behavior may be more complex than once thought because Cape bees remove only that brood containing beetle eggs, thus exercising discriminative and selective removal of affected brood only.

Although a suite of behavioral/environmental factors are probably responsible for overall Cape bee resistance to beetles, the data clearly show that Cape honey bees can

detect and remove brood infected with beetle eggs. Hygienic behavior likely contributes to Cape bees' success in thwarting potential damage caused by beetles. Indeed, that I found over 33 beetle eggs/infected cell, suggests that had the bees not removed the infected brood, the colonies would be quickly overrun by beetle larvae as occurs among European-derived honey bees in North America.

Related kinds of hygienic behavior towards other pathogens already exist in European bees (cf. Boecking and Spivak 1999; cf. Spivak and Boecking 2001), and preliminary data suggest that hygienic behavior toward beetle eggs is also present in European colonies. Therefore, resistance to beetles by European colonies may be improved because the behavior is amenable to selective breeding programs (Harbo and Harris 1999). In conclusion, the data suggest that hygienic behavior does not target any one brood-infecting pathogen but is instead a more general response to a suite of brood conditions that may ultimately weaken or destroy a colony. This behavior may, therefore, be considered a super-organismic immune response probably found in all *A. mellifera*.

Table 10.1. Data on treatment and control brood removal by Cape colonies and on the infection rate of cells containing punctures (punctured cells containing beetle eggs).

colony	treatment		control		infection rate*	
	no. cells with punctures	no. cells removed	no. marked cells	no. cells removed	no. cells with punctures	no. cells containing eggs
1	29	26	20	0	22	20
2	30	22	20	0	30	25
3	16	15	20	0	30	30
4	19	17	20	0	14	13
5	79	69	15	1	30	26
6	12	12	20	0	30	25
7	10	10	20	0	30	29
8	21	20	20	0	na	na
9	16	14	20	2	na	na
10	21	19	20	0	na	na

*Only 7 colonies were tested for infection rates so data for colonies 8-10 were not collected and are therefore not available (na)

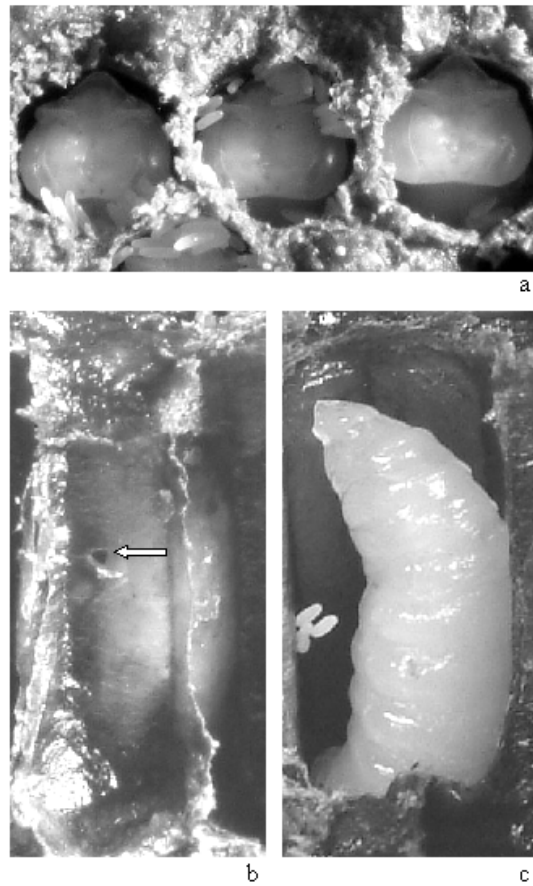
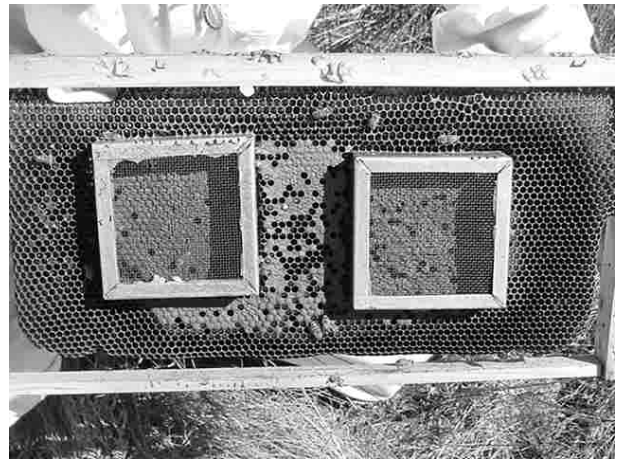


Figure 10.1. Small hive beetle oviposition through cell cappings (mode 1) and walls (mode 2). Figure “a” shows oviposition directly through cell cappings (removed)(Chapter 3). Beetles also puncture cell walls (arrowed in b). When the cell wall is removed, beetle eggs are seen around the honey bee pre-pupa (c). Alternatively, the punctures may be made closer to the bottom of the cell and the eggs laid under the pupa. Photography by James Greaves.

Section III: Controlling Small Hive Beetles



Chapter 11: Efficacy of Modified Hive Entrances and a Bottom Screen Device for Controlling Small Hive Beetles

Abstract - This 2-part study was designed to test if hive entrances reduced with polyvinyl chloride (PVC) pipe reduce the ingress of small hive beetles into honey bee colonies and if screen-mesh bottom boards alleviate side effects associated with restricting entrances. For the first study, colonies with pipe entrances (1.9-cm ID) had significantly fewer adult beetles (46.9 beetles/colony) than open colonies (107.7 beetles/colony). Pipe entrances did not directly affect the amount of sealed brood in a colony or the temperature inside colonies. There was a tendency for reduced brood in colonies with pipes. In the second study, forty-eight colonies distributed equally between 2 locations each received 1 of 6 experimental treatments: (1) conventional solid bottom board and open entrance, (2) ventilated bottom board and open entrance, (3) conventional bottom and 1.9-cm ID pipe entrance, (4) conventional bottom and 3.8-cm pipe entrance, (5) screen bottom and 1.9-cm pipe entrance, and (6) screen bottom and 3.8-cm pipe entrance. Results were inconsistent between apiaries. In apiary 1, colonies with 3.8-cm pipe entrances had fewer beetles than colonies with open entrances, but this benefit was not apparent in apiary 2. Pipe entrances tended to reduce colony and brood production in both apiaries, and these losses were only partly mitigated with the addition of screened bottom boards. Pipe entrances had no measurable liability concerning colony thermoregulation. There were significantly fewer frames of adult honey bees in colonies with 3.8-cm or 1.9-cm pipe entrances compared to open entrances but more in colonies with screens. There were more frames of pollen in colonies with open or 3.8-cm pipe entrances than 1.9-cm entrances. I conclude that the efficacy of reduced hive entrances in reducing ingress of beetles remains uncertain due to observed differences between apiaries. Further, there were side effects associated with restricted entrances that could be only partly mitigated with screened bottom boards.

Introduction

Since the introduction of small hive beetles into the United States, little progress towards developing beetle control methods has been made. In-hive applications of coumaphos-impregnated plastic strips (Check-Mite+) can be used to treat beetles, but control is not consistent (Elzen et al. 1999; Hood 2000; Wenning 2001). Further, coumaphos does not provide extended control because the strips are not registered to remain in colonies continuously. Treating soil around infested colonies with permethrin (GardStar® 40% EC) is recommended (Hood 2000; Pettis and Shimanuki 2000) because beetles pupate in soil (Lundie 1940; Schmolke 1974). However, this treatment is not always effective (Hood 2000; Wenning 2001), killing few beetles unless application is correctly timed (Pettis and Shimanuki 2000).

Mr. J.M. Sikes of Richmond Hills, Georgia suggested that colony invasion by adult beetles may be reduced by sealing and replacing the regular hive entrance with a 1.9-cm inside diameter (ID) polyvinyl chloride (PVC) pipe located 7.6 - 10.2 cm (Figure 11.1). The present, 2-part study was designed to test if screened bottom boards (used for control of *Varroa destructor* Anderson and Trueman in honey bee colonies: Pettis and Shimanuki 1999; Ostiguy et al. 2000; Ellis et al. 2001) and PVC pipes of 2 different diameters (Figures 11.1 and 11.2) can alleviate side effects associated with restricting entrances while rendering efficacious beetle control.

Materials and Methods

Experiment 1

Twenty Langstroth style honey bee colonies, consisting of single deep hive bodies, were established in Warren County, Georgia where there had been no reports of beetles. Each colony received 4 frames of drawn comb, 5 frames of foundation, and 1 division board feeder. One queen and an average of 1.14 kg of bees (range of 1.12 - 1.15 kg) were introduced into all colonies. Colonies were fed 1:1 sugar/water every 2 - 7 d for 5 weeks prior to the start of the experiment. One week after colony set-up, the regular entrances of 10 hives were blocked and tightly sealed with a piece of wood, and new entrances consisting of 2-cm, ID PVC pipe were installed 7.6 - 10.2 cm above the bottom board (Figure 11.1).

Five weeks later, all colonies were moved to an apiary in Richmond Hills, Georgia where there were established beetle populations (>50 beetles per colony, based on visual estimates). All cracks or holes in the colonies were caulked and the lids taped to the hive bodies. The experimental colonies were left in the apiary, unmanaged, unopened, and available to invading beetles until the experiment was dismantled.

The experiment was dismantled on days 58 - 59. Colonies were removed from the experiment if they had died. For each colony the temperature of the interior brood nest and ambient temperature outside the colony were determined with a hand-held digital thermometer. Colonies were then taken to an area of the apiary where bees were shaken from the frames and adult beetles collected for counting, the area of sealed brood (cm²) measured, and colony debris on the bottom board collected for weighing.

Data analysis

The effects of pipe entrance on adult beetle numbers, amount of sealed brood, temperature inside colony, and temperature deviation from ambient were analyzed with a randomized design analysis of variance (PROC GLM SAS 1992) recognizing residual error as the test error term. Because considerable variation in the amount of sealed brood among colonies was noted, brood was tested as a covariate for all variables of interest and retained for the one for which it significantly contributed to the model (temperature inside colony). Additionally, the effects of brood on inside colony temperature were tested with linear regression analysis (PROC REG SAS 1992).

Experiment 2

The experiment was conducted in Richmond Hills, Georgia from March to May 2002. Forty-eight Langstroth honey bee colonies consisting of single deep hive bodies were created as splits from existing colonies. An effort was made to minimize the number of beetles present in the new colonies (<5 beetles per colony). All Langstroth boxes were new and previously unused at the beginning of the study. Each colony was assigned one of 6 treatments and given 4 frames of bees, 3 frames of brood, 1 frame of honey, 6 frames of foundation (all new frames) and a laying queen. Assigned treatments consisted of: (1) a conventional solid bottom board and open entrance (control), (2) a ventilated bottom board consisting of 2-mm wide mesh plastic screen and open entrance, (3) conventional bottom and 1.9-cm ID polyvinyl chloride pipe (PVC) entrance, (4) conventional bottom and 3.8-cm ID PVC pipe entrance, (5) screen bottom and 1.9-cm ID PVC pipe entrance, and (6)

screen bottom and 3.8-cm ID PVC pipe entrance (Figure 11.2). All pipe entrances were 10.2-cm long and inserted through the hive body 7.6-10.2-cm above the bottom board; colonies receiving pipes had their regular entrances blocked shut so that ingress and egress of bees was limited to the pipes. Bottom screen mesh size was chosen based on beetle biometry described by Ellis et al. (2002); the goal was to permit exit of falling varroa mites while denying entry to beetles. The mesh size used in this study (2-mm) was smaller than that most often used for the control of varroa mites (3-mm) because beetles are small enough to move through 3-mm mesh screen. Alcohol samples of approximately 300 bees were taken from each colony to estimate beginning varroa mite populations.

All cracks or holes in the colonies were caulked and the lids taped to the hive bodies. The treatments were equally distributed between two apiaries separated by about 10 km apart and containing >50 colonies, each having existing beetle populations of >50 beetles per colony (based on visual estimates). Colonies were left unmanaged and available to invading beetles. Each treatment was replicated 4 times in each location for a total of 8 replicates per treatment (2 locations \times 6 treatments \times 4 reps = 48 colonies). During both weeks 4 and 8, one new (never used), pre-weighed, medium-depth Illinois super was added to experimental colonies so that each colony had 2 supers by the end of the study. Colonies were re-sealed after each super addition.

The experiment was terminated on day 70. Dead colonies were removed from the study. These colonies did not succumb to beetle pressures; rather they were unable to establish after the initial colony setup. Data collected from all colonies included weighed alcohol samples of approximately 300 bees (used to determine ending varroa mite populations and bee weight); net weight gain (kg) of medium supers (for colony production estimations); ending number of beetles (determined by aspirating and counting); number of deep frames of bees, pollen, and sealed brood (with visual estimates as per Skinner et al. 2001); and presence/absence of a laying queen. Bee weight was determined by weighing the jars of alcohol before and after the addition of bees; the difference between both weights (which was the total weight of bees in the jar) was divided by the number of bees in the jar to give individual bee weight. Unlike in experiment 1, there was no water accumulation in the colonies so this variable was not analyzed in this experiment.

After data collection, all experimental colonies were moved to Oconee County, Georgia and put in one location of maximum sunlight. Three days later the temperature of the interior brood nest was determined with a hand-held digital thermometer.

Data analysis

The data were analyzed in a randomized design analysis of variance recognizing entrance type (open, 1.9-cm pipe, 3.8-cm pipe) and bottom screen (present or absent) as main effects and apiary location as block (except for colony temperature for which there was no location effect). There were interactions between the main effects and location for beetles per colony, net gain of honey supers, and colony brood production. As a result, the data for these variables could not be pooled and were therefore analyzed by apiary (Table 11.2). The test error term was residual error. Means were compared with Duncan's test and differences accepted at $\alpha \leq 0.05$. All analyses were conducted using the software package SAS (1992).

Results and Discussion

Experiment 1

There were treatment effects for the number of adult beetles ($P = 0.0004$). There were no treatment effects for cm^2 sealed brood or temperature inside colonies. The covariate brood significantly affected the temperature inside colonies ($P = 0.0112$) but not the number of adult beetles or temperature deviation from ambient. Treatment means are presented in Table 11.1.

There were treatment effects on the number of adult beetles found within colonies ($F = 19.7$; $df = 1, 17$; $P = 0.0004$). Colonies with pipe entrances had significantly fewer beetles (46.9 beetles/colony) than colonies with conventional entrances (107.7 beetles/colony). The effect of brood on the number of adult beetles within colonies was not significant ($F = 0.0$; $df = 1, 16$; $P = 0.9629$). Therefore, differences between entrances account for the differences found in adult beetle populations. It is possible that adult beetles have difficulty entering colonies with PVC pipe entrances due to problems maintaining footing on plastic pipes. Alternatively, and perhaps more likely, the bees are able to guard smaller entrances better thus protecting the colony from potential beetle invaders.

Treatment did not significantly affect cm^2 sealed brood ($F = 3.2$; $df = 1, 17$; $P = 0.0940$) although there is a pronounced numeric difference (Table 11.1). Colonies with open entrances had almost 2.5 times as much brood (358.2 cm^2) as colonies with pipe

entrances (142.0 cm²). Even though this difference was not significant, it suggests a liability associated with beetle control measures that involve reduced colony entrances.

Treatment did not significantly affect colony nest temperature deviation from ambient ($F = 4.3$; $df = 1, 17$; $P = 0.0526$). Numerically, however, the deviation was smaller (ie., more similar to ambient) for colonies with pipe entrances (Table 11.1), suggesting that these colonies have greater difficulty regulating their temperatures independently of ambient conditions. This could pose a problem when outside temperatures are extreme.

The covariate brood was found to significantly affect the temperature inside colonies ($F = 8.2$; $df = 1, 16$; $P = 0.0112$), and the relationship was explained by the positive linear model $y = 0.007x + 27.2$ where $y =$ nest temperature (°C) and $x =$ cm² brood, $r = 0.59$. The more brood in a colony, the higher the nest temperature. Others (Ritter and Koeniger 1977; Kronenberg 1979; Delaplane and Harbo 1987) have reported a positive relationship between nest temperature and brood.

On average, 7.2 ± 2.7 grams of debris were found in colonies with pipe entrances. No measurable debris was found in open, conventional entrances. This suggests that bees living in hives with reduced entrances have greater difficulty maintaining general hive sanitation. Five of the 10 pipe colonies had debris. Additionally, four pipe colonies had flooded bottom boards, indicating poor water drainage. These problems are further justification for incorporating some type of screened floor in hives with reduced pipe entrances.

Experiment 2

In apiary 1 there were significantly more beetles in colonies with open entrances than in colonies with 3.8-cm pipe entrances (Tables 11.2 and 11.3). Colonies with 1.9-cm pipe entrances were not different from colonies with the other two entrance types. In apiary 2, beetle numbers were also affected by entrance, but the trend was different; there were significantly more beetles in colonies with 1.9-cm entrances than either 3.8-cm pipe entrances or open. The contrasting results for apiaries 1 and 2 suggest that other factors (such as apiary location, nectar flow, etc.) may be crucial in finally establishing the efficacy of reduced entrance devices in controlling beetles. Indeed, factors like nectar flow may influence colony build-up and colony strength, which would directly contribute to the efficacy of pipe entrances in slowing beetle ingress as stronger colonies would likely guard

the reduced entrances better. The success of reduced entrances in limiting beetle ingress reported in the first experiment may have been an artifact of particular season, larger numbers of invading beetles, overall colony health, etc.

An effect of screen on beetle numbers was apparent only in apiary 2 where there were more beetles in colonies without screens than in those with (Table 11.3). I cannot posit an explanation for this effect, especially since the trend was reversed in apiary 1. I do, however, believe the screen mesh used in this study did not allow increased beetle ingress because the mesh size was smaller than published data on beetle biometry (Ellis et al. 2002). If one were to use a smaller mesh size, the potential attributes of such screens toward varroa mite control might be compromised. I noted that beetles often congregated outside the colony under the screen mesh. Presumably this is in response to colony odors dissipating through the screen below the hive. It is possible that future beetle control methods, in the form of below-hive trapping devices, could take advantage of this behavior.

In both apiaries the net gain of honey supers was affected by entrance type (Table 11.2). In apiary 1, net gain was higher in open entrances than with 1.9-cm pipe entrances (Table 11.3). The 3.8-cm entrance group was not different from the other two. In apiary 2, net gain was higher with open entrances than either 3.8- or 1.9-cm entrances (Table 11.3). Thus, in some conditions the proposed IPM strategy may involve a cost to colony productivity. However the 3.8-cm entrance is clearly preferable over the 1.9-cm and in one apiary it did not significantly reduce yield. Nevertheless, it seems prudent to limit use of the candidate IPM strategy to non-production seasons.

Concerning brood production, there was a significant effect of entrance in apiary 2 (Table 11.3); colonies with open entrances had more frames of sealed brood than colonies with either 3.8- or 1.9-cm pipe entrances (Table 11.3). Although not significant, the trend was the same in apiary 1. Thus I conclude that there is a cost to brood production with this candidate IPM strategy, as suggested by the first experiment. There was a significant effect of screen in apiary 1 in which colonies with screens had significantly more frames of brood than colonies without. Although not significant, the trend was the same in apiary 2. Mean values in Table 11.3 show that brood production in colonies with reduced entrances was increased with the addition of a bottom screen. Thus, bottom screens may partially offset the negative effect of reduced entrances on brood. A positive effect of screens on brood has been reported in earlier work (Pettis and Shimanuki 1999; Ellis et al. 2001).

Absence of interactions between main effects and apiary location permitted me to pool apiary data for bee weight, percentage beetles female, frames of adult bees, frames of pollen, and change in the number of varroa mites per adult bee; colony temperature was analyzed without location effects (Table 11.4). There was a significant effect of screen on bee weight, with heavier bees in colonies with screens than without (Table 11.5). There was no effect of screen or entrance on colony temperature. It is noteworthy that the candidate IPM strategy of restricted entrances had no measurable liability concerning thermoregulation by bees. The percentage of beetles female was affected only by apiary location, with significantly more female beetles in apiary 2 ($71.4 \pm 7.2\%$) than apiary 1 (53.2 ± 2.9). In both apiaries there were greater numbers of female beetles than males. Female-biased sex ratios have been reported by others (Schmolke 1974; Neumann et al. 2001a; Ellis et al. 2002; Chapter 4). Because the percent female was not affected by bottom type, there is no reason to believe that female beetles (which are bigger than males and perhaps unable to cross the screen as well as males) were excluded from hives with screens more than males.

Frames of adult bees were affected by screen and entrance, with more bees in colonies with screens and significantly fewer bees in colonies with 3.8-cm pipe entrances or 1.9-cm entrances compared to open entrances (Table 11.5). There was also a significant interaction for this variable between screen and entrance, apparent in Table 11.5. In colonies without bottom screens there was a more pronounced decline in bee population with the addition of a reduced entrance, and the compensation afforded by the larger of the two entrances (3.8-cm) was modest. In colonies with screens, on the other hand, the addition of a reduced entrance reduced bee population only with the smaller 1.9-cm entrances; population with 3.8-cm entrances was actually higher than in the open entrances (Table 11.5). Thus, although there is an overall cost to adult bee population with reducing colony entrances, this cost can be offset in 3.8-cm pipe entrances if the beekeeper simultaneously uses a screened bottom board.

Frames of pollen were affected by entrance, with significantly more pollen in colonies with open entrances or 3.8-cm pipe entrances than 1.9-cm entrances (Table 11.5). Thus, there appears to be a cost to pollen storage with entrances reduced below 3.8 cm. Furthermore, there was a significant interaction between main effects, apparent in Table 11.5. In colonies without a screen bottom there was an overall sharper drop in pollen with the addition of a reduced entrance. With screened colonies the cost to pollen storage of a

reduced pipe entrance was moderated, with pollen in fact higher in 3.8-cm pipe entrances than the open group. As I found for adult bee populations, there is a cost to stored pollen with reducing colony entrances, but this cost is offset in 3.8-cm pipe entrances with a screened bottom board.

Because bottom screens are used in varroa mite IPM protocols (Pettis and Shimanuki 1999; Ostiguy et al. 2000; Ellis et al. 2001), I examined changes in number of varroa mites per adult bee. I found no effects of entrance or screen on this variable (Table 11.4), probably due to the low number of varroa mites present in the study. It remains inconclusive whether the screen mesh size used in this study, which is smaller than that conventionally used for the control of varroa mites, inhibits beetle ingress while permitting the exit of falling varroa mites.

I conclude that more studies must be done on reduced hive entrances in order to determine their efficacy in impeding beetle ingress. The data suggest that reduced hive entrances may slow beetle ingress in some instances, but that their success is limited by other factors internal or external to the colony. For the practice to work optimally, it is necessary to close superfluous gaps or holes in bee hives which is, no doubt, costly in time and labor to the beekeeper. Further, reduced entrances cause harmful secondary effects on brood and bees that are only partly mitigated by screened bottom boards. In spite of these challenges, non-chemical controls such as the one indicated in this study are an important step toward a more environmentally sound management program for beetles.

Table 11.1. Treatments administered to honey bee colonies placed in a beetle-infested apiary included conventional, open entrances or entrances reduced to a single pipe.

entrance	sealed brood (cm ²)	no. adult beetles	temp (°C) inside colony	temp deviation (°C) from ambient
open	358.2 ± 119.0 (9)a	107.7 ± 11.9 (9)a	29.7 ± 1.2 (9)a	5.7 ± 0.8 (9)a
pipe	142.0 ± 44.2 (10)a	46.9 ± 7.3 (10)b	28.1 ± 0.8 (10)a	2.9 ± 1.1 (10)b

Data are mean ± standard error; *n* is given in parentheses. Column means followed by the same letter are not different at the $\alpha \leq 0.05$ level.

Table 11.2. Analysis of variance testing effects of reduced hive entrances (E) and bottom screens (S) on the average number of small hive beetle adults per colony, net colony production (kg), and colony brood production (frames).

Apiary 1				
variable	source	df	<i>F</i>	<i>P</i> > <i>F</i>
beetles per colony	E	2	4.8	0.0228
	S	1	0.8	0.3989
	E×S	2	6.3	0.0089
colony production	E	2	3.6	0.0501
	S	1	0.03	0.8627
	E×S	2	1.2	0.3231
colony brood production	E	2	4.7	0.0586
	S	1	6.5	0.0440
	E×S	2	1.8	0.2478
Apiary 2				
beetles per colony	E	2	15.1	0.0002
	S	1	38.3	<0.0001
	E×S	2	18.0	<0.0001
colony production	E	2	9.5	0.0017
	S	1	0.01	0.9243
	E×S	2	6.0	0.0107
colony brood production	E	2	11.5	0.0020
	S	1	3.2	0.1013
	E×S	2	2.4	0.1348

There were significant interactions with the main effects and location, so these variables were analyzed by location. Terms were tested against residual error.

Table 11.3. Effects of reduced hive entrances and bottom board design on the average number of small hive beetle adults per colony, net colony production (kg), and colony brood production (frames).

Apiary 1				Apiary 2			
beetles				beetles			
	solid	screen	entrance totals		solid	screen	entrance totals
open	27.3 ± 3.4 (4)	61 ± 10.6 (4)	44.1 ± 8.2 (8)a	open	3.8 ± 2.8 (4)	3.3 ± 0.8 (4)	3.5 ± 1.3 (8)b
1.9	30.3 ± 4.2 (4)	28.5 ± 4.1 (4)	29.4 ± 2.7 (8)ab	1.9	40 ± 7.2 (3)	1.3 ± 0.8 (4)	17.9 ± 8.3 (7)a
3.8	30.3 ± 15.6 (3)	13.8 ± 2 (4)	20.9 ± 6.9 (7)b	3.8	11.5 ± 4.6 (4)	1.5 ± 0.3 (4)	6.5 ± 2.9 (8)b
bottom board totals	29.2 ± 4.1 (11)a	34.4 ± 6.9 (12)a		bottom board totals	16.5 ± 5.3 (11)a	2 ± 0.4 (12)b	
production				production			
	solid	screen	entrance totals		solid	screen	entrance totals
open	3.2 ± 1.5 (4)	1.9 ± 1.1 (4)	2.5 ± 0.9 (8)a	open	17.7 ± 4.9 (4)	7.9 ± 2.7 (4)	12.8 ± 3.2 (8)a
1.9	0 (4)	0 (4)	0 (8)b	1.9	0 (3)	0.9 ± 0.5 (4)	0.5 ± 0.3 (7)b
3.8	0 (3)	1.8 ± 1.3 (4)	1 ± 0.8 (7)ab	3.8	0.01 ± 0.01 (4)	9.6 ± 3.6 (4)	4.8 ± 2.4 (8)b
bottom board totals	1.2 ± 0.7 (11)a	1.2 ± 0.6 (12)a		bottom board totals	6.4 ± 3.1 (11)a	6.1 ± 1.8 (12)a	
brood				brood			
	solid	screen	entrance totals		solid	screen	entrance totals
open	2.7 ± 0.8 (2)	2.8 (1)	2.7 ± 0.5 (3)a	open	1.8 ± 0.1 (4)	1.7 ± 0.2 (4)	1.7 ± 0.1 (8)a
1.9	0.4 ± 0.1 (3)	1.6 ± 0.6 (3)	1 ± 0.4 (6)a	1.9	0.4 ± 0.1 (2)	1.2 ± 0.05 (3)	0.9 ± 0.2 (5)b
3.8	0 (1)	2.6 ± 0.4 (2)	1.7 ± 0.9 (3)a	3.8	1 ± 0.2 (2)	1.2 ± 0.6 (2)	1.1 ± 0.3 (4)b
bottom board totals	1.1 ± 0.6 (6)a	2.1 ± 0.4 (6)b		bottom board totals	1.2 ± 0.2 (8)a	1.4 ± 0.1 (9)a	

Colonies were fitted with either a conventional solid bottom board (solid) or a screened bottom consisting of 2-mm plastic mesh (screen). Additionally, colony entrances were either open conventionally (open) or reduced to a single pipe of either 1.9- or 3.8-cm diameter. There were significant interactions with main effects and location (Table 11.2), so these variables were analyzed by location. Values are mean ± standard error; number in parentheses = *n*. Bottom board totals and entrance totals followed by the same letter are not different at the $\alpha \leq 0.05$ level. Means were compared using Duncan's test.

Table 11.4. Analysis of variance testing effects of reduced hive entrances (E) and bottom screens (S) on average weight per bee (mg), average internal colony temperature (°C), percentage of beetles female, amount of adult bees (frames), amount of stored pollen (frames), and change in the number of *V. destructor* per adult bee.

variable	source	df	<i>F</i>	<i>P</i> > <i>F</i>
bee weight	L	1	0.1	0.7392
	E	2	0.4	0.6675
	S	1	10.5	0.0029
	E×S	2	2.5	0.1026
	L×E×S	5	0.9	0.4923
colony temperature	E	2	0.5	0.6380
	S	1	1.3	0.2584
	E×S	2	0.9	0.4034
% beetles female	L	1	4.7	0.0374
	E	2	1.0	0.3989
	S	1	1.5	0.2340
	E×S	2	0.2	0.8566
	L×E×S	5	0.6	0.70
amount adult bees	L	1	2.7	0.110
	E	2	13.0	<0.0001
	S	1	7.3	0.0106
	E×S	2	7.3	0.0023
	L×E×S	5	0.8	0.5736
amount stored pollen	L	1	0.04	0.8496
	E	2	8.4	0.0011
	S	1	0.01	0.9425
	E×S	2	4.7	0.0159
	L×E×S	5	0.9	0.5121
change in no. varroa mites	L	1	0.01	0.9430
	E	2	1.3	0.2866
	S	1	0.22	0.6443
	E×S	2	1.12	0.3379
	L×E×S	5	0.94	0.4674

The experiment was blocked on two apiary locations (L), except for colony temperature. The interaction L × E × S was never significant; so all terms were tested against residual error.

Table 11.5. Effects of reduced hive entrances and bottom board design on average weight per bee (mg), average internal colony temperature (°C), percentage of beetles female, amount of adult bees (frames), amount of stored pollen (frames), and change in number varroa mites per adult bee.

bee weight				temperature			
	solid	screen	entrance totals		solid	screen	entrance totals
open	133.9 ± 5.6 (8)	135.5 ± 4.2 (6)	134.6 ± 3.6 (14)a	open	34.1 ± 0.4 (8)	34.3 ± 0.4 (8)	34.2 ± 0.3 (16)a
1.9	121.1 ± 4.8 (7)	141.1 ± 4.5 (7)	131.1 ± 4.2 (14)a	1.9	35 ± 0.2 (4)	34.4 ± 0.6 (8)	34.6 ± 0.4 (12)a
3.8	117.2 ± 5.1 (7)	141.6 ± 7.5 (8)	130.2 ± 5.5 (15)a	3.8	35.1 ± 1 (5)	33.8 ± 0.5 (8)	34.3 ± 0.5 (13)a
bottom board totals	124.5 ± 3.3 (22)a	139.7 ± 3.3 (21)b		bottom board totals	34.6 ± 0.4 (17)a	34.2 ± 0.3 (24)a	
% female				bees			
	solid	screen	entrance totals		solid	screen	entrance totals
open	53.9 ± 11.5 (7)	60.7 ± 11.6 (8)	57.5 ± 8 (15)a	open	6.7 ± 1 (8)	5.1 ± 0.7 (8)	5.9 ± 0.6 (16)a
1.9	50 ± 3 (7)	64 ± 7.7 (6)	56.5 ± 4.2 (13)a	1.9	0.6 ± 0.2 (7)	3.7 ± 0.5 (8)	2.3 ± 0.5 (15)b
3.8	68.6 ± 10.1 (7)	71.7 ± 9.7 (8)	70.3 ± 6.8 (15)a	3.8	2.4 ± 0.8 (7)	5.6 ± 0.9 (8)	4.1 ± 0.7 (15)c
bottom board totals	57.5 ± 5.2 (21)a	65.6 ± 5.7 (22)a		bottom board totals	3.4 ± 0.7 (22)a	4.8 ± 0.4 (24)b	
pollen				change in no. varroa mites			
	solid	screen	entrance totals		solid	screen	entrance totals
open	0.9 ± 0.2 (8)	0.5 ± 0.1 (8)	0.7 ± 0.1 (16)a	open	0.04 ± 0.04 (7)	0.006 ± 0.003 (8)	0.02 ± 0.02 (15)a
1.9	0.1 ± 0.1 (7)	0.3 ± 0.1 (8)	0.2 ± 0.05 (15)b	1.9	-0.001 ± 0.0006 (7)	0.03 ± 0.03 (8)	0.01 ± 0.01 (15)a
3.8	0.4 ± 0.1 (7)	0.6 ± 0.1 (8)	0.5 ± 0.07 (15)a	3.8	-0.02 ± 0.01 (7)	-0.0003 ± 0.0003 (8)	-0.01 ± 0.006 (15)a
bottom board totals	0.5 ± 0.09 (22)a	0.5 ± 0.06 (24)a		bottom board totals	0.005 ± 0.01 (21)a	0.01 ± 0.01 (24)a	

Colonies were fitted with either a conventional solid bottom board (solid) or a screened bottom consisting of 2-mm plastic mesh (screen). Additionally, colony entrances were either open conventionally (open) or reduced to a single pipe of either 1.9- or 3.8-cm diameter. Values are mean ± standard error; number in parentheses = *n*. Bottom board totals and entrance totals followed by the same letter are not different at the $\alpha \leq 0.05$ level. Means were compared using Duncan's test.



Figure 11.1. A colony with a 2 cm PVC pipe entrance placed 7.6 – 10.2 cm above the bottom board of a Langstroth-style hive body. The regular entrance is blocked, and all gaps or holes in the hive are sealed. Colonies with pipe entrances had significantly fewer adult small hive beetles; however there appear to be associated problems with reduced brood production, impaired thermoregulation, excess floor debris, and poor water drainage.



Figure 11.2. A colony fitted with a 3.8-cm PVC pipe entrance and screened bottom board (screen not visible) to restrict entry of small hive beetles while compensating for a corresponding loss of hive ventilation.

Chapter 12: Hygienic Behavior of Cape and European Honey Bees Toward Small Hive Beetle Eggs Oviposited in Sealed Bee Brood

Abstract – In this study, I tested for the presence and efficacy of hygienic behavior by Cape honey bees in South Africa and European honey bees of mixed origin in the United States toward beetle eggs oviposited in sealed bee brood. I set forth a practical assay that can be used to test for the existence and level of hygienic behavior toward beetle eggs expressed by a single honey bee colony. I also looked for colony differences in removal rates of infected cells within each subspecies to possibly identify colonies within each location that display superior hygienic behavior. Finally, I determined the infection rate (presence/absence of beetle eggs) of brood cells containing punctures made by beetles and the number of beetle eggs oviposited in each infected cell. There were no colony differences within subspecies for the removal of control (capped brood), punctured-control (capped brood cells which were punctured with a pin), and infected brood (capped brood cells which were punctured by beetles). For both subspecies, the bees removed significantly more infected brood than either control or punctured-control brood; there was no difference between the amount of infected brood removed by each subspecies. Beetles oviposited significantly more eggs per cell in Cape colonies than in European ones but they did not oviposit in more cells in colonies of either subspecies. The proportion of infected brood in colonies of both subspecies was not significantly different from the proportion of infected brood removed by each subspecies. The data suggest that both Cape and European honey bees selectively remove only that brood which has been oviposited on by beetles.

Introduction

Honey bees express hygienic behavior (the detection of parasitized/diseased brood, uncapping of the wax covering over the brood cells, and removal of the infected larvae or pupae) toward diseased brood and the expression of this behavior often minimizes depredation caused by a host of parasites and pathogens (cf. Boecking and Spivak 1999; cf. Spivak and Boecking 2001). Rothenbuhler (1964a), who pioneered the study of hygienic behavior, demonstrated that European honeybees can detect and remove brood killed by *Paenibacillus larvae* (American Foulbrood) and subsequently others have shown detection and removal of *Ascosphaera apis* (chalkbrood)-killed and even *Varroa destructor* (varroa mites)-infested brood (Gilliam et al. 1983; Spivak and Gilliam 1993; cf. Boecking and Spivak 1999; cf. Spivak and Boecking 2001).

Female small hive beetles oviposit in bee brood sealed (capped) with wax (Chapters 3 and 10) and the removal of 'infected' brood may be one component that contributes to the overall success of natural host colonies (African honey bees) at limiting beetle-associated depredation (Chapter 10). Failure to remove brood in which beetles have oviposited could easily lead to a population buildup of beetle larvae (I have found as many as 120 beetle eggs oviposited in one brood cell), which in turn damage host colonies by devouring honey, pollen, and bee brood (Elzen et al. 1999; Hood 2000; Chapter 4).

In this study, I tested for the presence and efficacy of hygienic behavior by Cape bees in South Africa and European bees of mixed origin in the United States toward beetle eggs oviposited in sealed bee brood. I set forth a practical assay that can be used to test for the existence and level of hygienic behavior toward beetle eggs expressed by a single honey bee colony. I also looked for colony differences in removal rates of infected cells within each bee subspecies to possibly identify colonies within each location that display superior hygienic behavior. Finally, I determined the infection rate (presence/absence of beetle eggs) of brood cells containing punctures made by beetles and the number of beetle eggs oviposited in each infected cell.

Materials and Methods

Experiments on Cape honeybees were conducted at a Rhodes University research apiary outside of Grahamstown, South Africa (a geographic area predominantly inhabited by Cape bees) in March - May 2003. The complimentary studies on European honeybees

of mixed origin were conducted at The University of Georgia's research apiary near Watkinsville, Georgia USA in July - August 2003. Ten colonies of Cape honeybees and 9 colonies of European honey bees (housed in standard Langstroth-style hives, of equal strength, and having nearly identical reserves of brood, honey, pollen, and adult bees) were used for the study. All colonies had previously been exposed to beetles.

For each colony, a frame of capped brood was removed and twenty randomly collected adult beetles (anesthetized in a small vial surrounded by crushed ice for approximately 4 - 5 minutes) were placed on a 10×10 -cm area on the comb (treatment) in a sheet metal push-in cage ($10 \times 10 \times 2.5$ cm; $l \times w \times h$), the face of which was screen mesh to allow for ventilation (Figure 12.1). The combs used contained approximately 60-90% capped brood. The selected brood was > 6 days from eclosing (determined by randomly uncapping and removing brood in the test area) so that no brood from the test area would emerge during the study. A second cage without beetles was pushed into the brood frame as a control. Both caged sections of brood were placed in the center of the bee cluster in each colony.

Twenty-four hours later, both cages were removed and the adult beetles from the treatment cage were recollected. Beetle oviposition punctures in the capped cells were noted (Chapter 3). A transparent sheet of plastic was placed over the infected brood and all cells containing punctured cappings were marked (infected brood treatment). Similarly, twenty uninfected brood cells (no punctures in the cappings) from under the control cage were marked (control). A second control was created by puncturing the cappings of 20 brood cells with a minuten insect pin to simulate beetle oviposition punctures (punctured-control). The punctures were positioned around the capping perimeter to minimize damage to the pupae (pin-killed pupae are removed by bees: cf. Boecking and Spivak 1999). The infected and control brood were replaced in the center of the bee cluster. After forty-eight hours they were examined and marked cells from which infected or control brood had been subsequently removed by the bees were counted. The procedure was replicated three times for each Cape and European colony.

The infection rate of treatment cells containing punctures made by beetles was also determined. For each of six Cape and seven European colonies, twenty adult beetles were confined to one frame of capped brood as before and the frames were replaced in the colonies. Twenty-four hours later, cells from each frame having punctures in their cappings were opened to determine the presence/absence of beetle eggs (about 30 cells per colony in Cape colonies were opened and all punctured cells in European colonies were

opened). The total number of cells punctured and oviposited in by beetles was divided by the total number of punctured cells to determine the infection rate. For each infected cell, the number of beetle eggs was determined.

Data analysis

Differences between colony removal rates of control, punctured-control, and infected brood were analyzed by treatment within bee subspecies using one-way ANOVAs. Because colonies within both subspecies did not differ with respect to the amount of any treatment brood removed (ie. no colonies within subspecies were ‘more hygienic’ than others), colony replicates were averaged for each colony for use in further analyses. The proportion of removed brood was analyzed by ANOVA recognizing brood condition (control, punctured-control, or infected) and honeybee subspecies (Cape or European) as main effects. Differences in the infection rate of cells and in the number of beetle eggs per brood cell were both analyzed by honey bee subspecies (Cape or European) using independent sample t-tests. Further, the infection rate of brood was compared to the removal rate of infected brood for both subspecies using independent sample t-tests. Where analyzed data were proportions (as in the proportion of removed brood and the infection rate), the data were transformed using $\arcsin\sqrt{\text{proportion}}$ to stabilize the variance prior to analyses. All differences were accepted at $\alpha \leq 0.05$.

Results

Colony-level removal of infected brood

There were no colony differences among Cape honey bees for the removal of control ($F = 1.1$; $df = 9, 20$; $P = 0.4364$), punctured-control ($F = 0.6$; $df = 9, 20$; $P = 0.7510$), or infected ($F = 0.8$; $df = 9, 20$; $P = 0.6602$) brood. Further, there were no colony differences among European honey bees for the removal of control ($F = 0.6$; $df = 8, 18$; $P = 0.7359$), punctured-control ($F = 0.3$; $df = 8, 18$; $P = 0.9373$), or infected ($F = 1.2$; $df = 8, 18$; $P = 0.3647$) brood. Mean removal rates for colonies of both bee subspecies are reported in Table 12.1.

Hygienic behavior of Cape and European bees

There were no subspecies effects for the total proportion of brood removed ($F = 0.1$; $df = 1, 51$; $P = 0.7716$). Overall, Cape bees ($0.24 \pm 0.06, 30$; mean \pm standard error, n)

removed the same proportion of all tested brood as did their European counterparts (0.23 ± 0.05 , 27). There were treatment effects ($F = 336.4$; $df = 2, 51$; $P < 0.0001$) and treatment \times subspecies interactions ($F = 16.9$; $df = 2, 51$; $P < 0.0001$) for the proportion of brood removed. For both subspecies, the bees removed significantly more infected brood than either control or punctured-control brood (Table 12.2); there was no difference between the amount of infected brood removed by each subspecies (Table 12.2). Further the amount of control and punctured-control brood removed by Cape bees was not different from the amount of control brood removed by European bees (Table 12.2). The amount of punctured-control brood removed by European bees was different from that of all other treatments (Table 12.2). Colonies of both bee subspecies also uncapped some infected pupae (<5%), but did not remove it.

Infection rate and number of eggs per cell

There was no significant difference between Cape and European honey bees for the infection rate of cells punctured by the beetles ($|t| = 1.5$; $df = 11$; $P = 0.1642$). The proportion of infected cells in Cape colonies (0.68 ± 0.04 ; 6) was similar to that in European ones (0.56 ± 0.06 ; 7). Further beetles oviposited significantly more eggs per cell in Cape colonies (14.5 ± 1.4 ; 122) than in European ones (7.3 ± 0.4 ; 312) ($|t| = 7.0$; $df = 432$; $P < 0.0001$). The proportion of infected brood in Cape bee colonies was not significantly different from the proportion of infected brood removed by the bees ($|t| = 0.2$; $df = 14$; $P = 0.8367$); the same held true in European colonies ($|t| = 0.1$; $df = 14$; $P = 0.9393$).

While rearing beetles *in vitro* for use in this study, I observed the process by which beetles puncture and oviposit in capped brood cells. Female beetles use their mandibles to bite small holes through the cell capping. They then position the tip of their abdomen flush with the puncture and insert their ovipositor to begin laying eggs. This process usually lasted >5 seconds each time (probably depending on the number of eggs the females were ovipositing per cell).

Discussion

In European colonies, beetles puncture cell cappings and oviposit even in the presence of bees (Chapter 3) but it is not yet known if they do the same in African colonies. This mode of oviposition may be an important reproductive pathway for the

beetle (Chapter 10) since exposed beetle eggs are quickly removed from colonies (Neumann and Härtel 2003). Lundie (1940) and Schmolke (1974) suggest that beetles oviposit in cracks and crevices around the hive. However hatching larvae would have to crawl to the combs while bypassing the bees and studies have shown that free-roaming larvae are removed from African colonies (Neumann and Härtel 2003). Therefore, direct oviposition into brood cells may be preferred (Chapter 10). As a result, the hygienic removal of infected brood may be an important resistance mechanism toward beetle depredation.

The data indicate that both Cape and European honey bees remove brood which has been oviposited on by beetles. If this behavior were essential to the overall immunity of Cape bees toward beetle depredation, then one would expect to find the behavior either much reduced or absent in European bees. This clearly was not the case. The data did not demonstrate a difference between the level of infected brood removal for each subspecies. However, it remains possible that if a larger area of brood had been oviposited on, one may have seen differences between both subspecies with respect to the removal rate of infected brood.

Interestingly, both subspecies removed the same proportion of brood as that which was naturally infected, a finding also demonstrated for a second mode of beetle oviposition where beetles enter empty cells and oviposit through the cell wall into an adjacent cell (Chapter 10). In the present study, both subspecies removed an amount of brood equal to that of the normal infection rate, suggesting that they selectively open and remove brood only from those punctured cells actually containing eggs. Further, neither subspecies removed punctured-control brood at similar or higher rates to infected brood, suggesting that it is not the punctured capping which stimulates the removal of cell contents.

What stimulates bees to remove beetle egg-infected cells remains unclear. Pathogen-killed brood may be easily recognized and removed by the bees (Rothenbuhler 1964a; cf. Boecking and Spivak 1999); however, the oviposition tactics of beetles do not kill the brood. Despite this, both bee subspecies were able to detect and remove infected brood. Therefore, it is very likely the presence of beetle eggs in a cell or an oviposition chemical deposited by female beetles that causes the bees to remove the cell contents.

If bees cue into the presence of beetle eggs, there may exist a minimum number of eggs per cell that elicits the removal of the cell contents. If so, then one would expect that colonies in which beetles lay fewer eggs per cell would be most unlikely to detect and remove infected brood. This study did not allow one to determine if an egg threshold

exists, but beetles clearly laid fewer eggs per cell in European colonies perhaps increasing the bees' chances of missing infected cells in these colonies. As a result, putting fewer beetles under each cage may have encouraged beetles to oviposit fewer eggs per cell as competition for oviposition sites could have lead to the high number of eggs per cell seen in this study. Using fewer adults may make the test more sensitive to detecting differences in the removal rates between both subspecies (if such differences exist).

Why beetles puncture some cells but do not oviposit in them is unclear. This, however, may indicate that they cue into a certain development stage of the brood or into a chemical produced by the brood before they oviposit. Interestingly the infection rate of beetle-punctured cells in Cape colonies was higher than that in European ones. This may indicate the absence/reduction of a chemical oviposition-stimulant in non-native hosts.

One objective of this study was to determine if colonies differed with respect to the level of hygienic behavior they displayed (colony variation for hygienic removal of varroa is often high - cf. Boecking and Spivak 1999). However, differences in the level of hygienic removal of infected brood for colonies of either subspecies were not detected. This may indicate that the assay was not sensitive enough to tease out differences between the colonies or that individual colonies were not replicated enough to detect differences (the likely reason). However, because other factors (such as environmental conditions, colony size, etc.) are often responsible for the level of hygienic expression (cf. Boecking and Spivak 1999), one may have to control for these when trying to determine if the level of hygienic expression towards beetle oviposition varies between colonies.

Regardless, that all tested colonies of both bee subspecies removed infected brood is striking, especially since reports indicate that only few colonies (<10%) in nature express hygienic behavior (cf. Boecking and Spivak 1999). This further suggests that the level of removal stimulants in the brood (eggs, oviposition chemicals, etc.) may have been unnaturally high. This demonstrates a need to look at what beetle stimuli elicit removal of brood so that one may manipulate these levels experimentally. If done, it may be possible to 1) further determine if the expression of hygienic removal of infected brood differs between African and European subspecies of honey bees and 2) select for this behavior as a natural defense against beetle depredation in areas where the beetle is introduced.

Table 12.1. Colony data for the removal of control, punctured-control, and infected brood. Colonies within each subspecies did not differ with respect to the amount of brood removed within each treatment type.

col	Cape honey bees			European honey bees		
	control	punctured-control	infected	control	punctured-control	infected
1	0.02 ± 0.02	0.02 ± 0.02	0.41 ± 0.14	0.03 ± 0.03	0.15 ± 0.09	0.59 ± 0.10
2	0.03 ± 0.02	0	0.73 ± 0.13	0.02 ± 0.02	0.08 ± 0.08	0.73 ± 0.03
3	0	0	0.74 ± 0.14	0	0.12 ± 0.04	0.67 ± 0.03
4	0	0.02 ± 0.02	0.71 ± 0.07	0.02 ± 0.02	0.25 ± 0.18	0.51 ± 0.08
5	0.03 ± 0.02	0.02 ± 0.02	0.57 ± 0.15	0.02 ± 0.02	0.23 ± 0.21	0.51 ± 0.12
6	0.08 ± 0.04	0.02 ± 0.02	0.79 ± 0.14	0	0.07 ± 0.07	0.42 ± 0.12
7	0	0.05 ± 0.03	0.67 ± 0.11	0	0.10 ± 0.08	0.58 ± 0.08
8	0.10 ± 0.08	0.02 ± 0.02	0.69 ± 0.07	0	0.03 ± 0.02	0.60 ± 0.10
9	0.07 ± 0.07	0.02 ± 0.02	0.65 ± 0.05	0	0.08 ± 0.04	0.46 ± 0.09
10	0.07 ± 0.04	0.03 ± 0.02	0.71 ± 0.12	na	na	na

Data are mean ± standard error, $n = 3$ for all data. Data within columns are not different at the $\alpha \leq 0.05$ level. Data were collected for only 9 European colonies so data for the tenth colony is not available (na).

Table 12.2. The removal rate of control, punctured-control, and infected brood by Cape and European honey bees.

treatment	Cape honey bees	European honey bees
control	0.04 ± 0.01, 10a	0.01 ± 0.004, 9a
punctured control	0.02 ± 0.005, 10a	0.12 ± 0.02, 9b
infected	0.67 ± 0.03, 10c	0.57 ± 0.03, 9c

Data are mean ± standard error, *n*. Data followed by the same letter are not different at the $\alpha \leq 0.05$ level.

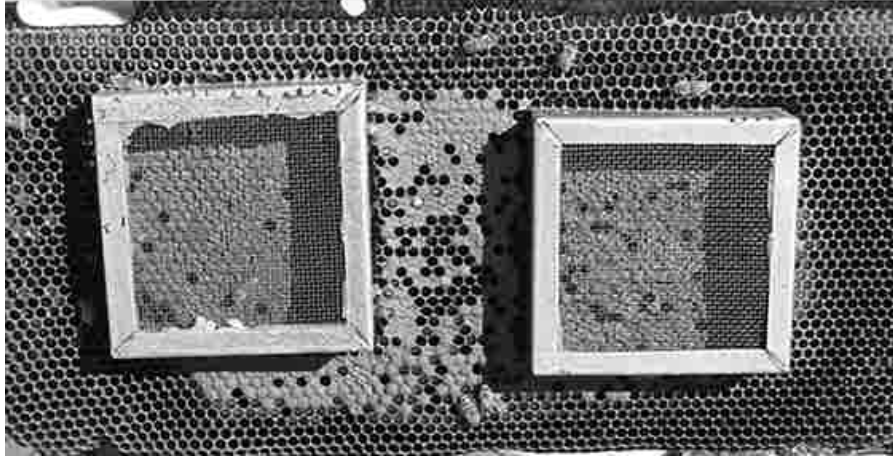


Figure 12.1. The metal push-in cages that were used to confine adult beetles to sections of brood. The face of the cage was screen mesh (for ventilation). One cage contains beetles (infected) and the other cage is empty (control and punctured-control).

Chapter 13: Susceptibility of Small Hive Beetles to Fungal Pathogens?

Abstract - In this study, otherwise-healthy, small hive beetle larvae were exposed to beetle larvae that had died during pupation and were colonized by fungi via 1) ingestion of honey bee brood inoculated with an emulsion of the dead larvae or 2) contact with the dead larvae post-feeding. Larval mortality was determined in a preliminary assessment of the unidentified pathogen's potential as a biological control agent. Finally, the fungi colonizing the dead larvae were identified. Similar numbers of beetle larvae eclosed when feeding on either the control or treatment brood. However, the number of eclosing beetles was significantly lower for healthy larvae that had contacted pathogen-killed larvae post-feeding than for larvae that had not. Two species of *Aspergillus* were discovered on the cadavers, *A. flavus* and *A. niger*. Both are soil fungi known to attack insects. Three additional fungi, all saprotrophic, were also found on the surface of the cadavers. They include: *Clonostachys rosea*, *Gliocladium catenulatum*, and *Mucor plumbeus*. Further investigations must be conducted to ascertain which pathogen caused increased mortality of beetle larvae.

Introduction

Those studying small hive beetles have tested a suite of chemical, mechanical, and genetic control measures against all beetle life stages. These include in-hive applications of coumaphos-impregnated plastic strips (Check-Mite+)(Elzen et al. 1999; Hood 2000), ground drenches using permethrin (GardStar 40% EC)(Hood 2000), reducing colony entrances with polyvinyl chloride pipes (to impede beetle ingress)(Chapter 11), in-hive trapping devices (Hood and Miller 2003), and hygienic behavior of honey bees toward beetle eggs oviposited in sealed bee brood (Chapter 12). However, studies into the efficacy of some of these measures (in-hive traps, hygienic behavior) are in their infancy and necessitate further investigations while the remaining control measures (coumaphos strips, ground drenches, reducing colony entrances) have proven either inefficacious or inconsistent.

Perhaps the most overlooked, possible beetle-control arena has been that of a biological one. This likely owes to a lack of research in the beetle's endemic range (sub-Saharan Africa) where if biological control agents exist, they are yet to be identified. Lundie (1940) first reported a potential biological control agent for the beetle when he noticed high beetle mortality while rearing beetles *in vitro*. He inferred that an unidentified fungus caused the increased beetle mortality. Similarly, I noticed that mortality of beetle pupae in various soil containers *in vitro* was markedly high and the pupae appeared to be succumbing to a fungal infection although the exact cause of death could not be verified. I therefore decided to investigate the possibility of the existence of beetle-associated pathogens.

When searching for biological control agents, one must 1) establish if there is an increased mortality when exposed to a suspected pathogen and 2) identify and 3) purify strains of the pathogen for further studies into host mortality and specificity. Here, I report data regarding the first two steps of this process. In this study, I exposed otherwise-healthy beetle larvae to diseased larvae (larvae that had died during pupation and were colonized by fungi which may or may not have been the causative agents) via 1) ingestion of bee brood inoculated with an emulsion of the dead larvae or 2) contact with the dead larvae during the wandering phase (Schmolke 1974). Larval mortality was determined in order to conduct a preliminary assessment of the pathogen's potential as a biological control agent. Finally, the fungi colonizing the dead larvae were identified in order to determine if any of them could have been responsible for the increase in larval mortality.

Materials and Methods

Experiments were conducted at Rhodes University in Grahamstown, South Africa, July - September 2003. Two rearing chambers were established for the first study, each housing about 40 adult beetles, food (a comb of honey, pollen, and bee brood) and one of two different treatments. Treatments consisted of food sprayed with distilled water (control) or sprayed with distilled water mixed with about 150, ground-up corpses of beetle larvae that had presumably died because of the pathogen (inoculated). Adult beetles were allowed to oviposit in the rearing chambers and the resulting larvae fed on and contacted the treated food sources (presumably ingesting the pathogen but certainly contacting it).

Once the larvae had finished feeding and had reached the wandering phase (Schmolke, 1974), thirty larvae from the control chamber were put into a plastic container in about 950 ml of loose, moistened soil (about 10% moisture, determined by weighing a sub-sample of the moist soil and then oven-drying the sample until constant dry weight). This was repeated 12 more times for a total of 13 soil containers each having 30 control larvae. Likewise, this procedure was repeated for larvae in the inoculated chamber except 14 soil containers were used, each having 30 larvae fed the inoculated brood. The soil containers were monitored daily and kept at constant light and temperature ($24.9 \pm 0.2^\circ\text{C}$, mean \pm standard deviation). Adult beetles were collected upon eclosion.

For the second half of the study, I collected otherwise-healthy larvae (reared *in vitro* as before on untreated bee brood) that had finished feeding and had reached the wandering phase. These larvae were assigned one of two different treatments for two time periods: (1) an empty plastic container (11 \times 11 \times 9 cm) for 4 hours (control 'a'), (2) an empty plastic container for 24 hours (control 'b'), (3) a plastic container having about 150 corpses of beetle larvae that had died to the pathogen for 4 hours (treatment 'a'), and (4) a plastic container having about 150 corpses of beetle larvae that had died to the pathogen for 24 hours (treatment 'b').

Thirty larvae from the control 'a' container were put into each of seven soil chambers (created as before). This was repeated for larvae in the treatment 'a' container. Larvae in the control and treatment 'b' containers were distributed (30 larvae per container) over 8 soil containers for each treatment instead of 7. The soil containers were treated as before and adult beetles were collected upon eclosion.

About 100 pupae that were colonized by the fungi were collected from the soil and sent to the Biosystematics Division of the ARC-PPRI, South Africa for fungal identification. About one third of the pupae were plated directly onto potato dextrose agar supplemented with antibiotics (Pendistrep 20/20 and Novopen) and water agar. The rest of the pupae were surface sterilized and then plated onto agar as before. Surface sterilization included dipping the pupae into either a 40% formaldehyde or 3.5% sodium hypochlorite solution for 6 seconds and then rinsing the pupae with distilled water for 10 seconds. Surface sterilization removed any fungi that would have colonized the pupae after death. The cultured fungi were identified by a professional mycologist.

Data analysis

Independent sample t-tests were used to compare the mean number of eclosing beetles that had been exposed (via contact and presumably ingestion) to either the control or pathogen-infected brood during their larval stage. The number of eclosing beetles that had contacted one of two treatments [control (healthy larvae wandering in empty container) and inoculated (healthy larvae wandering amongst larvae that had died due to the target pathogen)] post-feeding and during the wandering stage was analyzed by treatment and time exposed to the treatment (4 or 24 h) using ANOVA. All analyses were conducted using Statistica (Statistica 2001) and all differences were accepted at $\alpha \leq 0.05$.

Results

Feeding exposure

There were no treatment effects ($|t| = 0.5$; $df = 25$; $P = 0.6158$) between the number of eclosing beetles that had eaten either the control (28.6 ± 0.5 ; 13) or inoculated (28.9 ± 0.3 ; 14) food. No fungi-infected cadavers were collected from any of the 13 control chambers while only 2 were collected from the 14 treatment chambers.

Post-feeding exposure

There were no time effects ($F = 1.3$; $df = 1, 26$; $P = 0.2681$) or treatment \times time interactions ($F = 2.2$; $df = 1, 26$; $P = 0.1524$) for the number of eclosing beetles for the second study. There were, however, treatment effects ($F = 50.0$; $df = 1, 26$; $P < 0.0001$). The number of eclosing beetles was significantly lower (Table 13.1) for larvae that had

contacted pathogen-killed larvae than for larvae that had not (Table 13.1). Indeed, mortality of beetle pupae was about 32% when contacting pathogen-killed larvae before burrowing into the soil as opposed to a 4% natural mortality in the controls. Soils in which the larvae were pupating were filtered and the dead pupae were collected. Treatment larvae collected from the soils had all been colonized by various fungi (Figure 13.1); no dead control pupae were colonized by the fungi.

Fungi identification

Two species of *Aspergillus* were discovered on the cadavers, *A. flavus* and *A. niger*. Both are soil fungi known to attack insects. Three additional fungi, all saprotrophic, were also found on the surface of the cadavers. They include: *Clonostachys rosea*, *Gliocladium catenulatum*, and *Mucor plumbeus*.

Discussion

Biologically, *Aspergillus* is one of the most successful genera of all fungi (Barron 1968), and either *A. flavus* or *A. niger* could have been responsible for the documented increase in larval mortality in the post-feeding larvae as both are soil fungi known to attack insects (Domsch et al. 1980). Indeed, it is very likely that one or both of these fungi were the causative agents; however, in this preliminary study, cause-of-death could not be established with absolute certainty. The larvae could have died to other infections (either viral, bacterial, etc.) and then subsequently been infected by the fungi, but this is probably not the case (I. Rong, personal communication). In contrast, *C. rosea*, *G. catenulatum*, and *M. plumbeus* are all saprotrophic and likely colonized the larvae after death (I. Rong, personal communication). That these fungi were cultured from larvae that had been surface sterilized indicates that the larvae had been dead some time before being retrieved from the soil. Regardless, due to microbial succession a primary pathogen may have been overlooked (I. Rong, personal communication).

Beetle mortality was only significantly higher when the larvae were exposed to the causative agent post-feeding. Indeed, there was no difference between mortality of beetle larvae feeding on inoculated or control food. It is possible that the method of inoculation used to inoculate the food was insufficient to transmit the spores to the food. Further, fungi from the genus *Aspergillus* and *Mucor* must enter the cuticle through a wound such as a cut or an abrasion (Ferron 1985). When feeding beetle larvae undergo various molting

stages, the fungal spores adhering to the cuticle can be shed with the cast skin (Ferron 1985). Because of this, larvae that are no longer going to undergo further molts would be the most likely candidates for infection.

Classical biological control works under the assumption that most living species are attacked by natural enemies, whether predator, parasite, or pathogen, which may regulate the species' population density (Rosen 1985). However, some of the predator/parasite/pathogen species may not be host specific and these agents should be investigated with considerable caution. Perhaps the biggest concern with using the species of *Aspergillus* shown here to cause beetle larvae death is that both are also responsible for fungal diseases in honey bees although the diseases are considered of minor importance (Bailey and Ball 1991). *Aspergillus flavus* causes 'stonebrood' in honey bees while *A. niger* has been shown to kill worker, drone, and queen pupae (cf. Schmid-Hempel 1998).

The lack of host specificity for both *A. flavus* and *A. niger* would limit in-hive applications of either fungus for the control of beetle larvae. However, it is possible that the fungi could be sprayed on the ground around colonies in order to infect burrowing larvae. In fact, this may be the preferred method as I have shown in this study that feeding larvae do not contract the disease. If sprayed around the colony, the efficacy of the fungi could possibly be improved if one uses diatomaceous earth (which abrades larval cuticles, giving the fungi entrance sites into the larvae).

Besides a lack in host-specificity, there are further risks associated with using fungi from the genus *Aspergillus* as biological control agents. Fungi from this genus (especially *A. flavus*) produce toxins that are known to be carcinogenic (Ferron 1985) and for this reason, *A. flavus* has never been used as a biological control agent (Ferron 1985). When using fungi as biological control agents, one must consider the risk of human infection and/or physiological toxicity (Ferron 1985).

Further investigations must be conducted to conclusively determine which pathogen caused the increase in mortality of beetle larvae. Regardless, this preliminary study serves to stimulate the search for biological control agents of small hive beetles in their endemic range. The results from this study suggest that the beetles, like many other soil-pupating insects, are susceptible to fungal infections and this knowledge may one day be used to control them in their introduced range. It is also possible that existing fungi already used for the biological control of insects (such as *Beauveria bassiana*: Ferron 1985; Schmid-Hempel 1998) may be employed successfully against the beetles.

Table 13.1. Effects of exposure time (h) and treatment on the number of eclosing small hive beetles.

treatment	exposure time (h)		treatment averages
	4	24	
control	28.7 ± 0.5(7)	29.1 ± 0.6(8)	28.9 ± 0.4(15)a
inoculated	22.0 ± 0.6(7)	18.9 ± 2.0(8)	20.3 ± 1.2(15)b
time averages	25.4 ± 1.0(14)a	24.0 ± 1.7(16)a	

Treatments are control (healthy beetle larvae wandering in empty container) and inoculated (healthy beetle larvae wandering amongst other larvae that had died due to the target pathogen). Data are number of eclosed beetles, mean ± standard error (*n*). Treatment averages and time averages followed by the same letter are not different at the $\alpha \leq 0.05$ level.

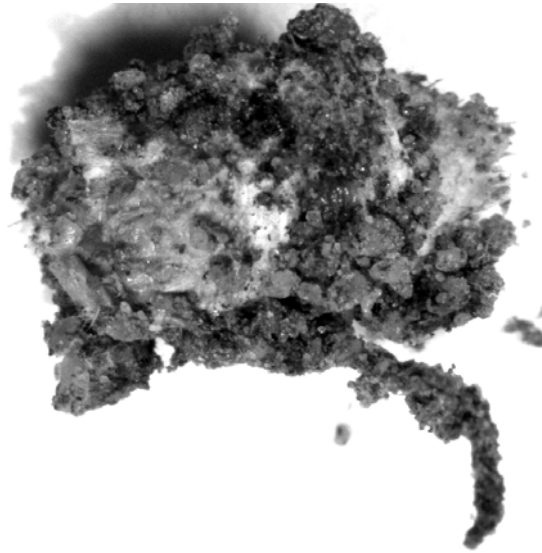


Figure 13.1. A small hive beetle pupa that was colonized by fungi. The pupa was collected from the soil after it failed to eclose.

Chapter 14: Discussing the Research Implications

Framework

Understanding the intricacies of the biology, behavior, and control of small hive beetles is a delicate art. Weaknesses in our knowledge are no longer due to a lack of information on the pest but instead reside in the fact that the total body of literature has not been synthesized in a fashion suitable to make predictions. Indeed, the goal of scientific accomplishment is one of prediction making; studying a phenomenon exhaustively so that one can generate plausible theories on the nature of that phenomenon. It is, therefore, obligatory that I spend the remainder of this dissertation weaving together the great body of literature that exists on the small hive beetle with pertinent information on social insect symbionts contributed by scientists in other disciplines. This holistic approach will allow many beetle-associated predictions to be made but it will also stimulate the generation of testable theories, which should be the goal of every scientific endeavor.

In order to accomplish this goal of predictive theory generation, I must first revisit the biology and behavior of small hive beetles in order to place their existence in and dependence on honey bee colonies in perspective. Because of this, I will begin this chapter with a discussion on beetle behavior and biology in order to characterize its ecological niche. In doing so, I will be able to comment on probable biological constraints of the beetle and its spread and impact outside of its native range. Most of this synthesis is made possible by the original data provided in this thesis.

In addition to the obvious biological interests surrounding it, the beetle remains an applied problem to beekeepers. I will, therefore, dedicate sections of this chapter to discussing control options for the pest, especially in the context of an integrated approach. As no one control proposal has yet proven a ‘silver bullet’, I will discuss all research control methods previously considered by myself and others and suggest ways these can be

integrated to achieve maximum efficacy. Further, new data on beetle biology and behavior have contributed novel ideas concerning both testable and plausible alternative controls not yet considered. These I will also discuss.

What I hope to accomplish with this discussion is an amalgamation of information that will contribute to a more thorough and appropriate understanding of small hive beetles. I begin this endeavor by considering the beetle's ecological niche where I explore its similarities to other symbionts of social insects, and its own possible 'symbiotic' relationship with its honey bee hosts.

Ecological Niche

Small hive beetles are not unique among insects in their relationship to their hosts; there are multitudes of other insects that inhabit colonies of various social insects. In order to understand the ecological niche filled by the beetle, it is vital to understand what other researchers have discovered concerning insect symbiosis in general. I begin by summarizing the nomenclature associated with symbionts of social insects and then relate this nomenclature specifically to small hive beetles (which will ultimately highlight weaknesses in today's categorical classifications of symbionts). I will also draw parallels between the beetle/honey bee relationship and that of other social insect symbionts. This synthesis will clarify many ambiguities associated with small hive beetles and will ultimately place the beetle in its appropriate niche.

Small hive beetles as symbionts

Symbiosis is a prolonged and intimate relationship between organisms belonging to different species with the association being obligatory or of some permanence (Wilson 1971, 1975; Kistner 1979). Individual species (like small hive beetles) may be considered symbionts if any combination of the following criteria are met: 1) they have been repeatedly captured with a definite host, 2) the association can be imputed by the kinds of morphological adaptations the species possesses, 3) the habits in relation to the host are known, or 4) by morphological similarity to species whose habits and associations with a host are known (Kistner 1979). Categories 1, 2, and 3 accommodate small hive beetles.

Depending on the host in question, invading arthropods can be considered 'sphecophiles' (social wasp symbionts), 'myrmecophiles' (ant symbionts), 'termitophiles' (termite symbionts), or 'melittophiles' (social bee symbionts)(Wheeler 1928; Wilson 1971,

Kistner 1982). Small hive beetles are, therefore, melittophiles although this term is not used as widely as are ‘myrmecophiles’ and ‘termitophiles’ (Kistner 1982). This probably results from an overall lack of true symbionts in social bee colonies (which I discuss later).

Wasmann, in a series of experiments (cf. Wasmann 1889 - 1925), was first to suggest a simple classification that divides symbionts into 5 behavioral categories. Based on these divisions, small hive beetles can be considered symphiles as they are accepted to some extent by their hosts as though they were members of the colony (Wilson 1971; Kistner 1979). Although small hive beetles are symphiles (being placed in this category by their ability to solicit food from guard bees), they also prey on bees’ offspring (and are therefore synecthrans – persecuted guests that possess a mechanism for eluding the host while eating some of the host’s colony, ie. brood, refuse, etc.) and are not fully integrated into the honey bee colony (as can be seen by the obvious aggression that bees direct at the beetles). Because of this, the beetles are able to fit into more than one of Wasmann’s categories, casting doubt on the universality of his categorical divisions, an assertion also made by Wilson (1971) and Kistner (1979).

Wilson (1971) and Kistner (1979) have suggested alternative levels of symbiosis including: commensalism (when the relationship benefits one species while neither benefiting nor harming the other), mutualism or “true” symbiosis (both partners benefit), or parasitism (where one species benefits at the expense of the other)(Wilson 1975). Small hive beetles are certainly not mutualists, but they may, at times, be either commensalists or parasites in honey bee colonies depending on their behavior.

In their native range of sub-Saharan Africa, the beetles are usually commensalists, neither harming nor benefiting their hosts (Lundie 1940; Schmolke 1974). During this phase of their life, they simply inhabit bee colonies and scavenge on whatever food sources are available to them; reproduction in their native range is often restricted to weakened/diseased colonies or empty nests made available by absconding colonies (Hepburn and Radloff 1998). Because they can live as scavengers on refuse, the beetles are behaviorally similar to other nonsocial arthropods that are modified for a commensalistic existence within the nests of social insects (Wilson 1975).

In sharp contrast to their commensalistic lifestyle in their endemic range, the beetles can cause general depredation to host colonies in their introduced range (Chapter 2) and it is at these times they may be considered parasites (I discuss this concept further below). Social parasitism is further divided categorically and as a result, the beetles may best be considered inquilines (species that spend their entire life cycles as a parasite within

a social insect society)(Wilson 1975; Kistner 1979), although they fail one major qualification of this definition: they do not complete their entire life cycle in the host colony (they pupate in soil).

Kistner (1979) was not entirely satisfied with the system laid out by Wasmann (1894 - 1925) and Wheeler (1910); instead he further considers symbionts as either 1) integrated or 2) non-integrated into the host colony. Integrated species are incorporated into their hosts' social life while nonintegrated species are not; instead, they are adapted to the nest as an ecological niche (Kistner 1979). Kistner would consider small hive beetles to be nonintegrated food thieves that have learned only one aspect of the bees' communication (trophallaxis); he openly considers *Amphotis marginata* (Coleoptera: Nitidulidae) the same, which behaviorally is similar to small hive beetles (as will be discussed later).

Although the aforesaid nomenclature is background to the ensuing discussion, it makes obvious the need for revising the nomenclature into more definitive categories, a conclusion realized by both Wilson (1971) and Kistner (1982). The nomenclature remains ambiguous and unspecific, qualifying many species for acceptance into more than one category (as it does with small hive beetles). To take this ambiguity further, there has been discrepancy in the literature about which ecological niche small hive beetles fill in a honey bee colony; and this ambiguity has been perpetuated by inconsistent nomenclature. It is, therefore, prudent to further discuss the status of the beetles as either a colony scavenger, predator, or a social parasite.

The discrepancy over which ecological role the beetles fill has successfully kept it from being studied in its native range of sub-Saharan Africa prior to the 1930's. Before then, it was generally held that the beetles were only scavengers in honey bee colonies because they eat pollen, honey, and other intra-colonial debris (Lundie 1940). Scavengers live on whatever food sources are available and these food sources are often no longer alive or are generally unusable to the producer, a pattern which small hive beetles clearly follow. Scavenger lifestyles are common among nitidulids where members may feed on fungi, carrion, rotten fruits, flowers, etc. (Blackmer and Phelen 1995; Habeck 2002; Chapter 1).

However, extensive studies have shown that adult and larval beetles also preferentially consume bee brood of all stages (Lundie 1940; Schmolke 1974; Elzen et al. 1999; Hood 2000; Chapter 4); therefore, it is equally true to suggest that the beetles are predacious. Kistner (1982) states that it is common for social insect symbionts to partially

retain their predatory habits, even when fed by their host or adopting the lifestyle of a scavenger. Indeed, predators from cryptic niches may be the ideal invaders of the nests of social insects while scavengers compose the second most successful group (Kistner 1982). It is, therefore, clear to see why small hive beetles have adopted both niches.

Despite the obvious rewards of predatory behavior, small hive beetles are not obligatory predators because they can complete their entire life cycle on foodstuffs other than brood (Chapter 4). Indeed, they are not even obligatory scavengers in bee hives (in theory) since it has been adequately demonstrated that they can reproduce on fruit (Eischen et al. 1999; Chapter 4). However, that they can complete entire life cycles on fruit *in vitro* does not indicate that they will do so *in vivo*. In Chapter 4, beetles being fed kei apples (*Dovyalis caffra*) were given a choice to ‘eat or die’; many insects will feed on things *in vitro* that they would never be found on in the wild (M. Hill personal communication). Regardless, because the beetles can reproduce on fruits, their close ties to other members of the family Nitidulidae are confirmed.

To complicate things further, the beetles have even been called colony parasites in the scientific literature (Neumann et al. 2001a). Schmid-Hempel (1998) states that parasites are organisms living in or on another living organism, obtaining from it part or all of its organic nutrients, commonly exhibiting some degree of adaptive structural modifications, and causing some real damage to its host. Small hive beetles live neither in nor on bees so superficially they cannot be considered outright parasitic.

However, a peculiarity exists among groups of social insects in that they cannot be considered congregations of hundreds, thousands or even millions of individual organisms. Instead, social insect colonies are often considered ‘superorganisms’ (a term first used by Wheeler 1928, but later employed by a host of authors: cf. Wilson 1971; cf. Hermann 1979; cf. Hölldobler and Wilson 1990; cf. Moritz and Southwick 1992); indeed, Moritz and Southwick (1992) convincingly argue that honey bee colonies are superorganisms. This is significant because if one considers honey bee colonies superorganisms, then one can easily consider small hive beetles parasites because they live in a colony and obtain from it all (presumably) of their organic nutrient. This clearly is encompassed by Schmid-Hempel’s definition of a parasite although admittedly on a higher, non-intrinsic level. Although I refer to the beetles as scavengers or predators in most of this discussion, it is helpful to consider them parasites in some instances as this will lend significant contributions to our understanding of their influence over and affects on honey bee colonies.

Despite the discrepancies in symbiont nomenclature, the beetles live in close association with their honey bee hosts and it is this association that I will explore in detail in the following sections. Before this relationship can begin however, the beetles must gain entrance into the well-defended colonies of their host. Other symbionts accomplish this feat in a number of different ways and reviewing the ‘tricks’ that facilitate host-invasion may suggest similar means by which beetles access honey bee colonies.

Gaining entrance into honey bee colonies

Integrating into the life of a social insect colony affords obvious benefits (food, shelter, protection, etc.) and there are many ways that this feat may be accomplished. Because social insects regurgitate, allogroom, recruit, and perform other services in a manner unrelated to either dominance or personal recognition and kinship within the colony, there are multiple lines of entry into both the colony and the nutrient flow contained within (Wilson 1971).

In general, the penetration of insect societies by inquilines has been made possible by physiological and behavioral convergence toward their hosts; Wilson (1975) calls this ‘breaking the code’. Symbionts may enter nest any 1 of 3 ways: 1) chemical use, 2) body form (which may include Wasmannian mimicry), and 3) use of signals to ensure being fed by their hosts (Kistner 1979). Despite the fact that honey bees have specialized guard bees that scrutinize incoming individuals (including bees, but also other invaders - Ribbands 1953) and are highly defensive, we may assume that small hive beetles employ the means mentioned to forgo their host’s defenses.

It is unclear if the beetles employ appeasement substances, such as trichomes from which the host can feed or smell, or other chemicals to successfully integrate themselves in honey bee colonies but it would not be entirely erroneous to assume that such substances may exist. Further, that bee-beetle interactions are also modulated by chemical mimicry as occurs, for example, in the death’s head hawkmoth (Mortiz et al. 1991) remains a possibility. More research is needed to determine if beetles use chemical mimicry/appeasement to gain entry into host colonies.

Small hive beetles exhibit a defensive body form that may facilitate entry into host colonies. Many myrmecophiles and termitophiles of diverse origin exhibit a limuloid shape, which is generally perceived as a defensive form (Kistner 1979). Accompanying this is the reduction of the length of the legs and antennae, the overlapping of the borders of body regions, the reduction of the head size, the development of shields (which protect

the joints of the appendages) and usually a thickening of the exoskeleton. It is very obvious to any observer that small hive beetles also share similarities with other arthropods bearing a defensive form. When attacked by worker bees, the beetles are able to retract their legs and head fully under their bodies in order to protect their extremities from being bitten or stung (Neumann and Elzen 2003). Further, their bodies are highly sclerotized and are very difficult for bees to grasp (Lundie 1940; Schmolke 1974).

Small hive beetles, like other symbionts, are able to solicit regurgitation (trophallaxis) from their hosts (Chapter 6), a behavior Kistner (1975) terms ‘rogatory’, and this is probably the most fundamental way the beetles achieve recognition in a honey bee colony. Unlike with other symbionts (Wilson 1971), it does not appear that rogatory behavior helps the beetle to achieve complete recognition and adoption as colony members. Regardless, the beetle must achieve some level of neutrality within the colony in order to be fed. Trophallaxis, the exchange of liquids among members of the same colony, plays a pivotal role in the social organization of most species of social insects (Wilson 1971). Because small hive beetles are able to tap into this reserve implies that they have gained some level of recognition from their hosts (Chapter 6). I will refer to this trophallactic relationship for large portions of this chapter so it is important that its significance be understood before I continue.

In Chapter 6, I suggested that only minimum tactile stimuli (like that provided by antennation) are required by bees to cause them to regurgitate food to the beetles. However, there may be other important stimuli that encourage the release of food and the beetles would likely have to achieve mastery of these stimuli as well if they are to be successful solicitors. For example, colony odor is important in nestmate recognition and community life in general (Kalmus and Ribbands 1952). Free (1956) demonstrated that bee heads belonging to the same nestmates were favored over those belonging to bees from other colonies. This led him to hypothesize that odor is significantly important in trophallaxis (Kistner 1979 agrees) and he was able to demonstrate this by obtaining occasional responses with balls of cotton that had been rubbed against bees’ heads. This may suggest that beetles need to acquire the odor of a colony or individual bees before they can be successful solicitors and there is at least one means by which they could accomplish this.

It is possible that the beetles acquire a colony’s ‘scent’ when they are free-roaming in a colony and are being attacked by the bees. This would, at least in some fashion, spread the colony’s scent on the beetle. At least one beetle (*Myrmecaphodius excavaticollis*,

Scarabaeidae) exhibits a similar passive defensive behavior that allows them to acquire the cuticular hydrocarbons specific to their host species of ants. The adsorbed substances enable this beetle to become integrated into the host colony (cf. Hölldobler and Wilson 1990). If small hive beetles need to acquire a colony's odor before they can solicit for food (or even gain entrance into a colony), then it remains immediately obvious that those beetles entering a colony for the first time may not be able to solicit food as successfully as beetles that had been in the colony for some time. Further, beetles migrating to other colonies may need to acquire the new host's scent in a similar fashion. The importance of acquiring colony odor in this process could easily be determined by gauging the success with which newly-eclosed beetles solicit for food vs. the same success garnered by beetles inhabiting a colony for an established period of time.

There are further complications that must be overcome with food-solicitation if beetles are able to successfully enter a colony. Hölldobler (1970) showed that *Atemeles pubicollis* (Coleoptera: Staphylinidae) is not always successful in food solicitation from the ants; they received food only once in 5 solicitations compared with 1 in 2 for the ants. Further, the ants received about 2.5 times more food per solicitation than did *Atemeles*; however, because the beetle is more persistent than ants, they end up with more food than even the ant larvae. My general observations of the relationship between small hive beetles and honey bees suggest some similarities; beetles are not as successful at soliciting food from their hosts as bees are from themselves and they are not fed every time they solicit for food. Therefore, although they are often able to secure food, the mechanism by which they do so clearly is not failsafe.

Rotagory behavior should be regarded as a type of mimicry. Wasmann (1889; cf. 1925) proposed that the elaborate mimicry of ant forms (morphological forms) exhibited by many myrmecophiles is an important mechanism of social integration. Because Wasmann developed the concept, Rettenmeyer (1970) coined the term 'Wasmannian' mimicry to describe any physical mimicry whereby the symbiont looks or feels like their host. Others (Kistner and Jacobson 1975) extended the meaning of Wasmannian mimicry to describe behaviors, including all mimicry of social releasers (like trophallaxis and pheromones), which are used to dupe their hosts, an interpretation supported by Hölldobler and Wilson (1990). Therefore, the solicitation for food from the bees by small hive beetles is a form of Wasmannian mimicry.

Trophallaxis is usually exploitive (Wilson 1971) and Hölldobler (1967b) showed this to be the case with larvae of *Atemeles* and *Lomechusa* that live in nests of *Formica*.

This probably remains true for small hive beetles and honey bees as well. However, the possibility does exist that the beetles feed the bees, as similar behavior has been described for a brenthid beetle (*Amorphocephalus coronatus*) that lives with ants of the genus *Camponotus*. Le Manse and Torossian (1965) demonstrated that this beetle receives food from its host and then regurgitates it back to other host workers. This implies that some symbionts can be integrated into a colony by showing altruistic behavior (Wilson 1971). It is for this reason that the trophallactic relationship between small hive beetles and honey bees needs to be studied further.

The tapping of mouthparts to secure food is a signal used by nearly all social insects (Wilson 1971, Kistner 1979; cf. Hölldobler and Wilson 1990) and only myrmecophiles and termitophiles had been known to use this form of signaling. Therefore, that small hive beetles (a mellitophile) employ the same behavior makes this relationship unique among all mellitophiles. Regardless, small hive beetles (like other social insect symbionts) exhibit a number of behaviors that ensure successful infiltration of honey bee colonies. Once beetles gain entrance into host colonies, they try to access the rich stores of food in order to reproduce while in contrast, the bees try to limit beetle reproduction. It is this constant game of ‘tug-of-war’ that I will discuss next.

Beetle/bee symbiosis and the resulting implications

Most beetles (at the populations tested so far) inhabiting a colony do not freely roam throughout the colony (Chapters 7 and 8). Instead, most are confined to cracks and crevices throughout the hive (Schmolke 1974) and are restricted to these areas by a cohort of guard bees who use aggressive behaviors to keep the beetles confined (Chapters 7 - 9). Confinement behavior limits beetle access to the combs where they could feed and reproduce, despite which, the beetles are able to remain alive by coercing the guard bees into feeding them (Chapter 6).

In my general search of the literature, the confinement behavior of African honey bees appears to be unique among social insects. There are similar trophallactic interactions between social insects and their arthropod guests (which I have already discussed in part but will discuss further in the next section), but the confinement and guarding of nest intruders is seemingly unique to honey bees. This has great biological significance because it is a social insect behavior that has only recently been described (despite the fact that social insects, and honey bees in particular, remain some of the most studied organisms on

earth)(Hepburn and Radloff 1998; Neumann et al. 2001b; Section II) and therefore warrants further investigation.

Confinement behavior is likely an advanced adaptation toward nest intruders that neither termites, ants, nor other eusocial insects developed despite the fact that they host far more symbionts than do bees (Wilson 1971; Kistner 1982). Why this behavior is seemingly absent in other eusocial insects needs to be investigated further but it may be because it is a more-derived behavior. Equally possible is that the behavior does exist in other social insects but has not been discovered or described yet. Finally, small hive beetles (and other small nest intruders in honey bee colonies) may exert unique pressures on their hosts that few other symbionts do to theirs so the need for a confinement scheme could be paramount to honey bees.

It is worth stressing that it never appeared that the host bees were actively *imprisoning* the beetles but they were actively *guarding* the beetles (Chapters 7 and 8). In numerous demonstrations, beetles have been shown to naturally run from bee aggression and hide in cracks and crevices throughout the colony (Lundie 1940; Schmolke 1974). At this point, bees station guards around the beetle clusters and actively guard these confinement sites. I have never observed bees building a ‘prison’ before a beetle infestation, only to herd the beetles into that prison once they infiltrated the colony. Instead beetles enter a hive, run from bee aggression into cracks and crevices, and are actively guarded at these confinement sites (Chapter 8).

The above statements beg the distinction between ‘active’ and ‘passive’ guarding of small hive beetles. If bees were not purposefully (actively) guarding the beetles, then they would simply be attracted to the beetles as a nest intruder, but only after contacting them while meandering about the hive. In this instance, bees ‘guarding’ the confinement sites would be expected to continually bite at the confined beetles until they become uninterested in the beetles because of their inability to reach them (= ‘passive’ guarding: bees guard the beetles only when they come into contact with them). In contrast, bees ‘actively’ guarding should belong to a certain cohort that serves as prison guards (Chapter 9) as bees are known to exhibit polyethism. This behavior could include aggressive behavior (such as biting at the beetles) but would also include a state of inactivity where the bees’ defenses and aggression are turned off unless triggered by beetles trying to escape confinement (Chapters 7 and 8).

There is undoubtedly a specific cohort of bees that guard beetles (Chapter 9). I clearly demonstrated that bees around 18-20 days of age are the ones guarding beetle

confinement sites in both South Africa and the United States. Marked bees never began guarding beetles earlier (<10 days of age) or later (>25 days of age) than their peers and this suggests some sort of rigidity in the onset and termination of prison guarding. Further, bees at the beetle prisons do not constantly bite at the beetles but are only ‘activated’ (show aggression toward the beetle) when a beetle comes to the prison perimeter. These bees remain motionless at the prison entrance, with their prothoracic legs in the air (like that done at the colony entrance) for the majority of their guarding tenure unless approached by a beetle that wants a free meal or to escape.

The confinement of small hive beetles is not unique to the beetle’s natural hosts as honey bees of European-derived origin in the United States also confine beetle intruders (Chapters 7 and 8). If beetle confinement were unique to honey bee subspecies in Africa, then one would expect the behavior to be essential to the relative immunity of African bees to depredation caused by the beetles. However, because the behavior is not unique to African bees, one must ask what purpose beetle confinement serves, especially if it does not appear to control beetle-associated depredation in European colonies.

The data in Chapters 7 and 8 suggest that confinement behaviors of both European and Cape honey bees are remarkably similar and this is quite surprising since European bees in the US have only hosted beetles since the mid-1990’s. That the behavior is present in European bees strongly suggests that it is only 1) an initial defense of all *A. mellifera* subspecies against invading beetles, or 2) a more general defense by honey bees against small colony intruders. If the behavior were an initial defense limited only to small hive beetles (1), then one would not expect the behavior to be present in honey bees that are not the beetle’s natural hosts, unless of course, the behavior is quickly selected for over few generations. This, however, is likely not the case. Therefore, the only other conclusion is that the behavior is a more general defense by honey bees against small colony intruders (2) which includes, but may not be limited to, small hive beetles.

Viewing the bee colony as a superorganism and the beetles as parasites is especially helpful when trying to understand what determines the nature of the confinement phenomenon. If you consider the colony a superorganism, then you can assert that the confinement behavior is an individual colony’s (or organism’s) response to an invading parasite, much like an immune response. In this scenario, the beetle would be the invading pathogen and the guard bees would play the part of individual immune cells, genetically programmed to respond to the pathogen. To determine if this is a helpful

model, I will draw parallels between beetle confinement and an insect's response to an invading pathogen.

When small, foreign particles enter an insect, they are usually phagocytosed by granular hemocytes (Schmid-Hempel 1998), a process that is ineffective if a large number of foreign objects have invaded the host. In the latter instance, the hemocytes release a coagulum that becomes melanized and traps the foreign objects. However, there are cases where objects are too large to be engulfed and trapped (like the eggs and larvae of parasitoids), and such objects are encapsulated (Drif and Brehélin 1993; M. Hill personal communication). Encapsulation may occur within minutes to hours. First, the hemocytes aggregate around the foreign object, following which they form a tight capsule (which becomes melanized) around the object thus killing it (Schmid-Hempel 1998).

The confinement of beetles can happen much the same way. Guard bees (by being part of the immune response of the superorganism are the 'hemocytes' in this analogy) surround beetles that have retreated to cracks around the colony. The bees may then remain at the confinement sites or begin 'encapsulating' beetles by fortifying the sites with propolis (which may be likened to the coagulum released by the hemocytes). It is important to note that just because propolis is present at the confinement sites does not imply the bees put it there with the intent of confining beetles (see Chapter 8). Propolis is commonly used in most *A. mellifera* colonies as a water repellent, sealer, caulk, etc. (cf. Hepburn and Radloff 1998). Regardless, the confinement of beetles keeps them out of the combs where they feed and reproduce; much the same way hemocytes limit the feeding of parasitoid larvae within an individual insect.

If the confinement of beetles is a superorganismic immune response, then it may be possible that additional infestations of beetles will elicit a more rapid response by the bees. Recent work has found evidence for an 'immunological memory' in individual insects (Faye and Hultmark 1993), which may be applicable to our analogy here. In this instance, colonies having already hosted beetle invasions may be more immune to subsequent ones. This is only a theory however, but its validation may clarify some of the ambiguity surrounding the confinement behavior of honey bees.

There remain uncertainties when considering the confinement of beetles as a superorganismic immune response. The main difficulties lie in the facts that 1) the pathogen (the beetle) is able to gain nutrition (sequester food) from the immunoresponsive cells (the guard bees) and 2) the role of propolis in the behavior is not fully understood. However, if the confinement of beetles is considered a general

superorganismic immune response to small nest intruders, then it would be very easy to understand why the behavior is present in beetle-naïve European bees.

Similarities with other arthropod/social insect relationships

Many scientists have dealt with the close association between arthropods and social insects (Wasmann 1889 – 1925; Wheeler 1910; Wilson 1971, 1975; Kistner 1979, 1982; Hölldobler and Wilson 1990) so there is little need for me to discuss all of these associations. Instead, I will focus more on those relationships that are similar, in nature, to that shared by small hive beetles and their hosts. This section appropriately follows the previous discussion on the relationship between beetles and honey bees because it places their relationship in context with those shared by other arthropod symbionts of social insects.

As I have already discussed, the trophallactic relationship (rotagory behavior, Kistner 1975) that small hive beetles enjoy with their hosts is not unique. Some mites live on food regurgitated by their ant hosts either by positioning themselves between two ants that are feeding or by soliciting food directly by stroking the mouthparts of workers with long, antenna-like forelegs (cf. Hölldobler and Wilson 1990). A phorid fly, *Metopina formicomendicula*, lives in the nests of the thief ant *Solenopsis* (= *Diplorhoptum*) *fugax* and strokes the head and mouthparts of the ant with its forelegs to solicit regurgitation (K. Hölldobler 1928). Some staphylinid beetles, (including *Atemeles*, *Lomechusa*, and *Xenodusa*) can also trick ants into feeding them (cf. Wilson 1971). Hölldobler (1973) demonstrated that *Dinarda dentata* (Staphylinidae) begged for food from adult *Formica sanguinea* and many other examples abound in the literature (cf. Kistner 1981; cf. Hölldobler and Wilson 1990). Termites also feed their guests (Kistner 1979). Emerson (1935) reported that *Thyreoxenus parviceps* is fed by workers of its host *Nasutitermes costalis*. There are, indeed, many examples of insects that solicit food from their social insect hosts.

Members of the beetle family Nitidulidae are known to associate with social insects, the most studied of which are ants (Navarrete-Heredia 2001). One species of Nitidulidae (*Amphotis ulkei*) has been reported with *Formica schaufussi*, *F. integra*, and *Crematogaster lineolata* from the United States (Schwarz 1890). The genus *Epuraea* contains scavengers in bumble bee and social wasp colonies (Scott 1920); indeed, nitidulids frequent the nests of bumble bees (Kistner 1982). Cumber (1949) reports *E. depressa* from the nests of 5 different English species of *Bombus* (raising a question about

host specificity). Scott (1920) found their larvae, which are scavengers, in a nest of *B. derhemellus* and managed to rear them to adults. Further, *E. unicolor* has been recorded from the nests of *Paravespula vulgaris* and *Polistes germanica* (Spradbery 1973). Lea (1910, 1912) records *Brachypeplus auritus* from the nest of *Trigona carbonaria* from Australia and states that other species of the genus have been taken from the nest of a domestic bee as well as another unidentified wild bee.

Members from the nitidulid genera *Amphotis* and *Claviger* can even solicit food from their ant hosts (Donisthorpe 1927; Park 1964); therefore, rogatory behavior is not a nitidulid behavior unique to small hive beetles. Perhaps one of the more interesting relationships between nitidulids and ants derives from the nitidulid genus *Amphotis* and this relationship is remarkably similar to that shared by small hive beetles and honey bees. For this reason, it is important that I discuss the relationship between *A. marginata* (the ‘highwayman’ of the local ant world) and its host *Lasius fuliginosus* because it may help us better understand the relationship shared by small hive beetles and their hosts.

Amphotis marginata does not live within the nests of the ants (Kistner 1979); instead, it frequents the feeding trails of its host (Hölldobler 1968). It, like small hive beetles, is able to solicit food from its host, and this is done in a manner remarkably similar to that done by small hive beetles. I quote below an excerpt taken from Hölldobler and Wilson (1990) concerning this relationship:

(*A. marginata*) occupy shelters along the foraging trails of the formicine ant *Lasius fuliginosus* during the day. At night they patrol the trails and successfully stop and obtain food from ants returning to the nest. Ants that are heavily laden with food are easily deceived by the beetles’ simple solicitation behavior. The *Amphotis* adult induces an ant to regurgitate food droplets by using its short antennae to tap the ant’s labia and rapidly drum on her head. Soon after the beetle begins to feed, however, the ant seems to realize she has been tricked and attacks the beetle. The beetle then is able to defend itself simply by retracting its appendages and flattening itself on the ground.

Small hive beetles, like *A. marginata*, use their antennae to tap the bees’ labium and this behavior dupes the bee into feeding the soliciting beetle. However, the bee also seems to recognize that it has been tricked shortly after the beetle begins feeding and, like *L. fuliginosus*, it begins to attack the beetle. It is, therefore, easy to see that rogatory

behavior has manifested itself in the nitidulids more than once and may yet be present in other unstudied species.

Despite the fact that many symbionts live with other social insects, the relationship between hive beetles and honey bees is seemingly unique among all social bees and wasps. Those studying the symbionts of social insects suggest reasons ants and termites host many symbionts while bees do not and the obstacles that must be overcome to be successfully integrated into social bee colonies. These concepts are discussed below

Biological significance

Thousands of species, representing at least 17 orders, 120 families, and hundreds of genera are involved in symbiotic relationships and by far, the greatest diversity is found in termite and ant colonies (Wilson 1971). Kistner (1979) calculates the ratio of myrmecophiles/ants within a colony to be 1:1117 but this can vary greatly depending on the size of the nest. He also estimates the ratio of termitophiles to termites within a colony to be between 1:5000 and 1:25000; there are more termitophiles than there are species of termites (Kistner 1979).

Despite the relative abundance of termitophiles and myrmecophiles, small hive beetles remain the only true symphiles in social bee colonies and this is biologically significant. The only known exception is *Echthrodope africana*, a perilampid wasp whose larvae are ectoparasites on the pupae of allodapine bees of the genus *Braunsapis* in Africa (Wilson 1971). Kistner (1982) suggests that *E. Africana* is a Wasmannian mimic of the larvae of its host. No other Wasmannian mimics of wasps or bees are known although Batesian mimics are frequent (Kistner 1982).

There are certainly fewer guests of social wasps and bees (Wilson 1971). Not only is this the case, but the guests that do exist generally have far less pronounced adaptations for symbiotic life (the exceptions are mites, beetles, and flies that live as scavengers and brood commensals - Wilson 1971). Those studying symbionts of social insects have set forth a number of hypotheses for the apparent lack of symbionts in social bee colonies. It is important to briefly review these ideas because, despite all of the obstacles, small hive beetles have 1) filled an ecological niche in honey bee colonies that has been vacated and/or neglected by other arthropods and 2) adapted in ways that other honey bee colony invaders have not. I begin by looking at factors that possibly influence the number of symbionts a colony hosts and I discuss how small hive beetles may have overcome these obstacles.

It is generally accepted that a colony's population size profoundly influences the number of symbionts taking refuge within its boundaries and termites and ants can have much larger colonies than honey bees (Wilson 1971; Kistner 1982). Large colonies exhibit: long colony life, high microhabitat diversity, and low symbiont extinction rates that reinforce one another to produce a higher diversity and abundance of symbiotic species (cf. Hölldobler and Wilson 1990). However, honey bee colonies are not altogether small (despite what Kistner 1982 argues), have high microhabitat diversity (brood chambers, food storage areas, etc.), and are well-protected so these three factors, in my opinion, provide little if any barrier to invading small hive beetles.

Kistner (1982) believes that the number of social insect nests per unit area also plays an important role in symbiont diversity. He maintains that a solitary termite (or ant) nest produces few termitophiles while in a field where there are many termite nests, the nests usually host more symbionts. Because bee and wasp colonies never aggregate close together (except *Apis dorsata*), they do not attract as many symbionts (Kistner 1982). However, small hive beetles are remarkably mobile (Lundie 1940; Schmolke 1974) and anecdotal evidence suggests that they may migrate great distances in host-seeking endeavors. Therefore, it should not matter if colony density per unit area is small because the beetles are mobile enough to overcome this difficulty.

Another barrier for small hive beetles is that honey bees, by nature, nest in arboreal locations and their nests are tightly sealed; thus few arthropod species are pre-adapted for the penetration of such nests (Wilson 1971). Honey bee nests are often reinforced by propolis and may have narrow, tightly guarded nest entrances. In order to penetrate such nests, an arthropod would have to prefer arboreal life, dark/tight spaces, and tolerate higher temperatures and lower humidities (Wilson 1971). By nature, these qualifications are only good for a limited number of arthropod groups that live in tree holes, standing dead branches, and deeper layers of bark (Wilson 1971). Any review of the family Nitidulidae will clearly show that members of this family are remarkably pre-adapted for such a life (Kirejtshuk and Lawrence 1999; Habeck 2002) and small hive beetles are no exception.

Not only can honey bee nests be cryptic and well-defended, but the detritus on which scavengers feed is scarce inside the nests because workers bees continually clean the nest (contrary to what termite and ants do)(Wilson 1971; Kistner 1982). Further, bees produce smaller amounts of refuse because pollen and nectar are highly concentrated food sources. Small hive beetles have overcome this problem by 1) being facultative predators of bee brood (eliminating the need for eating only pollen) and 2) ovipositing directly onto

concentrated food sources (Lundie 1940; Schmolke 1974; Chapters 3 and 10). The lack of detritus on which to feed may also explain why beetles are limited to reproducing in weak or absconded colonies in their native range and why they have developed rotatory behavior. The relatives of the small hive beetles are sap feeders (Habeck 2002) so it is easily seen why they were able to shift to a diet of honey/nectar supplied by their host.

Kistner (1982) also believes the effectiveness of defense probably plays a role in the number of symbionts a colony will host. That wasps and bees sting is well known; but most ants that host many symbionts have stings that are not as effective. While termites cannot sting, the biting power of soldiers is well known. However, as I have discussed in the section 'Gaining entrance into honey bee colonies', small hive beetles exhibit a defensive form (a hard exoskeleton and the ability to retract their heads and legs under their bodies) that allows them to be harassed (bitten, stung) by bees without being damaged (Neumann and Elzen 2003).

Small hive beetles possess a number of predisposed conditions that make them obvious candidates for life in honey bee colonies. They have overcome greater difficulties than symbionts adapted for living with ants and termites but the payoffs afforded beetles living in honey bee colonies are greater (concentrated food sources such as brood, pollen, and honey stores).

Counting the Costs

Although small hive beetles fill an ecological niche that is often overlooked by those working with the pest, the beetles remain an applied problem to the beekeeper. Beetle-associated problems manifest themselves in a number of ways. The negative effects may be observable to beekeepers/scientists in that one may clearly see how the beetle is compromising colony health. However, these effects may also be 'unobservable' in that they cannot be readily observed but can only be known by experimentation. The latter group of effects is the one least considered in the literature but it may be the most costly of all detrimental effects associated with hosting beetle populations.

In this section, I discuss the common symptoms of hosting small hive beetles; these symptoms are readily 'observable' to anyone working with the beetle. However, I also discuss those effects never previously considered to show how beetles may damage colonies in other ways ('unobservable' effects). I conclude with a more ecological discussion of the selection pressures exerted on bees by the beetles.

‘Observable’ beetle effects on colonies of honey bees

I have already discussed, in some detail (Chapter 1: Impact in Introduced Regions), what the known effects of small hive beetle infestations in honey bee colonies are. These effects are those most commonly reported in trade journals around the world and they are the most visible side effects of hosting beetles. It is, however, important that I discuss these, albeit briefly, so that I might use this information for supporting my hypotheses in later sections.

Anecdotal evidence suggests that in their introduced range, small hive beetles possess the ability to destroy entire apiaries. At times, strong colonies may be just as susceptible as weak ones but reports suggest that it is more common to see ‘weak’ colonies succumb to infestations than strong ones. Intra-colonial damage can partially be attributed to the feeding habits of adult and larval beetles because as a result stored honey is fouled (Elzen et al. 1999; Hood 2000). This presumably happens because beetles defecate in the honey, which many hypothesize promotes the fermentation of honey (Elzen et al. 1999; Hood 2000). Fermenting honey can be a serious problem for the beekeeper because larvae may be present in honey supers that have already been removed and are ready for harvesting. In these instances, larvae may cause a loss in production.

Besides fermenting honey and the associated problems, beetle larvae have voracious appetites and are predators of bee brood (Chapter 4). Feeding on bee brood may, therefore, result in less brood being produced and consequently fewer adult bees. If infestations are high enough, bee populations may be compromised and entire colony death may follow. Alternatively, high populations of beetles may induce European (or African) colonies to abscond (Chapter 2), although the number of beetles per frame of bees probably has to be high to cause this.

Queen and package bee producers also feel the effects of beetles. If beetles are causing a reduction in colony productivity, then producers may produce fewer bees and queens. Because producers ship live bees and queens through the mail, hive beetles can be shipped with the packages as there is currently no way to exclude beetles from the packages. Further, beekeepers offering pollination services must have strong colonies in order to properly pollinate a target crop because smaller colonies are not as efficient at pollinating crops as are large colonies (Delaplane and Mayer 2000). Therefore, if beetles are causing a reduction in colony populations, a reduction in pollination efficiency will

result. Therefore, both the beekeeper (the one supplying the pollination services) and the farmer (the one paying for the pollination services) are losing profit due to the beetles.

‘Unobservable’ beetle effects on colonies of honey bees

Previously unconsidered effects

In Chapter 2 of this dissertation, I demonstrated a negative correlation between the number of small hive beetles in a colony and the flight activity (number of incoming bees per minute) of that colony. The data indicate that hosting beetle populations may reduce foraging activity and therefore colony production. These effects are not likely to be noticed by the beekeeper. A survey of beekeepers/scientists in beetle-infested areas concluded that adult beetles never damage host colonies (Wenning 2001) and this is contrary to what I show in Chapter 2. Therefore, the presence of beetles may reduce foraging activity, an effect that can only be demonstrated through experimentation.

Beetles do not always remain in their original host colony. In Chapter 2 I demonstrated that many of the beetles I had introduced into the colonies were not in those colonies at the end of the study. The missing beetles were probably not dying as beetles can live 6 months or longer (Chapter 4) and the duration of the experiment was only 15 days. So, it is likely that the beetles were 1) either attracted to other colonies or 2) leaving the area to locate additional hosts. By migrating from colony to colony, beetles may mechanically transmit other bee pathogens between colonies. Beetles, as a result of being in a colony for some length of time, may acquire fungal spores, bacterial, or viral particles on their bodies (especially since they often access brood areas (Chapters 7 and 8) where bee diseases abound). If beetles carrying pathogens migrate from an infected colony to a non-infected one, then it remains possible that they are able to horizontally transmit the pathogens to the uninfected colony.

The final effect I wish to discuss is that of a cumulative one with other colony pathogens. If more than one parasite infects a host, resistance against each may be genetically covarying (Schmid-Hempel 1998). There is, for example, a negative correlation between the capability of encapsulating eggs of a parasitic wasp and defense against a fungal disease among aphid clones (cf. Schmid-Hempel 1998). Further, immunity against trypanosomes in cockroaches increases susceptibility to infection by mermithid nematodes (cf. Schmid-Hempel 1998).

Similarly, we may expect that colonies infected with beetles will be more susceptible to other pathogens. Consider, for example, varroa mites, which are well known

in the honey bee community (Webster and Delaplane 2001). The introduced ranges of both varroa and small hive beetles overlap in the United States. Florida, for example, has experienced the most severe beetle-associated depredation to date and is recently experiencing a resurgence of varroa-associated problems (P. Elzen personal communication). Therefore, although beetles may not be sufficient to cause apiary-wide destruction in some instances, they may be able to do so when entire apiaries are severely stressed by varroa (and vice versa). One may expect similar interactions between beetles and other bee parasites/pathogens.

Both ‘observable’ and ‘unobservable’ depredation elicited by beetle presence in colonies remains of great concern to beekeepers and scientists alike. However, considering this interaction on an ecological level, there remain additional ways that beetles may harm, compromise, or even ‘change’ their host colonies and this is worth considering further.

Costs of mounting immune responses

There is little doubt that host resistance to a parasite reduces the effect of that parasite, but the development and maintenance of the immune system itself is costly and may impose negative effects on other fitness components of the host (Schmid-Hempel 1998). The immune response may be dangerous or may involve a direct cost to the host in terms of energy and nutrients necessary to mount such a response (Schmid-Hempel 1998). Because this is a general phenomenon, we may expect that mounting a response to small hive beetles may compromise colonies in similar ways (ie. the ability to collect and/or store food, regulate their populations, and maintain intra-colonial homeostasis).

Data presented in Chapter 2 indicate a negative correlation between intra-colonial beetle populations and a loss of flight activity in colonies of European, but not Cape, bees (discussed above). This may indicate European bees are mounting an immune response against the beetles that in some way compromises their ability to forage. Although flight activity was not lower in Cape colonies hosting large populations of beetles, infested Cape colonies did experience a loss in pollen stores. Regardless, European colonies also experienced a reduction in the amount of brood, number of bees, and honey stores when infested and this probably reflects a negative cost of mounting an immune response. In contrast, this may indicate that immune responses are more efficient and less costly in natural hosts (Cape bees) than in unnatural ones (European bees), as should be expected.

Other costs may occur when mounting immune responses against invading parasites. In *Drosophila*, for example, increased encapsulation responses are associated

with a loss in other fitness components (Schmid-Hempel 1988). For bumble bees (*Bombus terrestris*), foraging activity is associated with reduced levels of encapsulation when they were implanted with a test parasite (Schmid-Hempel 1988). In both instances, encapsulation refers to immune responses toward immature parasitoids within individual insects. However, if this behavior serves as a model for the confinement of beetles by honey bees, then we may also assume that confinement efforts cost bees in other ways.

Also possible is that mounting a defense against beetles will make the bees more susceptible to other diseases. Other research on honey bees supports this. Lines of larvae resistant to American foulbrood (AFB) have reduced growth rates when compared to lines susceptible to the disease (Sutter et al. 1968 cited in Schmid-Hempel 1998). This clearly indicates that immunity to AFB costs larvae in other areas of general fitness and the same may be true with beetles as well.

There is a cohort of bees within a colony that is responsible for the majority of beetle confinement allocations (Chapter 9) and the employment of such workers is probably costly (because the workers are being diverted from what they would normally be doing). It benefits a colony to produce only a few workers specifically ‘designed’ to be resistant to parasites, as it requires more resources to do this (Schmid-Hempel 1998). If the same is true in honey bee colonies, then with a given amount of resources, only a limited number of resistant workers (but more of the susceptible ones) can be produced. In theory, more susceptible workers will be advantageous if parasitism is absent, since they retrieve more resources, but this is different if parasites are present (Schmid-Hempel 1998). Colonies living in good habitats should produce many workers but not invest much in defense against parasites. In other words, colonies may choose to produce many low-quality workers (that are more susceptible) or a few high-quality ones (that are immune) and still achieve the same immunity (Schmid-Hempel 1998). It is readily appreciated that below certain parasite levels in the habitat, defense does not pay because the expected costs exceed the expected benefits (Schmid-Hempel 1998).

Selection pressures on honey bees

It is commonly held that parasites exert selection pressures on their hosts that will enhance the reproductive potential of the parasite (Schmid-Hempel 1998). Although this is a general phenomenon, it is relatively untested in science. Because small hive beetles possess the ability to completely destroy their hosts (at least in their introduced ranges), their hosts are under huge selection pressures for resistance to the beetle. Besides acquiring

resistance to beetles, host colonies may actually acquire behaviors that facilitate the reproduction of beetles. So this results in an adaptive ‘tug-of-war’ where colonies become more resistant, and beetles become ‘better’ at what they do. I will begin exploring these possibilities by discussing similar phenomena in varroa mites.

Varroa are ectoparasites that position themselves between plates of the exoskeleton and feed on the bees’ hemolymph and they have been considered the biggest threat to species of *Apis* worldwide (Webster and Delaplane 2001). The spread of varroa in a colony is facilitated when the colony is prevented from carrying out its normal activities so that the density of individual bees within the nest is high (vertical transmission: transmission of mites from nestmate to nestmate)(cf. Schmid-Hempel 1998). This is characteristic of other honey bee parasites as well (Bailey and Ball 1991). Because the presence of mites disrupts a colony’s homeostasis, more bees remain in the nest and the spread of varroa is facilitated (Schmid-Hempel 1998). So, we have an example where a behavioral change in the host elicited by the parasite benefits the parasite.

Likewise, varroa also spread by horizontal (hive to hive) transmission through ‘drifting’ workers that ‘erroneously’ enter foreign nests. Therefore, horizontal transmission of mites is facilitated if the infested colonies are prone to drifting and this has been shown to be the case (Sakofski 1990). The usual explanation for this is that the infected bees are likely in a ‘bad’ condition and their behavior is abnormal (Schmid-Hempel 1998). But, in terms of mite transfer between colonies, drifting favors the horizontal transmission of mites to other colonies so it would actually benefit the mites if bees drift. In this light, drifting may not represent an ‘error’ committed by the worker but rather the result of a behavior being ‘rigged’ by the parasite to its own advantage (Schmid-Hempel 1998).

Robbing, absconding, and swarming behaviors have also been invoked as explanations for the rapid spread of mites, such as with the tracheal mite (*Acarapis woodi*) in Mexican honey bee populations (Eischen et al. 1990). Again, all of these factors facilitate the horizontal transmission of tracheal mites between colonies so we possibly have another situation where the parasite may be responsible for some of the often-overlooked behaviors in honey bees. It is, therefore, reasonable for us to expect that small hive beetles affect colonies in much the same way. Such differences should be easily reflected in the behavioral repertoires of the beetles’ natural (African *A. mellifera*) and new (European *A. mellifera*) hosts.

It is commonly appreciated that many subspecies of African honey bees have high absconding rates (Hepburn and Radloff 1998). This is usually attributed to nectar dearth,

colony stress, beekeeper management, etc. (Hepburn and Radloff 1998; Lipiński 2001); however, it may be possible that increased tendency to abscond is higher in colonies hosting beetles. It is well documented that beetle reproduction is often limited to absconding colonies in their native range (Lundie 1940; Schmolke 1974; Hepburn and Radloff 1998) because of the bees' ability to limit beetle reproduction otherwise (Chapter 10). If this is the case, then beetle reproduction is favored (indeed, may only be possible) if host colonies abscond and leave behind unprotected food stores. This is likely a selection pressure exerted on the bees by the beetles and could be another reason absconding is prevalent in African subspecies of honey bees.

As I noted in Chapter 3, I discovered beetles in an absconding cluster of European bees and observed beetles leaving the colony with absconding bees. Anecdotal evidence by other beekeepers suggests the same. Therefore, absconding (and swarming?) colonies may facilitate the horizontal transmission of beetles. However, before this can be asserted with confidence, it is important that the phenomenon of beetles leaving with absconding colonies and being found in post-absconding clusters be studied further.

In sharp contrast, European colonies are much less likely to abscond due to nest disturbances than their African counterparts (cf. Chapter 2). Despite this, I showed that large populations of adult beetles are sufficient to cause European colonies to abscond (Chapter 2). It is not 'natural' for European bees to have high absconding rates; therefore, that they do when large numbers of beetles are present could be direct evidence that the beetles do exert some selection pressures on their host colonies.

Another aspect in which African and European honey bees differ is in their use of propolis (cf. Chapter 2). African bees are known to use more propolis than European ones (Dietz 1992; Hepburn and Radloff 1998). Propolis is used by both bees as a caulking compound, for water-proofing, and for a number of other purposes (Schmidt and Buchmann 1992; cf. Ellis and Hepburn 2003; Chapter 2). However, that African bees tend to use greater quantities of propolis in their nests may be related, in part, to selection pressures exerted by their natural parasites. Indeed, African bees (and Cape bees in particular) almost completely seal their colony entrances, reducing them to a tiny opening, and this could be an effort to limit the amount of space through which invading arthropods can enter, thus making it easier for bees to guard the entrance (Ellis and Hepburn 2003). Further, propolis has been used in the confinement schemes of host colonies (Neumann et al. 2001b; Chapters 7 and 8) so hoarding propolis may benefit African bees in their general

defense against beetles. In sharp contrast, the use of propolis is far less pronounced in European bees which may, in part, be due to a lack of selection pressures from beetles.

As I discussed in Chapters 10 and 12, the removal of disease- and pathogen-infested brood (hygienic behavior) is common among many species of *Apis*. Hygienic behavior is genetically linked (Rothenbuhler 1964a, b; Lapidge et al. 2002) and its expression is usually higher in the natural hosts of various bee diseases and pathogens than in unnatural ones (such as the varroa mite: cf. Boecking and Spivak 1999). Because of this, it is very likely that the presence of pathogens and diseases confers some selection pressure on natural hosts. If this is true, then it is reasonable to expect that the expression of hygienic behavior of African bees toward beetle eggs oviposited in bee brood (Chapters 10 and 12) may have resulted, in part, from selection pressures exerted by beetles. Likewise, the behavior of removing beetle larvae from colonies by African bees (Neumann and Härtel 2003) may have also resulted from pressures exerted by beetles. I realize that hygienic behavior is a universal trait among *A. mellifera* (indeed, European honey bees also show hygienic removal of beetle eggs: Chapter 12) so if beetle presence has boosted hygienic expression in African bees, it may have only done so at small levels.

Rates of absconding, propolis use, and hygienic behavior are not the only quantitative differences between African and European subspecies of honey bees that may have been influenced by the presence/absence of beetles. African honey bees are known to be much more aggressive than their European counterparts (Hepburn and Radloff 1998). This leads to another hypothesis that parasites (small hive beetles) may influence defensive and nesting behavior of their hosts (Schmid-Hempel 1998). Therefore, general African bee aggression may be due, in part, to selection pressures exerted on them by invading beetles.

It is important to stress that beetles and other parasites need not be solely responsible for all of the observed behaviors of their hosts but they may well influence a great deal of their phenotypic expression. European bees are equally likely to use less propolis, abscond less, and be less aggressive than African bees because these traits are all considered negative by beekeepers, who in turn have bred against them for centuries. Regardless, there is little doubt that beetles can influence and exert selection pressures on their hosts.

Selection pressures from honey bees

It seems unbalanced to only address the selection pressures of beetles on their hosts when the reciprocal may happen as well. It cannot be doubted that honey bees confer

selection pressures on small hive beetles. The outcomes of many of these pressures have already been discussed, in part, in this chapter (see ‘Gaining entrance into honey bee colonies’); they may include, but are not limited to, the defensive body shape of the beetles, the degree to which beetles oviposit in cracks and crevices and in a disguised manner in combs, the development of rotatory behavior, high reproductive capacity, and the ability of the beetles to invade clusters during cold temperatures. This is by no means an exhaustive list but I believe they are the major implications of the data presented in this dissertation and therefore warrant further emphasis.

I do not wish to address how the defensive body shape and the development of rotatory behavior developed because I have discussed these in some detail already in this chapter (see ‘Gaining entrance to honey bee colonies’). I will, however, focus on the remaining points outlined above to demonstrate how pressures exerted by honey bees could have resulted in today’s observed beetle behaviors.

Beetles employ a number of tricks that help them oviposit on nutrient-rich food sources (Chapters 3 and 10). Studies have shown that bees will remove beetle eggs that are left unprotected in the hive (Schmolke 1974; Neumann and Härtell 2003); as a result, beetles that are better able to conceal their eggs should be the ones most likely to reproduce. Because bees are able to detect and remove beetle eggs from sealed bee brood (Chapters 10 and 12), it would benefit beetles to lay eggs in cracks and crevices around the colony. Further beetles laying large numbers of eggs also benefit since their reproduction is limited by their hosts; this may explain why the number of beetle eggs laid per cell was high (Chapters 10 and 12).

It would also benefit beetles to be able to reproduce quickly and efficiently, as they have to compete with ants and other predators/scavengers in their native range when presented with food reserves left behind in post-absconding colonies. Because bees often remove (abort) their own larvae before absconding (Chapter 2), it may be necessary to reproduce in large quantity and very quickly (an observation made in Chapter 4). If these pressures from bees are not present in the beetles’ introduced ranges, one would expect to see explosive reproduction in otherwise-healthy bee colonies (which has been the case).

As I will discuss later, it seems apparent that beetles are closely tied to their honey bee hosts. Beetles, in fact, probably spend very little time outside of colonies (except during host-seeking exercises and pupation) so they undergo the same cycles through which their hosts go. This is not a problem for most of the year but temperate zone honey bees almost completely change their behavior during winter, when they cluster to keep

warm and live solely on food stores. Because there is no colony-wide thermoregulation outside of the cluster, beetles have had to develop ways to survive winter. One such way is that beetles overwinter as adults by infiltrating honey bee clusters in order to keep warm (Pettis and Shimanuki 2000; Chapter 3), a behavior possibly induced by host pressures.

Again, it is very likely that honey bees have influenced the geno- and phenotypic expression of beetles in ways not mentioned above. Admittedly, more research is necessary in this area to understand the intricacies of these relationships and how one factor affects the others.

Why are beetles only a problem in European colonies?

In general, lines of honey bees differ in response to viral (Bailey and Ball 1991) and a variety of nonviral diseases (Schmid-Hempel 1998). It would be easy to rest on this difference and not investigate the avenue of African bee resistance and European bee susceptibility any further; however, there must exist reasons that explain why organisms that are closely related can have varying susceptibilities to parasites and pathogens.

The ability of European subspecies of honey bees to deal with beetles may depend on factors that influence the immunity/susceptibility of other insects to pathogens, namely age and health status of the colony, general host condition, environmental factors (Schmid-Hempel 1998), pathogen levels in the host colony, presence/absence of beetle natural enemies, colony geno- and phenotype, and genetic biodiversity. However, colony age and health status is seemingly as variable in African populations of honey bees as in European ones so these two likely play little role in colony defense against the beetle.

Environmental factors, such as climate, rainfall, temperature, etc. may influence the virulence of beetles in their introduced range. For example, much of Africa is arid or semi-arid (except equatorial Africa) while the introduced range of beetles is predominantly temperate. It is possible that beetle fecundity is tied to environmental conditions, which I clearly show to be the case in Chapter 5. The data presented in this chapter indicates that beetle larvae will not burrow into the ground (to pupate) if the ground is not moist. So, beetle reproduction may be limited to climates where precipitation is frequent. Anecdotal evidence from beekeepers in Florida suggests that beetle problems are usually worse after periods of high precipitation. Other examples of how beetle biology is linked to the environment will be discussed later.

It is also important to remember that parasites/pathogens can act synergistically, where the presence of one increases the susceptibility to the other (Schmid-Hempel 1998).

This could be a substantial reason that beetles do little damage in Africa while they do more in the United States and Australia. Much of Africa remains free of many of the diseases commonly affecting colonies of European honey bees in other parts of the world (like American foulbrood, for example). In sharp contrast, European bees often host many parasites and pathogens, some of which are natural in colonies of European bees but others that are not. Because of this, the immune responses in many European colonies may already be taxed by other parasites/pathogens, giving beetles the opportunity to take advantage of an already compromised ‘immune system’.

Species that are introduced into a non-native area have one major advantage that allows them to reproduce in numbers that they may not have been able to do before; they have escaped their natural enemies (Huffaker and Messenger 1997). Natural enemies can constitute a range of things from parasites and predators to pathogens and/or other disease-causing organisms (Rosen 1985). Within their native range of sub-Saharan Africa, beetle populations may have been controlled by natural enemies such as parasitic wasps or ground-dwelling fungi that kill pupating beetles (Chapter 13). When the beetles escaped their endemic range, they may have been released from these natural enemies. With their natural enemies no longer present, limiting beetle populations is left to the natural defenses of host colonies, which may not be enough. Although such natural enemies have not been identified yet (except for the generalist fungi discussed in Chapter 13), a closer look in the beetle’s native range may prove beneficial.

Colony geno- and phenotype may also partially explain the differing susceptibilities in host colonies of African and European honey bees. Geno- and phenotypic differences would best exhibit themselves in behavioral responses of each bee toward the beetle. African bees, for example, are notably more aggressive toward free-roaming beetles than their European counterparts (Elzen et al. 2001) possibly indicating that they handle beetles more aggressively than do European bees. In contrast, confinement behaviors of both bees toward beetles are very similar, quantitatively, at least at the beetle levels studied in Chapters 7 and 8. However, it may be possible that once beetle populations reach higher levels, confinement behaviors of both bees may begin to substantially diverge.

Other behaviors may be under the control of colony geno- and phenotypes. These behaviors include, but are not limited to, hygienic behavior toward beetle eggs (Chapters 10 and 12), removal of beetle larvae from the colony (Neumann and Härtel 2003), and propolis hoarding (which may correlate with confinement behavior). In this dissertation, I

have shown that the removal of beetle eggs from capped bee brood is similar in European and African honey bees at high beetle levels but could differ if there are fewer eggs per cell (Chapter 12). Although European bees do remove some cells containing beetle eggs, they may fail to remove all of the infected cells and this could lead to a quick build-up of larvae. Infected cells can contain many eggs (Chapters 10 and 12) so it would only take overlooking a few cells before beetle larvae populations explode in the colony.

Genetic biodiversity, by which I mean the diversity of genes within a geographical area or within a population, is simply not as high in honey bees in the United States and Australia as in those in Africa because honey bees are not native to either area. Further, both areas have stringent laws governing the importation of bees from other areas so their genetic biodiversity is not likely to increase any time in the near future. Genetic biodiversity has direct consequences on a population's ability to adapt to pressures exerted on them by parasites and it is often appreciated that the more 'genetically diverse' a population is, the more likely it is to handle parasite pressures. This, and the fact that honey bees in the United States are managed as virtual 'monocultures', must contribute to the beetle's virulence there.

I do not believe that this list of factors is solely responsible for European bee susceptibility to beetles; I only list these as probable causes of which we are currently aware. There must be other reasons for the differing susceptibilities between both bees as well and these will, undoubtedly, be illuminated in future studies. The good news is that parasites, when not interfered with by man, should inevitably adapt toward lower levels of virulence; otherwise, they would eliminate their hosts and go extinct themselves (Schmid-Hempel 1998). So, given time, we would expect beetle virulence to decrease in its introduced range and anecdotal evidence already suggests that this is happening in the United States.

Ecological Implications

Ecology, the study of the interactions between organisms and their environment, is an important discipline because it allows one to make predictions about a given organism (its success, spread, etc.) in its native environment. Further, understanding the biology (Section I) and behavior (Section II) of an organism in its native range (Chapters 2, 4, 5, 6, 7, 8, 9, 10) will allow one to predict the same in its introduced range. In this dissertation, I present data collected on the small hive beetle in both its native and introduced ranges and

the amalgamation of this data will allow me to predict how successful the beetle will be in the areas where it has been introduced, but also in areas where it may be introduced in the future.

Data presented in Section I of this dissertation concerns the biology of the beetle and this data has highlighted at least some weaknesses in its biology. These ‘weaknesses’ may, in fact, not be viewed as weaknesses at all but as indications of the beetle’s need for regulated environmental conditions. Understanding the beetle’s biological limitations will help us make predictions on its potential spread outside of its native range and potential effects on non-target species and/or organisms. For these reasons, I explore ecological implications that can be inferred from the data presented in this dissertation and by others.

Biological constraints

Nitidulid beetles have diverse feeding habits, which include feeding on fungi, carrion, rotten fruits, plant saps, etc. (Borror and White 1970; Scholtz and Holm 1985; Kirejtshuk and Lawrence 1999; Habeck 2002; Picker et al. 2002). Further, many species of nitidulids live in colonies of social insects (cf. Kistner 1982; cf. Hölldobler and Wilson 1990); so it comes as no surprise that small hive beetles live in honey bee colonies where their diverse feeding ability allows them to consume pollen, honey, and bee brood (Elzen et al. 1999; Hood 2000; Chapter 4).

Despite the prevalence of a wide-range of diets among nitidulids, small hive beetles seem to be limited to feeding on foodstuffs found within honey bee colonies. There is, however, a major exception and this includes the documented fact that beetles can feed and reproduce on various fruits *in vitro* (Eischen 1999b; Chapter 4). That beetles can feed and reproduce on fruits has further implications that will be discussed later (see ‘Non-target effects’ below), but for now this anomaly warrants discussion in this section as I deal with biological constraints of the beetle.

That beetles can reproduce on fruits has caused some concern to beekeepers providing pollination services and/or managing colonies for honey production. However, there is ample evidence suggesting that although beetles may reproduce on fruits *in vitro*, they are likely limited to reproducing on foodstuffs found in bee colonies *in vivo*. Supporting this is that beetle reproductive success when feeding on foodstuffs found within bee colonies is significantly higher than when feeding on fruits (Chapter 4). Despite the fact that many beetles remain confined while in honey bee colonies, the potential to

reproduce when the opportunity presents itself is so much greater within a colony than outside a colony that it benefits beetles, reproductively, to remain in bee colonies.

In addition to being constrained by what they eat, beetle reproduction may be limited, in part, to climatic conditions (see ‘Why are beetles only a problem in European colonies?’ above). In Chapter 5 I was able to show that beetle larvae only burrowed into soil that was adequately moist (about 10% moisture by weight) and Schmolke (1974) demonstrated that beetles would not burrow into ‘very wet’ soils (soils which were thoroughly soaked). This data suggests that beetle pupation success may be greater in areas where rainfall is moderate, but not absent or extreme. Lundie (1940) also suggested that soil type might influence the pupation success of beetles however the data presented in Chapter 5 does not support this assertion. It is also very likely that pupation success of beetles may vary depending on temperature and chemical nature of the soil. Neither of these aspects has been studied; however, it is possible to determine if either is the case.

Experiments have shown that the hatch rate of beetle eggs positively correlates to humidity and that at lower humidities, relatively few eggs hatch (Pettis cited in Somerville 2003). This also suggests that beetles may thrive better in climates where the humidity is high (which is supported by the pupation data already discussed above and in Chapter 5). An amalgamation of the data strongly suggests that beetle virulence is minimized in dry climates because egg hatch rates and pupation success are compromised. Likewise, beetle distribution, or at least impact, may be tightly regulated by temperature. Beetles have been found in colder states in the United States (Chapter 1), however as of yet, beetles have failed to become established convincingly in these places or cause any significant amount of damage.

This is, by no means, an exhaustive list of aspects of beetle biology that may be regulated by environmental factors. Future studies into beetle biology and behavior may illuminate more weaknesses, or dependencies, in its biology (for example, pupation success may be related to the chemical constituency of soils). Regardless, the data presented in this dissertation and by others studying the beetles suggest that beetle impact and reproductive success are closely tied to environmental conditions.

Spread and impact outside of its native range

This section may seem unnecessary in a general discussion of the small hive beetle as the beetle has already spread far outside of its native range. The beetle’s introduced range currently (October 2003) includes the entire eastern half of the United States (Figure

1.4), very limited parts of Canada, and isolated areas of eastern Australia (Figure 1.5). However, a discussion predicting the beetle's spread outside of its native range is important because it will allow one to 1) know where the beetle may be introduced and 2) predict its impact on honey bee colonies in these areas although the latter may be difficult to do.

Areas that are potential ranges for introduction obviously include any area that may exhibit climate compatibility with any of sub-Saharan Africa, the beetle's endemic range (Hepburn and Radloff 1998). As limiting as this may seem, it is important to remember that sub-Saharan Africa includes diverse environments from deserts in the south and north to tropical rainforests found along the equator. Further, that the beetles survive in the northern United States where the climate is cooler than in sub-Saharan Africa indicates that the beetle can survive quite a wide range of climatic conditions. Therefore, beetles are likely able to survive in any ecosystem that is not considered 'extreme' (like deserts or polar areas).

What this means for the beekeeper and scientist is that we may rest assured that the beetle will not be limited any longer to those areas where it was first introduced. We may with confidence assume that the beetle will spread to encompass all of the United States, parts of Canada, down into Central America, and then finally into South America as all of these places share climates similar to those places the beetle is already found. Further, the beetle will likely spread around the populated areas of Australia but may not be able to push into the arid interior due to the desert-like conditions (and lack of beekeepers) there. From Egypt, the beetle may spread into the Middle East, and if so, this will likely be accomplished by beekeeper-assisted means. It should be assumed that all of these areas will eventually host beetle populations although it is currently impossible to know how long it will take for this to occur. The time it takes for beetles to spread to these areas will likely be dictated by the number of beekeepers (especially migratory ones) and honey bee colonies in the region, and the climatic/environmental conditions.

It is reasonable to assume that the beetle will spread to the areas discussed above because they are geographically connected to places where the beetle already occurs. What is harder to predict is where else the beetle will occur if it cannot get there over land. For example, Europe does not currently host populations of the beetle (as far as it is known) and beekeepers and normal beetle migration are very unlikely to transport the beetle there simply because the beetle is not found on the continent. What must be considered in these

instances is how the beetle reached the United States and Australia because this will allow us to predict how and if it will ever be found in Europe or Asia.

To summarize information on how beetles arrived in their introduced regions: we simply do not know. Presumably, the beetles would most successfully arrive in these places if they were traveling with honey bee colonies. This could only happen if a swarm of bees were ‘stowed away’ on a boat (in cargo for example). If, however, the bees were to die, it could be possible that adult beetles supplemented their diets with fruit included in cargo on ships. It is not likely that adult or larval beetles were simply feeding on fruit that just happened to get transported to the United States and Australia because it is unlikely that beetles feed on fruit *in vivo* simply because their reproductive success is so low on fruit.

Beetles survive up to 6 months when feeding on a diet of honey (Chapter 4) so it is not far-fetched that beetles lived on sugary substances on a ship transporting goods from sub-Saharan Africa to the United States and Australia. In support for this notion it must just be remembered that beetles were first found in coastal areas of the United States (like Charleston) and Australia (Sydney) and this may be direct evidence that they arrived in both locations through the international ports at each place. This mode of entry into the United States has already been proposed (Hood 2000) and is probably the most likely scenario. Also possible (but less likely) is that beetle pupae could have been transported from Africa in any soil-containing vessel. But again, import laws are very strict about such occurrences so the chances of this happening are minimized.

If beetles are able to travel in cargo ships from their native range to the United States and Australia, then it is very reasonable to assume that the same may happen in other parts of the world. Europe (especially the United Kingdom) is a major importer of goods from South Africa so it is probably only a matter of time before the beetle arrives there in a manner similar to how it arrived to the United States. Similarly, Australia exports a lot of goods to Asian markets so this would easily provide a way for beetles to arrive in continental Asia. Therefore, the potential for global spread of beetles seems both very real and equally inevitable.

Just because beetles will likely spread around the world over the next few decades does not mean that they will impact European honey bees in these areas as they have in the United States and Australia. Indeed, Australia has yet to be heavily affected by beetle-associated depredation (Somerville 2003) so it is clear that the presence of beetles in an area need not automatically be feared. Whether or not beetles will have a definitive impact

in a given area is likely dictated by the same factors that I discussed in ‘Why are beetles only a problem in European colonies?’ above.

It can easily be asserted that beetles will be a problem anywhere that is environmentally and climatically similar to the southeastern United States. Beetles have already proven that they thrive under similar conditions. Most of the southern United States and Europe should then be ideal places where the beetles may succeed. Equally as plausible is that temperate and subtropical Asia may be affected, as there are places in Asia climatically similar to the southeastern United States. However, many other factors must also be considered, such as the race of honey bee present, beekeeper vigilance, genetic biodiversity, and presence/absence of other honey bee parasites/pathogens.

It is true that, in general, European honey bees succumb to beetle pressures more often than do their African counterparts (Chapter 2); however, how susceptible different races of European bees are toward the beetle is not yet known. This is especially important because there are a number of European races and some may be virtually immune to beetle pressures. So, whether or not beetles impact a given area may depend on the type of bee inhabiting the area and that bee’s level of susceptibility. It is, therefore, prudent that beetle effects on various European subspecies be established in order to understand if there are more susceptible races.

Beekeeper vigilance and style of beekeeping may also play an important role in determining beetle impact in introduced regions. For example, commercial beekeepers in the United States effectively farm ‘monocultures’ of bees where tens-of-thousands of hives may be present in an area. As is often the case with monocultures, they remain extremely susceptible to invasion and devastation by new pests. However, ‘beekeeping-by-the-thousands’ rarely occurs in Europe, the Middle East, and Asia so ‘monocultures’ are not as common.

Further, the ‘neatness’ and ‘cleanliness’ of individual beekeeper’s operations may influence, to a degree, the impact of beetles (Hood 2000). Anecdotal evidence suggests that tidier beekeepers have fewer beetles; although this needs to be confirmed quantitatively. Regardless, this suggests that beetle impact may be tied to the beekeeper so it may be very difficult to predict beetle impact especially since it may entirely depend on ‘who’ we are talking about.

In the section above (‘Why are beetles only a problem in European colonies?’), I briefly considered the role of genetic biodiversity on a honey bee-race scale. Assuming that genetic biodiversity is greater in areas hosting native populations of *Apis*, then it may

be plausible to assume that beetle impacts in these areas may be further reduced because of the native bees' superior ability to adapt to pressures. Following this logic, beetles may not pose as great a threat in Europe and Asia where native populations of *Apis* abound.

Finally, the presence/absence of other honey bee parasites/pathogens in areas where beetles are introduced may affect beetle virulence in these areas. I have already discussed that there may be synergistic relationships between beetles and other parasites/pathogens (see 'Counting the costs' above). This simply implies that, for example, where varroa mites are present, beetles may be more virulent. The same may also be true in areas where other parasites/pathogens are prevalent. In order to better understand this hypothesis, one need only do a survey of bee 'pests' in a region. With this done, one may assume that areas hosting many bee-associated parasites/pathogens may be more inclined to suffer beetle-induced depredation. However, before this hypothesis can be considered plausible, the relationships between beetles and other parasites/pathogens must be studied in further detail to determine if they are synergistic or antagonistic.

What I have outlined so far in this section are two, seemingly opposing ideas. I asserted that 1) beetles will likely spread around the world to areas that are not considered climatically 'extreme' but that 2) they will not likely be a problem in all of these areas. Predicting where the beetle will end up remains easy; it is far more difficult to predict if the beetle will have an economic impact in an introduced area. This is because a number of factors likely contribute to beetle-virulence and all these factors must be considered accordingly. Regardless, my discussion of beetle impacts has only taken into consideration what beetles may do to *A. mellifera* around the world; I have not considered potential non-target beetle effects which are equally as tangible.

Non-target effects

Small hive beetles, being scavengers (Lundie 1940), possess the ability to negatively-impact non-target species. In 'Biological constraints' above, I went to great lengths to establish the fact that beetles are likely limited to life in honey bee colonies as these places afford the biggest reproductive potential for the beetles (Chapter 4). However, it is important to note that honey bees are not the only social insects that gather and store nectar and pollen; bumble bees (species of *Bombus*) of Europe and North America and stingless bees of Africa, Asia, and Australia (just to name two examples; both are members of the family Apidae) do as well. Similarly, there are even different species of *Apis* (mostly

confined to Asia) that may be susceptible to beetle invasions. For these reasons, it is very important to consider the beetle's potential impact on species other than *A. mellifera*.

Kistner (1979) states that host specificity is exhibited by parasites at some level or another and that the term is usually used to denote a closer host specification at the species or generic level. Because of the significant lack of mellitophiles, it is difficult to predict at what level small hive beetles are specific. Studies from other social insect symbionts may shed some light on the host specificity of beetles, but as we will see, they may generate more questions than answers.

Most termitophiles are host specific at the species level and only a few live on more than one host (Kistner 1979). However, the situation is a bit more complex with myrmecophiles. Even highly-integrated myrmecophiles with elaborate behavioral and glandular adaptations are found with several hosts (Kistner 1979). Therefore, host specificity of termitophiles is usually at the species level while host specificity of myrmecophiles is more often at the species group, or generic levels. This leaves us to question how host specific small hive beetles are going to be but there is further information that may assist in this consideration.

When parasites are passed through different hosts, their virulence is typically reduced; in contrast virulence is often increased when passing through similar hosts (Schmid-Hempel 1998). Therefore, we can assume with reasonable certainty that beetles will probably not be able to inhabit ant, termite, or even social wasp colonies; but the same conclusion remains unlikely when considering the move from honey bee colonies to other social bee colonies in the family Apidae. The main reason for this is that colony structure and food stores within this family are similar enough (pollen and nectar hoarding, the presence of brood chambers, etc.) to warrant concern.

To look at host-specificity from the vantage point of small hive beetles, it is important to consider the family Nitidulidae to which the beetle belongs. Members of the genus *Epuraea* can live in *Bombus* nests (Scott 1920; Parsons 1943; Cumber 1949) while the larvae of *Brachypeplus auritus* feed on wax and honey of wild *Trigona* colonies in Australia (Lea 1910, 1912; Lundie 1940; Habeck 2002). Indeed, nitidulids frequent colonies of bumble bees (Kistner 1982). Therefore, it is clear that nitidulids have already taken advantage of niches available in colonies of various members of Apidae.

Further, small hive beetles can reproduce in bumble bee colonies *in vitro* (Ambrose et al. 2000; Stanghellini et al. 2000) so the potential for them to do so *in vivo* exists. It is also very likely that beetles can reproduce *in vitro* in *Trigona* colonies as well but again, if

they will do so *in vivo* remains to be seen. Based on the data, it may be safe to assume that beetles are at least host specific at the family (Apidae) level, but without experimentation, it is difficult to extrapolate much further than that.

Parasite virulence is often increased when passing through similar hosts (Schmid-Hempel 1998), so if beetles can naturally infest and reproduce in bumble bee and *Trigona* colonies their impact in these colonies may be greater than those in honey bee colonies, especially since any natural defense against the beetle is likely absent or reduced in non-honey bee colonies. However, if honey bee colonies are present in an area, beetles will almost certainly choose to infest those colonies rather than colonies of bumble bees and *Trigona* because reproductive success is probably maximized in honey bee colonies. Further, bumble bee and stingless bee colonies are smaller than honey bee colonies, possibly making them less attractive.

However, just because reproductive potential is smaller in non-*Apis* colonies does not mean that beetles will avoid those colonies. *Meligethes aeneus* (another nitidulid) changes its egg production to match host quality (Hopkins and Ekbom 1999). These nitidulids, when moved from high- to low-acceptability plants, reduced their oviposition rate considerably and when moved in the opposite direction, the rate of oviposition increased after the switch. Hopkins and Ekbom (1999) suggested that adjusting oviposition rates to match host acceptability maximizes the average host quality for offspring even at the cost of a lower egg-laying rate. That other nitidulids respond to host quality by adjusting their egg-laying rate suggests that similar responses may occur with small hive beetles (infest a non-*Apis* colony and simply lay fewer eggs).

At this point, it remains speculation that beetles may host-shift to other members of the family Apidae. However, I believe that this is an important area of research that should be investigated in the near future. Researchers in the beetle's native range need to conduct surveys in native bee colonies to determine if beetles are present; the same also needs to happen in the beetle's introduced range. It is possible that the beetle has already switched hosts but that no one has noticed because of a lack of research. Further beekeepers in beetle-infested areas of the United States often relocate colonies to escape the beetle. As a result, a great number of beetles may be left pupating in the ground so eclosing beetles may infest bumble bee (or other bee) colonies in the absence of their natural hosts.

It is more unlikely that beetles will shift between members of the family Apidae than between members of the genus *Apis*. We already know that every race of *A. mellifera* exposed to the beetle has become a host, but will similar trends occur with other members

of the genus *Apis*? For example, *A. cerana* (the ‘Asian’ honey bee), like its African and European counterparts, is a cavity dweller. It seems inevitable that beetles will be able to shift from *A. mellifera* to *A. cerana* colonies as the biology of both species is similar. The same has already occurred for the varroa mite which shifted hosts in the reciprocal direction (from *A. cerana* to *A. mellifera*). Therefore, host shifts between the two species have already resulted in a serious threat to honey bees.

Perhaps even more worrying is that when varroa was introduced to *A. mellifera*, their virulence increased. Varroa rarely harm their natural hosts but cause substantial damage to *A. mellifera* so it is possible that beetles, if introduced to Asia, will cause greater decimation in *A. cerana* populations than they do in *A. mellifera* ones. Likewise, there are many other species of *Apis* present in Asia that may be susceptible. These include species such as *A. dorsata* and *A. florea* which are both open-nesting species of *Apis*. At this point, it is impossible to predict what impact the beetles may have on species of *Apis* other than *A. mellifera*, but the argument that beetles possess the ability to cause substantial damage if introduced into Asia is valid and worth considering.

As I have discussed before, this host-shift from honey bees to fruits will probably not occur. There are numerous reasons that I believe beetles will not be pests on fruits in the wild including a reduced ability to reproduce on fruits (as I have already discussed) and the obvious abundance of honey bees (or other native bees) in the wild. Further, beetles (as I discussed in ‘Counting the costs’) are very adapted for lives in honey bee colonies and in many cases these adaptations are highly-specialized ones. It would not only require a host-shift to begin attacking fruit, but it would also require behavioral, physiological, and morphological adaptations to overcome dietary obstacles that fruits present to beetles.

However, just because beetles will likely never host-shift to fruit does not mean that they will never be found on fruit. Again, I would like to revisit my example where a beekeeper managing bees in a heavily-infested area moves his hives to accomplish some type of beetle ‘control.’ In this extreme circumstance, beetles pupating in the soil may eclose and, when not finding a honey bee colony in which to inhabit, feed on fruits to supplement their diets. However, this is an extreme circumstance and the beetles will likely only *supplement* their diets while continuing to seek a host.

Avenues for Control

A discussion of the beetle's ecological niche, effects on honey bee colonies, and the resulting ecological implications serve to satisfy those researchers studying biological and behavioral phenomena, but in the end, the beetle remains an applied problem for beekeepers. Indeed, it is for this reason alone that in recent years, we have seen a resurgence in beetle research. Because of this, it is vital that I take all of the data presented in this dissertation and try to synthesize it in such a way that will make beetle control options available for the beekeeper.

This will by no means be a clear-cut endeavor as most of the beetle control measures tested to-date have failed (to put it conservatively). Regardless, I will discuss all of the currently available control measures in their appropriate category (chemical, biological, etc.) and then attempt to suggest ways that these can be integrated for maximum efficacy. The controls mentioned below will be a combination of those reviewed by Hood (2000), Mostafa and Williams (2002), and original ones reported in Section III of this dissertation. Additionally, all of the work reported in Sections I and II of this dissertation have contributed information towards developing control methods not previously considered. These too will be discussed. In the end, this section will serve to identify ways to control beetles that will produce the fewest environmental impacts, a novel goal for any pest control scheme.

Controls chemical

I have decided to discuss the chemical agents tested for the control of small hive beetles first because it is common practice to immediately look for some sort of chemical control when an insect pest first presents itself. This happened with small hive beetles and one can see that there is an obvious bias towards chemical control of the pest in the literature. However, as is widely known, chemical control always comes with a price, and these too will be discussed.

In this discussion, I consider two types of chemical controls, 1) synthetic and 2) natural. Synthetic controls are those that one traditionally considers 'pesticides', which are often synthetically produced (realizing, of course, that they may be produced in nature as well). Examples of these are organophosphates, pyrethroids, etc. 'Natural' controls are those that are often produced as byproducts of plant metabolism that may be used by the plant to limit herbivory. I also consider pheromonal controls in this category for lack of a better-fit elsewhere.

Synthetic controls

Lundie (1940) was first to show that chemicals could be used against beetles when he suggested that combs kept in storage may be fumigated with carbon bisulphide to limit beetle damage. Similarly, Morse (1997) suggested that one could use paradichlorobenzene (PDB) to fumigate stored supers in an attempt to limit beetle damage. PDB has been shown to keep beetles away from stored combs (Mostafa and Williams 2002) so it seems to be a practical method of limiting beetle damage in stored combs (which may contain pollen and honey residues that attract beetles). Park et al. (2002) demonstrated that household bleach was effective in controlling adult and larval beetles in the honey house. Further, they demonstrated that one can clean frames that had been infested previously with beetles with bleach and then reuse the frames in a colony.

Schmolke (1974) used B.H.C. (benzene hexachloride), Carbaryl and Chlordasol to treat the ground around infested hives. He found that these ground drenches were effective in killing both larvae and pupae. He also demonstrated that a salt solution, sprayed around the colony, was effective in killing beetle pupae. Similarly, Delaplane (1998) suggested using ground-drenches to control the beetle in the United States. Following this, Baxter et al. (2000) showed that permethrin (sold as Gard-Star 40%) applied around colonies achieved some success at killing beetle larvae and pupae.

There are problems with using soil ground-drenches. Firstly, they are not extremely effective unless timely applied (Pettis and Shimanuki 2000). Further, they need to be applied at a radius of 90-180 cm from the hive in all directions in order to maximize their efficacy (Pettis and Shimanuki 2000). Finally, no one knows how many times the treatment needs to be applied. During the reproductive season, beetle larvae continually leave colonies to pupate in the ground so one would seemingly need to continue treating the ground in order to maximize the chemical's efficacy. Ultimately, soil treatments may protect individual hives on site but do little to curb the spread of beetles (Hood 2000).

Coumaphos (under the trade name Checkmite +) is often used to control beetles within a colony. The chemical is impregnated into plastic strips, which are then cut in two and placed under a piece of cardboard on the bottom board of a colony. The *modus operandi* in this case is that beetles presumably want to run from bee aggression into cracks (Schmolke 1974) so they run under the cardboard and are killed by the pesticide. This chemical is used against adult and larval beetles but with varying degrees of efficacy. Coumaphos is even used to control beetle adults in package bees and queen cages (Baxter et al. 1999), but results vary.

There are also problems associated with using coumaphos, and the primary problem is that it is an organophosphate (OP). The Environmental Protection Agency in the United States is trying to limit the use of OP's and as a result, Checkmite + is only available through emergency release. Further, the product does not perform well under cooler temperatures (Mostafa and Williams 2002). Kochansky et al. (2001) demonstrated that coumaphos residues in wax can get into syrup and honey, a result often not tolerated by those governing food quality. Finally, beekeepers often apply the plastic strips in a manner not consistent with the label and may leave the strips in for extended periods of time. Because of all of this, coumaphos is, at best, a very marginal control solution for beetles.

Alternative chemicals have been tested for the control of the beetle. The US Department of Agriculture (USDA) tested the efficacy of 8 insecticides against SHB (Elzen et al. 1998). Five showed excellent toxicity against adult and larval beetles causing 100% mortality; these included 3 pyrethroids (YT-1605, YT-100B, YT-1105) and two organophosphates (YT-205, OP-2). A formamidine (YT-1903) showed poor toxicity as did a third organophosphate (YT-1701) and a neurotransmitter (YT-2501). However, because these tests were conducted by scientists from the USDA, the names of these chemicals remain undisclosed.

Besides the obvious lack of efficacy of all of the chemicals that I have mentioned thus far, there are greater problems associated with using chemicals to control beetles. Honey is marketed as a pure product and as a result, most countries have very strict regulations regarding chemical residues in hive products (including honey, pollen, and beeswax). Beetles also have the potential to become resistant to any chemical we might use to control them because of their high fecundity and mobility. Resistance implies that the pest has developed a capability to withstand a dose of pesticide that would ordinarily kill the majority of individuals in the population (Milani 2001). Because beetle life cycles are relatively short, they have the ability to develop pesticide resistance quickly so beekeepers will likely find themselves on a pesticide treadmill. This is not a good place to be.

Further, chemicals in general afford only temporary relief from pest problems but their overuse and misuse have resulted in serious worldwide consequences (Rosen 1985). The cost of chemical applications lowers the profitability of agricultural crops (honey and pollination in this instance)(Rosen 1985) and pesticides may kill natural enemies of the

pest, stopping any biological control schemes aimed at controlling beetles. In the end, pesticides simply are fraught with difficulties.

Natural controls

It is difficult to suggest the use of ‘natural’ chemical controls against small hive beetles primarily because so little is known about this avenue of control. Further, what constitutes a ‘natural’ chemical control remains unclear because of this category’s general ambiguity. However, there are at least two natural controls that have been employed successfully against other arthropods and they include 1) pheromones and 2) botanical extracts.

Aggregation pheromones have been described for a variety of nitidulid species and are often used as control agents (Petroski et al. 1994; James et al. 2000). The pheromones most used for the control of nitidulids are those produced by large specialized cells within the body cavity (Nardi et al. 1996); Neumann and Elzen (2003) suggest that similar pheromones may exist in small hive beetles. Preliminary data indicate that male beetles tend to infest a host before females, perhaps suggesting the existence of aggregation pheromones in male beetles (Elzen et al. 2000). However, the use of pheromones to control beetles has not been studied in sufficient detail, but other work with nitidulids suggests that it may be profitable to do so.

Using botanical extracts in beetle control schemes may also be a viable alternative. Botanical extracts are usually derived as secondary compounds (or by-products) of plant metabolism which the plants may use to discourage herbivory (D. Downie personal communication). The use of botanical extracts for the control of various arthropods has been gaining popularity because they offer a ‘natural’ alternative to traditional chemical pesticides.

Interestingly enough, botanical extracts have been used to control both varroa and tracheal mites in honey bees (cf. Ellis et al. 2001). Mixtures of these extracts (often including thymol, camphor, eucalyptol, and menthol) are widely used in Europe (Imdorf et al. 1999) and are beginning to be tested and used in the United States (Calderone and Spivak 1995; Calderone 1999; Ellis et al. 2001). Because of their documented efficacy against varroa and tracheal mites, such extracts may also prove harmful to small hive beetles. However the doses of these extracts necessary to kill adult beetles will probably also harm adult bees, but the vapors may be sufficient to kill beetle larvae and especially

eggs. Further, the odor of these chemicals may be sufficient to keep beetles from invading colonies or make it more difficult to locate host colonies by masking normal colony odors.

There are problems with using botanical extracts. Chiesa (1991) found evidence for increased adult bee mortality in thymol-treated colonies and Bunsen (1991) documented increased brood mortality in the presence of thyme. However, Mattila et al. (2000) failed to detect differences in brood mortality between non-treated colonies and colonies treated with the thymol-based acaricide Apiguard™. Regardless, testing botanical extracts for efficacy against beetles may prove beneficial in the future.

What scientists and beekeepers alike do not realize is that because none of the chemicals registered for the control of beetles thus far are efficacious at the levels that beekeepers need them to be, we have been presented with a wonderful opportunity to control a new pest using non-chemical means. So, instead of trying to find additional chemical controls for the beetle, we have been given a chance to develop and use a suite of non-chemical controls against a pest that has been able to overcome most chemical applications.

Controls cultural/mechanical

Cultural/mechanical controls are those controls that result from a change in practice with the intention of limiting, but not eradicating, a pest. Lundie (1940) first suggested cultural controls for the beetles. He stated that good sanitation (hygiene) around the honey house goes a long way in controlling adult and larval beetles, a suggestion that Hood (2000) substantiates. Practices such as removing honey, bits of comb, and cappings will minimize foodstuffs to which beetles may be attracted. It is also important to extract supers of honey quickly to reduce the damage that beetle adults and larvae do to standing, unprotected crops (Hood 2000). Pettis (cited in Somerville 2003) and Waite and Brown (2003) suggest that reducing the relative humidity to 50% in honey houses and other places where honey is stored inhibits beetle eggs from hatching.

In the apiary, Lundie (1940) suggests that it is prudent to eliminate, requeen, or strengthen weak colonies to reduce colony stress and possibly make the colony better able to deal with beetles. One should avoid other conditions that might lead to colony stress such as brood diseases, mite problems, wax moth activity, failing queens, excessive swarming and over-supering (Hood 2000). Other cultural controls such as reducing the comb-to-bee ratio may help bees protect the combs from beetles better (Lundie 1940; Schmolke 1974). Further, it is vital that one uses good equipment that has few holes as

holes allow increased beetle ingress. If one finds a dead colony in the apiary, one may just freeze the entire colony, instead of treating it with chemicals, to destroy beetle eggs/larvae (Schmolke 1974). The mangled frames can then just be washed out with warm water to remove beetle frass (Lundie 1940). Hood (2000) also suggests that abandoning old, established apiaries where beetles have over-wintered may provide some control against newly-eclosing beetles in the spring.

Schmolke (1974) devised 3 simple traps, to provide secure hiding places for the beetles, from which he could periodically remove adult beetles. Two of these traps fit over the hole in the inner-cover to trap beetles moving up through the colony. Schmolke (1974) fitted the third trap at the rear of the bottom board to catch beetles trying to hide from bees. Despite his efforts, Schmolke (1974) showed that his traps accomplished little and he hypothesized that the cold weather kept beetles from moving enough to enter the traps (it was winter when he tested his traps).

Hood and Miller (2003) tested an in-hive trapping device in which they used a variety of potential beetle attractants: alcohol, beer, ethylene glycol, mineral oil, honey and cider vinegar. Cider vinegar in the traps yielded the highest counts of dead beetles in the field but showed low lethality to beetles in lab tests. In contrast, mineral oil showed the complete opposite with low trap counts in the field, but high mortality in the lab. Studies like this are a positive step towards beetle control and such devices need to be tested further.

Elzen et al. (1999) tested beetle-trapping devices designed to work outside of colonies. They discovered that odors from hive products plus adult bees were attractive to flying adult beetles as this combination yielded the greatest number of beetles in the traps. In contrast, odors from hive products alone or bees alone were not sufficient to attract flying beetles. However, devices such as this may not be sensitive to small resident populations of beetles and if this is the case, by the time the traps are collecting beetles, beetle numbers in one's apiary may already be too great to control via trapping.

Moving on to research reported in this dissertation (Chapter 11), I showed that reducing colony entrances with PVC pipe was, in some circumstances, sufficient to decrease beetle ingress. However, there were some notable side effects of reducing pipe entrances (fewer adult bees and brood, less production, etc.), but in some instances these side effects were mitigated if one simultaneously used bottom screens. Despite some success using reduced hive entrances, the negative side effects may outweigh the benefits, especially if used in production seasons so it would probably be prudent to limit the use of

PVC pipe entrances to non-production seasons. In the end, more research is needed to firmly establish the efficacy of reducing colony entrances for beetle control.

On a more ecological level, Blackmer and Phelan (1995) were able to show that some nitidulids prefer woodland habitats while others prefer agricultural situations, thus indicating that many nitidulids exhibit habitat preference (Blackmer and Phelan 1995). It may be beneficial to test if small hive beetles also exhibit habitat preference because if they do, it may be advantageous to limit apiary sites to wooded areas or agricultural areas (depending on which the beetles show least preference for).

On the practical side, cultural/mechanical controls often result from ‘common sense’ practices. For example, it makes sense that one should extract honey under sanitary conditions; if not, beetles may be able to exploit this oversight. Cultural control boils down to sound management practices, good beekeepers, and good hygiene. If these qualifications are met, then colonies should be strong, which assures that they will be in a condition where their natural defenses against the beetle are maximized.

Controls genetic

Genetic control, in the context that I am referring to here, is made possible by enhancing the natural defenses of a host by selective breeding for the traits that confer resistance. To date, a number of potential avenues for the genetic control of small hive beetles have been identified. These include the confinement behavior of bees toward beetles (Chapters 6-9), general bee aggression toward free-roaming beetles (Elzen et al. 2001), removal of beetle eggs and larvae from colonies (Schmolke 1974; Neumann and Härtel 2003), and the hygienic removal of beetle eggs oviposited in capped bee brood (Chapters 10 and 12). All of these traits are present in European honey bees at some level and they may be selectable for enhancement in bee breeding programs.

In Chapters 6-9, I describe the complicated process by which host colonies of African bees confine and guard adult beetles. The extent to which this behavior confers resistance to the beetle is unknown especially since the behavior is also present in European honey bees. However, confining beetles away from the central nest where they feed and reproduce must limit beetle reproduction at some level and this needs to be investigated further. In Chapter 7 and 8, I suggest that the behaviors associated with the confinement of beetles expressed by both African and European bees are similar at low beetle populations but may begin to diverge at higher beetle populations. If true, then it

may be possible that if the behavior is enhanced in European bees via selection, beetle virulence may be lessened.

Elzen et al. (2001) demonstrated that African bees, in general, are more aggressive toward free-roaming beetles than their European counterparts. It does not take much to see how this aggression may reduce beetle reproduction by not allowing the beetles time to oviposit on rich food sources. Perhaps this is another genetically-linked behavior that is amenable to selection in breeding programs that would benefit European bees to have in their arsenal.

African honey bees remove exposed beetle eggs and larvae from their colonies (Schmolke 1974; Neumann and Härtel 2003) and it is not yet known if this behavior is present in European honey bees. The behavior is probably present at some level as it is common for bees to remove foreign objects from the hive. However, the behavior may be present at much-reduced levels in European honey bees. If this is the case, European honey bees may benefit from breeding programs that serve to enhance the behavior in the European genotype. Further studies are needed to determine if this is the case.

The final behavior that may lend itself to genetically controlling the beetle is the hygienic behavior that I discussed in Chapters 10 and 12. In these chapters I demonstrate that Cape honey bees are able to detect beetle eggs oviposited in capped brood via two different modes of oviposition (through the capping and through the side wall of the cell). I also showed that Cape bees are remarkably efficient at detecting and removing 'infected' brood; however, I also show the same behavior is present in European colonies at comparable levels. I suspect that one reason European bees were so efficient at removing infected brood was that the colonies were moderately strong and the patch of infected brood was proportionally low. If beetle populations were higher, the colonies were stressed, and the beetles oviposited in more brood, the European bees may not have removed the infected brood as efficiently.

Further, beetles were able to oviposit without the presence of bees, so they may have laid more eggs per cell than what is normal *in vivo*. This may be crucial because there may be a minimum number of eggs per cell that elicits the removal of infected brood. I say all of this to suggest that at the beetle levels studied, both Cape and European bees removed infected brood at similar rates but if other parameters were manipulated (such as increasing the number of beetles, manipulating the number of eggs per cell and colony strength) both bees may have shown differing rates of egg removal. Hygienic behavior is amenable to selective breeding programs (cf. Boecking and Spivak 1999; Spivak and

Boecking 2001) so because the behavior is already present in European honey bees suggests that it may be enhanced by breeding and used for the control of beetles.

I do realize that the above behaviors are only just beginning to be studied. It is, therefore, possible that none of them are responsible for conferring resistance to the natural hosts of the beetles. Because of this, it is vital to determine if each behavior is 1) crucial to controlling higher populations of beetles and 2) selectable in breeding programs of European bees. If neither of these criteria is met, then the behavior may be deemed insignificant. On the other hand, if both criteria are met for any of these behaviors, then breeding bees to enhance those behaviors for the control of the beetle may be advantageous. In the end, it is important to understand that breeding bees for resistance to beetles will not make them immune to the beetle; instead, it will help the colony cope with beetle populations, perhaps to a degree that beetles are no longer a problem.

Controls biological

Biological control is usually defined in one of two ways, 1) an applied field of endeavor or 2) as a natural phenomenon (Rosen 1985). The applied sense (utilization of natural enemies to reduce the damage caused by pests) is relevant to this discussion, but the applied sense relies heavily on the fact that it is a natural phenomenon (the regulation of pest number by other organisms)(Rosen 1985). If biological control is to be successful, it must have a firm basis in sound ecological principles and in vast practical experience. If these criteria are met, then biological control is a successful alternative to chemical control (Rosen 1985). Any search for biological control agents should begin in the beetle's endemic range of sub-Saharan Africa for it is here that the chances of finding such an agent are maximized.

Lundie (1940) did not find any beetle parasitoids in South Africa. He collected a number of beetle larvae from the floor of a honey house and kept them in a container to look for parasitoid emergence; after some time he found none (Lundie 1940). Lundie further tested *Microbracon brevicornis* (a braconid wasp that parasitizes wax moths) for parasitism on beetles and found that the females stung the larvae and fed from the puncture holes. However, no new parasites were reared. Lundie (1940) did manage to discover what he presumed to be a fungal pathogen that killed beetle larvae *in vitro*. However, Lundie did not pursue this subject any further and the pathogen was not identified.

Similarly, I also found increased mortality of beetle pupae in soil rearing chambers and the pupae appeared to be dying due to a fungal infection (Chapter 13). Although the

causative agent could not be identified with absolute certainty, it is thought that either one or both of two different species of *Aspergillus* (*A. flavus* and *A. niger*) were responsible for beetle mortality as both are soil fungi known to kill insect larvae (Ferron 1985). However, both fungi are also known to cause diseases in honey bee brood (Baily and Ball 1991) so they may not be ideal agents for the biological control of beetles. Indeed, for an agent to be considered a successful biological control agent, it must be host-specific (among other things) so neither fungi should be used for controlling beetles in honey bee colonies.

Fire ants have been shown to keep nitidulids away from rotting fruit (Vinson 1991). They may consume the nitidulids or exclude invasion by nitidulids. In fruit pans where ants were prevalent, adult nitidulids were present but at reduced numbers than in pans not exposed to ants. Further, larval numbers were lower in pans where ants were present. It is unlikely that ants will prove to be effective biological control agents as they are often predacious on a suite of insects, including honey bees. Despite this, ants may be able to clean out dead or empty hive bodies left by absconding bees. In these instances, ants may be able to reduce beetle reproduction to some extent.

More studies in the beetle's native range are needed to identify possible biological control agents. These agents may include, but are not limited to, predacious nematodes, parasitic flies/wasps, fungi, viruses, bacterial, protozoa, etc. Basic studies of the systematics, biology, and ecology of the pest and its natural enemies are an integral part of the field of biological control (DeBach 1964). It may be some time before researchers find a suitable biological agent for the control of beetles; but this should not limit the search for one. Rosen (1985) states that biological control should be an integral part of any control against a pest so the importance of searching for a biological control agent for beetles cannot be overemphasized.

Integrated pest management

Beekeepers must accept that no single control option described above or to be identified in the future will single-handedly provide absolute control against the beetle. If the control is to be a successful one, it will have to be integrated in nature. For this reason, it is pertinent that the available control options be considered in an integrated fashion, under the umbrella of IPM.

The goal of an IPM-based approach is simple, to avoid treatments (especially chemical ones) at low, tolerable pest levels and to interject with a treatment only when higher pest densities are reached (Hood and Delaplane 2001). If achieved, IPM limits (and

may even eradicate) the use of pesticides altogether, or at least until absolutely necessary. The ‘treat when necessary’ approach is much preferable to the recommended treatment schemes beekeepers currently subscribe to (Hood and Delaplane 2001).

IPM is not a new concept and it is highly valued in current-day pest management environments; however, the program is not always easy to employ and this may hold true in beetle control. For example, beetle levels can vary from region to region, depending on climate, colony health, etc., as does the level of beetles (economic threshold) that causes damage. Because of this, IPM is often region-specific and can only be applied under certain, pre-described conditions. Likewise, treatment thresholds must be revalidated periodically to ascertain if the threshold is too high or too low as thresholds may change with time (cf. Hood and Delaplane 2001).

Further, there are a number of practical considerations one must acknowledge before he/she tries to approach beetle control in an integrated fashion. There must be an established economic threshold for the pest. It does no good to have a bag full of treatments if one does not know when to apply them. Further, a way to sample beetle populations in order to know when beetle numbers are approaching the established threshold values must be developed. Finally, one must know how to successfully integrate all of the known controls in order to most efficiently combat the beetle.

Establishing an economic threshold

The key to successfully employing IPM against beetles will lie in the development of suitable guidelines for treatment recommendations commonly referred to as treatment thresholds. Hood and Delaplane (2001) define treatment thresholds as the ‘(pest) density at which control measures should be applied to prevent an increasing pest population from reaching the colony collapse level.’ Based on this definition, the threshold for beetles will always be below the level necessary to cause that collapse. If one were to delay treatment beyond the threshold, he would risk increased colony mortality and experience economic loss; in contrast, if the beekeeper treats below the threshold, he will experience increased management costs and other negative side effects (like beetle immunity to pesticides)(Hood and Delaplane 2001).

Determining a treatment threshold for beetles will not be as easy to do as it was for varroa mites. This is due mainly to 3 problems; 1) we, as of yet, do not know what ‘damage’ beetles actually do to colonies so it will be very difficult to determine what level of beetles actually cause economic damage if we do not know what the damage is; 2)

beetle adults are very mobile and anecdotal evidence suggests that a colony may be heavily infested one day, but have relatively few beetles the next day and for this reason, it will be difficult to attribute measurable damage to a given population of beetles; and 3) beetle adults are not the only life stage that attacks honey bee colonies, a true treatment threshold may have to consider the combined effects of adult and larval beetles.

In ‘Counting the costs’, I described what research presented in this dissertation and by others indicates that beetles damage is. However, it is very difficult to determine what exactly beetles do to a colony and as I have already stated, just because we do not ‘see’ damage, does not mean that it is not there. So before an economic threshold can be established, researchers must determine what beetle damage actually is and what level of that damage beekeepers are willing to accept. Further, it is important to know what level of beetle-associated damage is actually causing an economic loss and what is simply ‘damage’ on the aesthetic, but not economic, level. I have suspicions that economic damage manifests itself in ways beekeepers and scientists do not fully appreciate yet.

Unlike varroa mites, beetle adults can easily migrate from colony to colony on their own effort. As a result, a colony may host many beetles one day, only to be almost beetle-free the next (Wenning 2001). This presents quite a challenge to those trying to determine an economic threshold for the beetle. If colony ‘A’ is showing an appreciable amount of damage that is undoubtedly attributable to beetles, it is difficult to assign that damage to a given population of beetles if that population is fluctuating every day. It is important that researchers understand the biology and dynamics behind beetle migration from colony to colony to understand if it happens on an appreciable scale. Only after this is done will it be possible to establish a reliable economic threshold for the beetle.

Contrary to what others have suggested, beetle larvae are not the only beetle life stage that damages a colony (Chapter 2). For this reason, it will be impossible to establish an economic threshold for the beetle if one only approaches the effort in a way to determine a threshold for either beetle adults or beetle larvae. In order for an accurate threshold to be established, we must understand the relationship between beetle adults and larvae and understand their cumulative effects on colony production. Further, we must know what triggers beetle reproduction in a colony. If, for example, a given beetle population must be reached before reproduction is triggered, then that population may be considered a threshold. However, bees may be able to rid the colony of beetle eggs (Chapters 10 and 12) and larvae (Neumann and Härtel 2003). In this instance, what is the

threshold?...the number of beetles it took to elicit reproduction or the number of eggs and larvae past which bees no longer efficiently removed the offspring?

One last complication concerning establishing an economic threshold for beetles relies in potential synergistic interactions between the beetle and other honey bee pests (varroa mites for example). For example, if varroa mites are present in a colony then the threshold for beetles may actually be lower than if varroa mites are absent. As a result, one would have to treat for varroa continuously to assure that their presence is not affecting beetle impact. Therefore, it is vital that future research take synergistic relationships into account before an economic threshold be established.

Having said all of this, it remains of utmost importance that treatment thresholds be established for the beetle before we commit to relying on chemical control. There are major obstacles to overcome before such a threshold can be determined but understanding beetle biology (Section I) and behavior (Section II) will go a long way in helping us understand what course of action should be taken. In order to most effectively establish a threshold for the beetle, researchers are going to have to find a way to restrict beetles to a given colony, quantify and determine the various shapes beetle damage might take in a colony, understand the relationship between beetle adults and larvae, and understand the relationship between beetle adults and other parasites/pathogens of honey bees. If entomologists are able to do this (and I believe that they will) then a treatment threshold will soon follow.

Sampling for the beetle

We may now assume, for the sake of argument, that a treatment threshold has been established for small hive beetles; in short, we now know what intra-colonial population of beetles will result to economic damage in our colonies. I must now tackle the dilemma on how to determine if beetle populations have reached the threshold or not. Economic thresholds are virtually useless if a reliable sampling method is not determined (Hood and Delaplane 2001).

It is important to know that no one sampling method will likely be 100% reliable for determining the actual population of beetles in a colony. Similarly, this was found to be the case with varroa mites where a large range of mite populations within a colony represents a treatment threshold (cf. Hood and Delaplane 2001). Regardless, a sampling method for the beetle must be easy and simple to employ if one expects beekeepers to use it. Further, sampling must be cheap to do and the results should be easy to interpret by

relatively untrained people. If these criteria are met, then such a sampling method may be considered useful.

There will be, inevitably, some difficulties with discovering ways to sample beetles. As I have shown in Chapters 7 and 8, beetle movement about this hive is restricted especially at low beetle populations. So, sampling devices, when developed, may not be sensitive to low beetle populations. This will not present a huge problem unless low populations actually fall within the range of an established treatment threshold.

Further, one may have to develop sampling methods for both the adult and larval stages of the beetle because they are the ones that cause the most damage in a colony. However, one idea I have had that has not previously been considered is to try to determine a correlation between the number of beetle pupae in a given area of soil (say 0.5 x 0.5 x 0.5 m) around the colony and the number of beetles and larvae in the hive. This method would be very simple to do and would not cause one to have to enter a hive in order to sample beetles.

Regardless of the form a sampling method takes, one is going to have to consider the biology (Section I) and behavior (Section II) of the beetle when designing such a device. For this reason, it would be premature to make elaborate suggestions as to the form such devices should take. However, Hood and Miller's (2003) intra-colonial trapping device and Elzen et al.'s (2000) sampling device may be considerable steps in the right direction. Further, using coumaphos to kill beetles under a piece of cardboard may give a reliable estimation of the number of beetles in a colony. Regardless, it is vital that a reliable sampling method for the beetle be devised in order to establish if the beetle has reached the economic threshold and determine what course of action to do next.

Integrating current treatments

Once an economic threshold for the beetle is established and a reliable sampling technique to measure beetle populations is determined, then the beekeeper has to know how to use the tools that researchers have given him/her in order to effectively control the beetle. Under the various control categories that I outlined in this section, I listed all the known and tested avenues of control that have been discussed in the literature. As should be obvious from my discussion, none of these controls are significantly efficacious by themselves, but may gain efficacy when used in conjunction with other controls.

The goal of IPM is to eliminate or greatly reduce the use of pesticides in a control scheme (Hood and Delaplane 2003) and this should be relatively simple in the case of

small hive beetles as none of the chemical controls used thus far are highly effective. I must stress that it will be impossible, without research integrating the various aspects of beetle control that are available today, to determine an effective IPM suggestion, but, using the data outlined in this thesis and by others, an effective IPM approach to beetle control might take shape as follows.

First and foremost, it is important to use bee stocks that have shown some level of resistance to the beetle as outlined in Chapters 7, 8, 10, and 12 and by others (Neumann and Härtel 2003). Confinement of beetles, hygienic behavior towards beetles, and removal of beetle larvae may all be increased through selective breeding and it would be very important to use bees that have shown some level of resistance to the beetle. Using ‘genetically modified’ bees may slow the time it takes a colony to reach a treatment threshold.

It would be very important to sample beetle populations (possibly via devices by Elzen et al. 2000; Hood and Miller 2003) periodically throughout the year to determine if beetle populations are increasing during particular seasons. If they increase during production season, it may be necessary to use coumaphos-based insecticides in order to elicit a quick response. Further, it may be advantageous to use permethrin-based ground drenches to kill wandering larvae or pupating beetles. If beetle populations increase during non-production seasons, it may be advantageous to reduce colony entrances (as in Chapter 11) while simultaneously using a screened bottom board to reduce side effects.

Because Hood and Miller’s (2003) in-hive trap uses natural substances to attract and kill beetles (such as cider vinegar and mineral oil), it may be used year round to constantly tax beetle populations. Other devices that are designed to trap wandering beetle larvae may also be developed and used to slow population build-up. Such devices have an important place in IPM management today because they are non-chemical and have been shown to provide some knockdown of beetles.

Data presented in Chapter 5 show that beetle larvae need certain cues to cause them to burrow into the ground for pupation. It may be possible that chemical content of the soil (such as pH) plays an important role in pupation biology. This avenue needs to be investigated further. Regardless, the data (Chapter 5) suggest that colonies should not be kept immediately beside agricultural areas where the ground is kept moist and tilled (which are ideal environments for beetle pupation to occur). Further, Schmolke (1974) showed that drenching the ground with a salt solution may increase wandering larvae mortality and this may be a means that low populations are kept subdued.

A current-day, effective IPM program may be achieved through the development of a vigorous program of applied biological control (Rosen 1985). Biological control should be the backbone of any IPM program; indeed the value of natural enemies cannot be overemphasized and their conservation should be the first goal of IPM (Rosen 1985). For this reason, it may be possible to use species of fungi described in Chapter 13 as biological control agents although more studies certainly need to be done before their use. If biological control agents are ever used against beetles, then it is of paramount importance that chemical pesticide use be minimized to limit secondary effects on beetle natural enemies.

Admittedly, we are a long way from being able to control beetles in a way that current-day problems demand. Fortunately, the lack of efficacious chemical controls for the beetles may actually promote studies into other categories of control. One point worth emphasizing is that research on beetle control presented in Section III of this dissertation and discussed in the literature by others is going to have to be integrated if it is to achieve an appreciable amount of control. I realize that I have discussed beetle control in light of many weak control measures, but this emphasizes the important place IPM has in controlling beetles.

Conclusion

In the end, we have come a long way since Lundie's documented amazement at the lack of research on such a notable honey bee pest. The research reported in this dissertation and by other scientists has significantly contributed to understanding the beetle's biological and behavioral intricacies, which have gone relatively unstudied since the beetle was first described by Murray in 1867.

In this dissertation, I explored new avenues of beetle biology that contributed to an overall understanding of the beetle's natural history. I also unraveled behavioral interactions between small hive beetles and their honey bees hosts in both their 1) endemic and 2) introduced range. All of this data contributed relevant information that has helped, and will continue to help, discover ways that the beetle may be controlled. The amalgamation of all of the data presented in this dissertation led to an overall discussion of the beetle's ecological niche, ability to impact honey bee colonies in ways never considered, and the ability to predict the beetle's spread and impact around the world.

Postscript

In conclusion, the small hive beetle has been both biologically and practically fascinating to study. I often wonder if Lundie would have ever predicted what his beetle would be doing 60 years after he wrote his technical report. I expect the same will hold true for me 60 years from now.

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