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DEFENSIVE BEHAVIOR OF HONEY BEES: Organization, Genetics, and Comparisons with Other Bees

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■ **Abstract** One key advantage of eusociality is shared defense of the nest, brood, and stored food; nest defense plays an important role in the biology of eusocial bees. Recent studies on honey bees, *Apis mellifera*, have focused on the placement of defensive activity in the overall scheme of division of labor, showing that guard bees play a unique and important role in colony defense. Alarm pheromones function in integrating defensive responses; honey bee alarm pheromone is an excellent example of a multicomponent pheromonal blend. The genetic regulation of defensive behavior is now better understood from the mapping of quantitative trait loci (QTLs) associated with variation in defensiveness. Colony defense in other eusocial bees is less well understood, but enough information is available to provide interesting comparisons between *A. mellifera* and other species of *Apis*, as well as with allodapine, halictine, bombine, and meliponine bees. These comparative studies illustrate the wide variety of evolutionary solutions to problems in colony defense in the Apoidea.

INTRODUCTION

For many people, honey bee (*Apis mellifera* L.) defensive responses are a primary point of contact with the insect world. Fear of honey bee stings permeates the consciousness of both the lay and scientific public, driving public policy concerning management of this valuable source of food and pollination services (37, 92). In the New World prior to 1956, human exposure was to honey bees of European derivation (EHB). The introduction of the "African" honey bee (AHB), *Apis mellifera scutellata*, to Brazil (83, 127) and its subsequent spread through

tropical and subtropical New World habitats, along with the characterization of AHB as "killer bees" (47, 112, 137), have added to scientific and public awareness of honey bee defensive behavior. Indeed, more than 1000 people and tens of thousands of domestic animals have died owing to stinging incidents as AHB have spread. In Mexico alone, more than 300 people were killed between 1988 and 1993 (61).

Unfortunately, this public health problem has created a fear-driven public policy climate that is often antagonistic to both feral and managed populations of honey bees, regardless of actual hazards. Public discourse concerning honey bee management and the impacts of honey bee defensive responses to humans has too often been passionate and lacking in scientific objectivity.

We review the current scientific understanding of colony defense by honey bees. Knowledge of how defense is organized and of the genetic underpinnings of defensive behavior provides a more rational basis for public and scientific discussion. Our focus is on work published since earlier reviews appearing in books edited by Needham et al. (98) and Spivak et al. (127). Considerable scientific progress over the past decade has clarified the organization of honey bee defensive responses. We now have a better understanding of phenotypic and genotypic variability in defensiveness between colonies and among honey bee populations. Also, recent results better place defensive behavior within the overall division of labor in honey bee colonies.

We also discuss how defensive behavior of *A. mellifera* compares with that of other eusocial bees. Comparisons within *Apis* give insight into the evolution of defensive behavior within the context of the range of ecological conditions that the genus faces. Comparisons with non-*Apis* apoids give us a measure of the phylogenetic constraints on bee defensive behavior. For a full analysis and cross-species comparisons, defensive behavior for each taxon would be analyzed in terms of division of labor, variations in use of defensive weaponry, any special adaptations to cope with unique predators or parasites, and the nature of enemy identification and specification systems. Unfortunately, for most of these defensive characteristics *A. mellifera* has been the only species studied, and these studies have concentrated on ecotypes that have been transplanted from Europe to the Americas. However, we make comparisons, as the data allow, and suggest productive routes for further comparative work.

ORGANIZATION OF BEE DEFENSIVE BEHAVIOR

The Defensive Bee: Behavioral Sequences

Figure 1 shows the organization of EHB defensive behavior, but the elements are probably similar for all bees. Both guard bees, which patrol the colony entrance (7, 8, 12, 31, 128), and soldier bees (28), which participate in mass attacks on vertebrates, follow this basic sequence. Each event in the sequence represents a heightened defensive response. Once a bee has oriented the threat, it may be in antennal

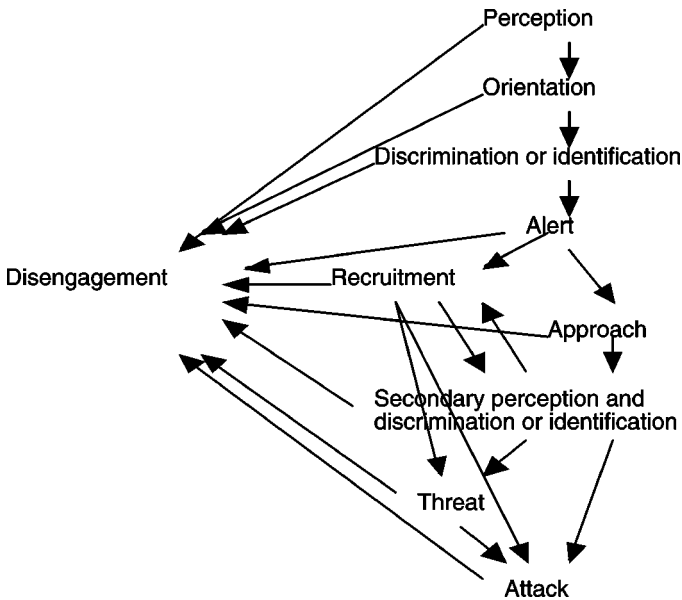

Figure 1 The sequence of events in a honey bee defensive response.

contact with the target; contact at this point in the encounter has an intimidating effect on many intruders, but it also allows verification of the identification of the target as a threat. Antennation is particularly important in discrimination of conspecific intruders, which may be easily confused with nestmates. This detailed breakdown of the events in a defensive response allows for independent analysis of the factors regulating each behavior.

Usually more than a single bee apprehend a threat to the colony. The presence of hundreds or thousands of other bees in close proximity means that under most circumstances many bees have the opportunity to perceive an intruder; the perception stage in Figure 1 is typically a shared event. In analyses of defensive behavior, investigators often find it difficult to separate the effects of an individual bee's responses to the threat from the effects of recruitment on their responses.

Although the sequence of events in an individual bee's defensive response is probably similar for all contexts, the effectiveness of different attack components varies substantially, depending on the threat. Some bees may be more likely than others to encounter a particular defensive context, leading to the possibility of specialization in defensive roles. The same physiological and chronological factors that prime bees to engage in defensive behavior also regulate other activities in the hive. Either by coincidence of location or physiological priming, individual bees have differential probabilities of encountering and managing different threats to the colony.

Defensive Culmination

Defensive behavior often culminates with an attack; most threats to colonies elicit pursuit, biting, and stinging behavior, although *Apis* species, and ecotypes within species, vary in how active a defense they use. *Apis* species can be divided into those that nest in cavities (commonly cited examples include *mellifera*, *cerana*, and *nulanensis*) and those with exposed nests (such as *andreniformis*, *florea*, and *dorsata*). Cavity nesting provides physical protection, which is achieved in the exposed-nesting species by having a "curtain" of workers covering the comb. Sting autotomy enhances the use of sting venom in honey bee defense by allowing continued venom delivery after the bee flies away, although this comes at the cost of worker mortality (45). The sting reflex is fully developed in bees older than 5–7 days (35), but the likelihood of actually using the sting shows considerable phenotypic variation (see below).

A. mellifera workers fly from the colony to chase, bite, and sting vertebrates, although AHB and EHB differ dramatically in their levels of response. *Apis florea* workers also may pursue an intruder (119). According to Seeley et al. (119), *Apis cerana* workers retreat into their nest cavity when threatened, flying out to sting only after persistent disturbance. The giant honey bee, *Apis dorsata*, is well known for aggressive stinging attacks, which are effective in deterring a wide variety of would-be predators.

Worker *Apis* sometimes employ unusual defensive tactics against other arthropods. For example, *A. mellifera* (125) and *A. cerana* (119) use blasts of air to dislodge ant intruders at nest entrances. These are produced when a worker turns to face away from the ant and fans its wings rapidly, a posture normally associated with ventilating the nest. Ants are excluded from *A. florea* nests by resin, which the bees deposit on the branches around their nests (119). *A. cerana* workers surround hornets in a tight ball and use their thermoregulatory capabilities to raise the temperature inside the ball to a lethal point for the wasp (102, 121). Balling and heating are apparently also exhibited by *Apis nuluensis* (85).

Hornets may also elicit "shimmering" and "body shaking" behavior from workers of *A. mellifera* (36), *A. florea* (105), and *A. dorsata* (80). In "shaking," bees massed at the nest entrance or curtaining exposed comb synchronously raise their abdomens, creating a ripple effect that impedes the hornet from landing (36, 119). Shaking *A. dorsata* and *A. nuluensis* expose their Nasanov glands, which suggests a function in defensive communication for the Nasanov gland in these species (80, 85).

"Shimmering" is an audible high-pitched hissing noise (perhaps piping) that is released by direct mechanical contact with the threat. Whereas alarm pheromone is the primary mode of communication and recruitment among defensive workers in *Apis*, shimmering stimulates other workers to "hiss." Shimmering serves as an alarm signal in *A. florea*; the hissing noise alerts workers to the presence of a threat and may deter small predators (120). *A. dorsata* workers also hiss when the nest is threatened (119), and in *A. nuluensis* tactile contact stimulates shimmering and visual cues cause shaking (85).

Worker *A. mellifera* imprison small hive beetles, *Aethina tumida*, by using propolis (plant resins) (51, 99). The beetles, which are easily detected by worker bees, are attacked and sometimes decapitated. In response to attack, beetles may withdraw their head and antennae, preventing physical damage, or seek to hide in crevices. If bees discover a hidden beetle, they encapsulate it in propolis; guard bees prevent its escape during the encapsulation process. Ironically, the imprisoned beetles induce their captors to feed them by behaviorally mimicking begging behavior of worker bees (51). Similarly, *A. cerana* may trap mites, *Varroa jacobsoni*, in cells using wax plugs. Generally, arthropods that are too small or durable to be stung, such as mites, may be targeted with grooming and/or biting behaviors or removal from cells, (9, 17, 21, 43, 89, 126). Wax moths *Galleria mellonella* and *Achroia grisella* are also victims of biting and dragging by bees within colonies (50). These behavioral responses, which occur inside the nest, involve the same principles of enemy identification that apply to intruders at colony entrances and may be behaviorally related to threats from outside the nest.

The highly eusocial meliponine bees lack a functional sting. This evolutionary fact greatly differentiates their defensive behavior from that of the *Apis* species. Meliponine species vary substantially in the aggressiveness with which they defend their nest (113), with many species relying on cryptic or high arboreal nest entrances as mechanisms for evading predators. Some meliponine species, such as *Trigona corvina*, have aggressive nest defense, using a combination of biting and mobbing by hundreds of workers to intimidate would-be predators. One species, *Oxytrigona mellicolor*, even exudes caustic chemicals from cephalic glands (114).

Comparisons of EHB with AHB

Few studies compare the defensive behavior of EHB with that of AHB, and the organization of defensive behavior in AHB is poorly understood. Defensive responses are more rapid in AHB than in EHB and involve a higher proportion of a colony's population. Schneider & McNally (116) studied AHB defensive responses in Africa [see (115a) for details of this study]. The available data suggest that AHB display a sequence of defensive events and organization of defensive behavior similar to that of EHB but have lower thresholds of response and have the capability to react to defensive stimuli far more intensively, faster, and in larger numbers. They may also be more efficient at recruiting nestmates. Hunt et al. (76) found that, when presented with alarm pheromone, AHB responded in greater proportions. Additionally, AHB colonies sting 4 to 10 times more frequently than EHB in a variety of stinging assays when colonies of each type receive the same stimulus (56, 57, 59, 60, 63). AHB colonies also pursue intruders with 10- to 30-fold more bees than EHB colonies (58, 106, 129).

Although many generations separate AHB and EHB lineages, genetically they are conspecific and it seems logical that the organization of their behavioral systems be similar. The extreme responsiveness of AHB to defensive stimuli may be a result of natural selection, which favored defensive bees in southern Africa, in response to the higher predation risks in that geographic area compared to those faced by

EHB. This assertion is speculative based on the current information available and remains to be confirmed in further studies (46).

Priming for Attack

Overlaid on the analysis in Figure 1 is the internal priming (physiological state) of the individual bee, which determines the likelihood that the bee will progress to the next level of response (71, 103, 111) rather than disengage. Juvenile hormone (JH) may play a role in priming workers for defense (103). The role of JH could be induction of neural responsiveness to threat-related stimuli or stimulation/disinhibition of responses by increasing neurotransmitter titers (perhaps octopamine) (65, 117). Alternatively, the effect of JH may be more causally removed. JH could have a role in pacing temporal polyethism resulting in a JH/defensiveness correlation. Some worker bees, particularly in EHB colonies, are not primed for defense and never express a defensive response, even if the nest is violently disassembled by a predator such as a bear or a human.

In colonies of the halictid *Lasioglossum zephyrum*, guard bees have the second largest ovaries; Bell (15) manipulated worker status in *L. zephyrum*, including guarding, by applying JH analogs to bees. *Bombus* workers are more similar to *Lasioglossum* than to *Apis* in that they do not undergo strong temporal changes in labor activity (101). As it does in both *Lasioglossum* and *Apis*, JH appears to play a role in caste regulation in *Bombus* (18), but studies of *Bombus* division of labor have not dealt with defensive bees per se. Meliponine workers seem to exhibit considerable flexibility in task allocation (3, 55, 86, 87) and may not have specialized defensive workers, although Breed & Page (27) observed individual *Melipona* workers persistently guarding nest entrances.

In *A. mellifera*, priming is also affected by meteorological factors; Southwick & Moritz (123) found that 92.4% of the nongenetic variation in defensiveness in EHB could be explained by variables such as air temperature. Woyke (140) found that season and time of day affect defensive responses in AHB.

Defensive Context

Defensive organization is also a function of context: the location and the identity of the threat. Context ranges from threats of small arthropods within the nest to attacks on the entire nest structure by large predators such as humans or bears. The extent of the defensive perimeter around the colony is also a contextual issue. EHB have a much smaller defensive perimeter than AHB, initiating attacks only within a few meters of the nest and pursuing intruders to approximately 50 m from the colony under most circumstances. AHB may initiate attacks on intruders one hundred or more meters from the nest and may pursue intruders for several kilometers (127). In some stingless bee species the defensive perimeter extends to floral resources; colonies maintain territories so that workers from a colony prevent non-nestmate conspecifics from sharing food resources (26).

ALARM PHEROMONE

Alarm pheromones aid bees in mounting an effective defensive response. The best-known apoid alarm pheromones are those of *A. mellifera*: This pheromonal mixture is released by bees that extrude their stings and from stings left by bees in a target. The sting in the target increases the likelihood that it will be stung again, as long as the target is moving (95). Specific behavioral effects of the sting apparatus include attraction (but see below), higher activity, and release of stinging behavior. The Koschewnikow gland and glandular areas of the sting sheath produce most honey bee alarm pheromone components (39, 54), which are then quickly volatilized on the hairs of the setaceous membrane (91). Meliponine bees also produce alarm pheromones, generally from the mandibular glands (113). Nerol is known to have alarm pheromone activity in *Trigona fulviventris* (79); this finding is of interest because of the suggestion that some *Apis* species expose their Nasanov glands when alarmed [(85); see above]. Johnson et al. (78) found that 2-nonanol and 2-heptanol are the primary alarm pheromone components of *Trigona silvestriana*.

For EHB, isopentyl acetate (isoamyl acetate, or IPA) is the principal active component of the alarm pheromone blend. This compound is responsible for the majority of sting-releasing activity. However, a large array of other volatile hydrocarbons appears in extracts of the sting apparatus (20). At least eight of these compounds cause agitation in caged bees and many cause recruitment from colony entrances, flight activity, or increased attraction to moving objects (134). Pickett et al. (104) identified a less volatile component, *cis*-11-eicosenol, as another effective alarm pheromone component for releasing stinging behavior and determined that this is the most abundant compound in stings. The amount of IPA released increases as a worker bee ages, reaching its highest level at about the time when the worker is old enough to perform guarding or foraging tasks (2). Other components also increase with age, but at different rates. For example, n-butyl acetate and 2-nonanol were not produced in significant amounts until after workers foraged. Similar results were reported for AHB (2, 136). More recently, Hunt et al. (76) found an unsaturated derivative of IPA (3-methyl-2-butenyl acetate, MBA) in stings from AHB guards in four of five AHB colonies sampled. This compound was present at up to one third the quantity of IPA in the same bees and was at least as effective as IPA for recruiting bees to emerge from colony entrances. When mixed with IPA, MBA had a synergistic effect on recruitment (76). As MBA was not previously reported, it may be specific to certain populations of AHB.

The role of 2-heptanone from worker mandibular glands in colony defense is less clear. This compound shows a much lower ability to attract bees from colony entrances and sting than does IPA (90, 132). When applied to flowers, 2-heptanone repels foragers, which suggests it might be partly responsible for the repellency of recently visited flowers, although this compound seems too volatile to adequately explain this phenomenon (132). There are doubts about the role of 2-heptanone in colony defense, but it is present on the mandibles and could serve to mark individuals that were bit by guards.

Strains of honey bees that sting more in defensive assays generally respond more quickly, and in larger numbers, to alarm pheromone, but perhaps different levels of IPA or other alarm pheromone components could account for this higher defensive behavior (44, 84). Collins et al. (42) found that AHB have higher levels for 9 of 12 alarm pheromone components compared to EHB. After controlling for age and geographic location, however, EHB actually had higher levels for two of those nine components and had twice as much IPA. In contrast, the strongest evidence that pheromone levels do not increase defensive behavior comes from mapping quantitative trait loci (QTLs, see below for a further discussion of QTLs and defensive behavior) for genes that influence pheromone levels. Seven potential QTLs were mapped in the bee genome indicating the location of genes that influenced the production of four alarm pheromone components in colonies that were derived from an AHB/EHB F1 queen. In general, these QTLs did not correspond to those that influenced colony stinging response in the same group of colonies [(72); see Genetics, below]. More defensive bees may simply be more sensitive to alarm pheromones; increased sensitivity to pheromones might be mediated by neuromodulators such as kynurenine, a serotonin precursor, or octopamine (65).

Núñez et al. (100) electrically stimulated individual restrained foragers to study the effects of IPA by measuring the stimulus threshold at which the sting was extruded. Surprisingly, in the absence of alarm pheromone, higher-response thresholds were found for AHB than for EHB. Care must be taken when interpreting results of assays using bees removed from the colony setting, and it is worth noting that results with a constant-current stimulator and unrestrained individual bees showed a lower-response threshold for AHB than for EHB for stinging a substrate (E. Guzmán-Novoa, D. Prieto-Merlos, J.L. Uribe-Rubio & G.J. Hunt, unpublished data). In the study by Núñez et al. (100), prior conditioning with IPA seemed to induce an endogenous opioid system in bees. Foragers exposed to the odor of mineral oil for 30 min, or to 25, 50, or 100 μl of IPA diluted 1:9 in mineral oil, were clamped to electrodes and subjected to varying electrical stimuli while the investigators rated sting extension. IPA reduced sting extension; this effect was reversible by application of a specific antagonist of opioids. A subsequent study indicated that a prior exposure to a lower level (3 μl) of the IPA/mineral oil mixture actually increased sting extension but a high level (125 μl) decreased the response (10). In contrast, 2-heptanone increased sting extension only when present at the high level. Mixing the two components resulted in unexpected nonadditive synergistic effects, which suggests that 2-heptanone was not simply less effective but that the two compounds have different functionalities.

It is still a mystery why honey bees have such a complex blend of compounds in the sting apparatus. In total, about 30 compounds in the sting apparatus are relatively volatile, oxygenated hydrocarbons that could potentially influence behavior (19). Some components may have different roles in different contexts or may act synergistically. Some pheromone components may have both short-term and long-term effects on behavior. AHB workers guard for longer periods when cofostered in colonies that contain a high proportion of bees with an AHB genotype (74).

Thus, one function of alarm pheromone might be to reinforce individual guarding behavior.

Wager & Breed (134), working with single alarm pheromone components and intact stings, suggest that defending bees localize large intruders mainly by visual cues. Their data support the hypothesis that alarm pheromones release searching activities at the hive entrance but are unimportant in target localization. When the bees localize an intruder, its motion and color play key roles in promoting defensive responses. This finding contradicts the often-repeated, but never previously tested, conclusion that worker bees receive orientation information from alarm pheromone (95).

BIOASSAYS OF INDIVIDUAL AND COLONY DEFENSIVENESS

Given the many behavioral expressions of defensiveness and the many contexts in which bees exhibit defensive behavior, no single bioassay adequately characterizes the defensive phenotypes of bees. The commonly used bioassays naturally divide into those that assess individual phenotype and those that yield a colony-level phenotype. Most of these assays have been developed for use with *Apis* but are probably useful, with modification, with any social insect.

Individual Phenotype Bioassays

Individual bioassays often assess nestmate recognition using a measure of the defensive response of a single bee to a non-nestmate intruder. Breed (23) reviews the techniques used in nestmate recognition bioassays. These assays follow the general model developed by Bell et al. (16) for use with *Lasioglossum* and have been applied in other halictids (94) and in meliponines (27). In *A. mellifera*, Breed et al. (25) and others (48, 49) used these bioassays to assess the efficacy of putative nestmate recognition pheromones. Less commonly, other aspects of an individual bee's responses are documented. These include time or concentration latencies of response to alarm pheromone and measures of sting use after provocation, such as an electrical shock (10, 100, 124), and metabolic expenditure after exposure to alarm pheromone or perception of a threat. Telzur & Lensky (131) used a pressure-sensitive device to measure abdominal movements, reflecting attempts to sting, after presenting a stimulus to the bee.

Group or Colony Phenotype Bioassays

Assessments of colony-level defensive phenotypes employ either a quantified physical stimulus, such as a weight that strikes the nest, or a quantified chemical stimulus, usually alarm pheromone, which is placed at the nest entrance (see Reference 127 for reviews of these assays). The result is a count of the number of bees responding at the colony entrance or stinging a leather target suspended

in front of the nest. Guzmán-Novoa et al. (63) replicated bioassay trials using this technique and an automated electronic technique developed by Spangler & Sprenkle (124); both assay types yielded highly variable results, with the stinging assay being somewhat more reliable than the automated assay. Guzmán-Novoa et al. (63) conclude that although current bioassays are reliable enough to discriminate gentle from highly aggressive colonies, better bioassays must be developed to assess intermediate defensive phenotypes. In a comparison of four assay methods, Guzmán-Novoa et al. (64) found that the most reliable assay was the "ratings" method, in which two investigators disturb colonies by opening, using a minimal amount of smoke, and removing frames for inspection. The tendencies of workers to run on the comb, to fly off the comb, to fly against the investigators' veils, and to sting the investigator's hands are threatened. Counts of stings in targets, responsiveness to alarm pheromone, and stinging behavior by bees confined in an observation arena ("box") were less repeatable when applied more than once to a set of colonies. Some studies have compared metabolic expenditure of a group of bees prior to perception of a threat of non-nestmate bee odor or of alarm pheromone with metabolic expenditure after perception; this removes bees from the field context but gives a precise quantitative measure (4, 5, 97). Group measures may be subject to nonlinear effects owing to interactions among workers (95).

ENEMY IDENTIFICATION

Enemy identification serves an important role in the self-defense of all organisms. Generally, self-defense must first involve discriminating self from nonself; cellular recognition systems and MHC-mediated immune systems are examples of mechanisms of enemy identification that function against internal parasites (103a). In social animals, such as the honey bee, discrimination of self from nonself extends to a group identity; workers must discriminate group members from nonmembers. An animal may identify external enemies using genetically encoded information, such as species-specific odors or visual patterns, or learned information from previous encounters with parasites or predators. Enemy identification is the filter that sorts genuine threats from irrelevant or incidental contact with other species.

Genetic information about enemies may be quite specific. Sweat bees (*L. zephyrum*) respond with vigorous defensive behavior to the odor of a mutillid parasite, *Pseudomethoca frigida*, without prior exposure to the odor and without the opportunity to learn to associate the odor with subsequent parasitism (12). In *Tetragonisca angustula* colonies (small, widespread Neotropical bees), guards hover near the colony entrance and direct their defensive activity to potential robbers, in particular foragers of *Lestromellita limao*, an obligate robbing species that has a distinctive lemony odor owing to its production of citral. While Wittmann et al. (138, 139) argued that *T. angustula* guards use this odor as a cue for attack. Bowden et al. (22) found that visual cues (size and color) were the primary stimulants of attack by guards. One or more guards attach to the wings, legs, and antennae

of incoming dark-colored flying insects, causing them to fall to the ground. Guards normally do not release their hold, and neither the attacker nor the guards often survive. This prevents recruitment of robbers to the nest. Whether the primary cues are visual or olfactory, the behavior of *T. angustula* guards, hovering outside the nest entrance, is a unique defensive adaptation. Kelber & Zeil (82) used this system as a model for studying how guards maintain their spatial positioning.

Genetic information may also encode general parameters that set a framework for learning. Learned enemy identification is used in nestmate recognition. Although the inclination to identify nestmates is genetically encoded, the specific identifiers are learned. The ability to discriminate nestmates from non-nestmates is nearly universal in both primitively and highly eusocial bees. This fact suggests that the need for defense against conspecifics or closely related species is a common selective pressure that shapes colony defense in many bee species. This is true, as well, in the eusocial insects in general (24); nestmate recognition is one of the characteristic attributes of eusocial colonies.

Self versus nonself discrimination among honey bee colonies is well understood (25, 32). Workers in each colony learn a colony-specific mixture of chemical compounds that is carried on the external surfaces of all workers. In *A. mellifera* these identification compounds are probably a mixture of fatty acids and alkenes; the mixture is homogenized among bees in the colony by contact with the comb (which is a source of compounds for the surfaces of the bees) and by contact with each other (25). Guards then use colony-specific labels to make discriminations between nestmates and non-nestmates, excluding them from the nest by biting and stinging intruders. However, other task groups, such as nurses and foragers, also may make such discriminations (31). Guards respond defensively to other arthropod species, such as spiders, ants, and wasps, at colony entrances; little is known whether these discriminations are made on the basis of visual or olfactory cues. Nestmate recognition functions in a manner similar to that in *L. zephyrum* (16) and in at least some meliponine species (27). The simplest discriminatory model would be for guards to attack all intruders that do not present the olfactory profile of the colony (24).

In honey bees, exclusion of non-nestmate conspecifics after olfactory discrimination is usually a defense against robbing of food stores from the nest. The strength of the defensive response varies with the magnitude of the threat; colonies have more guards and are more likely to engage in defensive responses when robbing among colonies is high. Workers of *A. mellifera capensis* parasitize colonies of *A. mellifera scutellata*; guards exclude these non-nestmate conspecifics, but not efficiently enough to prevent parasitism (14).

Drones illustrate an interesting exception to the general rule of exclusion of non-nestmate arthropods, as they are, apparently, generically acceptable in honey bee colonies (although they may be excluded from colonies late in the summer season in the temperate zone). This interchangeability of drones could be incidental or could be the result of selection for a mild form of parasitism in which drones obtain nutrition from unrelated workers and perhaps a mating advantage (108).

In many eusocial insects, unaffiliated queens pose risks as potential social parasites; for example, inquilinism is well known in ants (70). Sometimes chemical cues, which allow olfactory identification of nestmates, are circumvented by inquilines by chemical mimicry (77), whereas in other cases the parasite remains chemically distinct from the host (122). Potentially parasitic queens usually attempt to enter a colony, even though attacked by resident workers. In some cases selection favors tolerance of the parasite rather than fights to the death.

Among bees the most commonly cited example of social parasitism is *Psithyrus* queens parasitizing bumble bee, *Bombus*, colonies (52). *Psithyrus* queens sometimes are attacked by resident *Bombus* workers, but at other times they coexist peacefully in the same nest with *Bombus*. Even in the *Bombus-Psithyrus* relationship, in which the invasion of a *Bombus* nest by a parasitic *Psithyrus* queen can be disastrous for the *Bombus* queen, there is no evidence for specialized enemy identification; indeed, the *Psithyrus* queen may mimic *Bombus* social signals so well that in some cases she is met with no hostility (88). A lack of aggressive defense may allow both species to produce males and gynes in the same nest; this result may be better, for the bumble bee, than risking death in defense of the nest. Cartar & Dill (38) reported that workers of *Bombus occidentalis* and *B. melanopygus* from energy-rich colonies were likely to attack *Psithyrus insularis* intruders, whereas workers in energy-poor colonies merely threatened but did not attack intruders.

Another social parasite, *Sphecodes*, a genus of halictid bees that invades social and solitary halictid host colonies, does not chemically mimic its hosts; the hosts are simply killed by the parasite (122). *Paralictus*, another halictid parasitic genus, is excluded from colonies of *Lasioglossum imitatum* on the basis of olfactory information. Sometimes the parasite is able to circumvent the guard bee's defense and enter the colony, and once inside the colony it is less likely to be a target for defensive behavior (135).

Social parasitism could occur in honey bees; colonies produce queens in excess of the number required to replace queens lost in swarms or from old age. These excess queens might be parasitic threats to other colonies, but the efficacy of queen recognition in honey bees may have prevented the evolution of this mode of parasitism, as such parasitism is unknown in honey bees. Worker *A. mellifera* recognize their colony's queen by her olfactory signature, and EHB queens are interchangeable among colonies only if they are genetically similar (31).

AHB bee swarms sometimes overpower EHB guard bees, resulting in a takeover of the nest by a new swarm; this could be viewed as a form of social parasitism in which the conquering colony replaces the resident queen with their own queen. In this case the olfactory discriminatory mechanisms function, but the defensive capability of the targeted colony is inadequate to prevent the attack.

Honey bee guards do not respond defensively to dead or immobilized workers from other colonies, suggesting that visual (movement) or tactile cues are also important in enemy identification of non-nestmate bees. This lack of response to nonmoving intruders extends to dead arthropods of other species. In contrast,

L. imitatum workers respond to the odors of dead or immobilized workers or parasites. Like EHB, AHB respond to movement, dark colors, vibration, and alarm pheromone. When a visual stimulus (moving black leather object) is presented in front of colony entrances or when hives are disturbed by the impact of a projectile, AHB respond by stinging 10 to 20 more frequently than EHB (56, 57, 59, 61, 62).

Mammalian and avian predators, such as skunks, bears, armadillos, badgers, raccoons, honey buzzards, and bee eaters, seem to be identified by vibration, motion, and color. Breath from a vertebrate also elicits a response, although no tests have yet been performed to determine if this effect is due only to a mechanical (wind) effect or if bees respond to carbon dioxide or other chemicals in the breath (46). Wager & Breed (134) found that a moving target without alarm pheromone attracted more bees than a stationary source of alarm pheromone; beekeepers find that slow and deliberate movements, when manipulating a hive, are less likely to elicit defensive responses. AHB respond more to motion and color than to alarm pheromone. Bees follow a moving mammalian threat for considerable distances after a colony is disturbed. Dark coloration in combination with motion or vibration is attractive to defensive worker bees; AHB workers may be attracted by this combination of stimuli to animals or humans over 100 meters from their colony. EHB workers enforce a much smaller defensive perimeter (127).

A. dorsata workers seem to identify their major predators, such as the blue-bearded bee eater, *Nyctornis athertoni*, by motion and color (81). Hundreds or even thousands of "guards" leave the nest and pursue the threat. *A. dorsata* colonies live in aggregations, and in some cases other colonies join the attack, presumably in response to stimuli from the first colony (81). Pursuit of a threat by *A. dorsata* may extend for more than one kilometer.

Anecdotal accounts suggest that honey bees learn to identify the source of repeated threats, such as a beekeeper who disturbs a colony on a daily basis. Some casual reports suggest that a beekeeper who repeatedly visits a colony elicits a response different from that of another person visiting the same colony. Beekeepers attribute this to the odor, color, and shape of their clothing. If this observation were supported by experimental evidence, this would be another example of learned enemy identification.

DIVISION OF LABOR

Division of labor is the hallmark of eusociality and is responsible for the ecological success of eusocial insects. Defensive behavior is part of the overall division of labor in bee colonies. Nest defense is an important problem for solitary bees and the possibility of improved defense may be a key factor in selection for group-living in bees.

Most of what is known about division of labor in *Apis* colony defense derives from studies of EHB; even less is known about the organization of defensive responses in AHB colonies. Division of labor in honey bee and stingless bee colonies

(133) is age related, so the two primary foci of these studies have been identification of defensive activities and placement of those activities in the context of worker age. There are at least three distinct tasks associated with the defensive behavior of honey bees: guarding, pursuing, and stinging. Breed et al. (28) proposed that guards, stingers, and foragers are distinct genotypic groups of workers within a colony and therefore called the bees that respond to massive disturbances "soldiers." Breed et al. (28) thought that guards evolved primarily to deter robbing from conspecifics and probably other invertebrates, whereas soldiers evolved as a response to vertebrate predation. Numerous studies have demonstrated that guarding is a specialized task of the defensive behavior of bee colonies and is performed by few, genetically predisposed individuals (29, 31, 74, 109). However, it is not yet clear whether individuals that pursue and sting (soldiers) are a different group of specialized individuals within the colony.

Guarding

Abrams & Eickwort (1) found an advantage for defense from communality in the halictid *Agapostemon virescens*. Communal nests of the megachilid *Microthurge corumbae* are less subject to parasitism and predation owing to enhanced colony defense (53). For incipiently eusocial bees, such as the allodapine *Exoneura robusta* (also cited as *E. bicolor*), in which females may nest singly or in primitively social groups, the main benefit of cooperative nesting may be derived from more continuous guarding of the nest from possible predation or parasitism (33, 34). In this species the reproductively dominant female guards the nest entrance and uses her position to protect both the brood and her reproductive status (67). In contrast, there are two types of guards in *Xylocopa pubescens* (66, 68, 69). Young guards are "hopeful" reproductives; they may be waiting for an opportunity to challenge the queen in the nest, who also does all the foraging. Old guards are past their reproductive prime and may be guarding their own reproductive output, even though another bee has taken over reproduction.

In *Lasioglossum*, a halictid genus that contains both solitary and primitively eusocial species, nests are usually constructed as tunnels in the soil (11). The entrance is circular and barely large enough to admit one bee at a time. In these small (up to 25 or so worker) colonies the bees do not show evidence of temporal polyethism; instead, an intracolonial dominance hierarchy seems to be responsible for task determination. One bee with enlarged ovaries assumes most of the guarding duties; she obstructs the entrance to the nest, preventing non-nestmate bees and nest parasites from entering. This bee is the target of most of the queen's dominant behavior and may be one of the older workers in the colony (16). If the guard is removed, another bee assumes her role. Parallels between *Lasioglossum* guards and *Apis* guards include the manner in which the mandibles and sting are used in defense and in the ability of the *Lasioglossum* guards to identify non-nestmate threats to the colony. Studies of division of labor in bumble bees and stingless bees typically do not include guarding or other defensive roles in their ethograms

(133). This lack is probably due to the fact that defensive displays are highly context specific, requiring the presence of a threat, and because defense may involve only a small proportion of a colony's workforce.

In *Apis*, guard bees patrol the entrance of the colony as well as the periphery of the nest in open colonies, inspecting incoming bees and excluding individuals that do not belong to their nest; they may also alert other colony workers about intruders (23). The main purpose of guarding is to identify and remove foreign conspecific intruders (23, 31), but guards may also play a role in recruiting other bees to defend against larger intruders. Moore et al. (96) reported that some guard bees constantly remain at the colony entrance, whereas others fly and sting vertebrates near the colony. Guards that remain at the nest entrance may alert the colony by releasing alarm pheromones, whereas guards that fly out may orient the bees of the colony toward a vertebrate intruder. Few guards fly out in EHB colonies, as this is much more common in AHB colonies. Guzmán-Novoa et al. (58) found that, in cofostered colonies, guard bees of AHB origin were seven times more likely to fly out and sting than EHB guards.

A small proportion of a colony's worker population serves as guards; only about 10% of the workers perform guarding activities (74, 96). These are usually middle-aged workers (13 to 16 days old). Workers become guards after performing duties inside the nest but before performing foraging tasks (96). EHB workers guard briefly, for one to three days, in the majority of cases. Some EHB may guard for as long as six days (96), and an AHB guard may persist as long as 21 days (74).

AHB are not more likely to guard than EHB but are more persistent as guards. Comparisons between individuals in large colonies from planned matings showed that AHB guarded much longer than EHB. Ten percent of individual AHB guards were still guarding after six days, but only 1 of 250 EHB did so (74). In cofostered colonies composed of different sources of EHB and AHB, the time that bees spent as guards was about 50% higher for AHB. On average, AHB guarded for 4.7 days and EHB for 3.0 days (74). Additionally, Hunt et al. (74) found significant genotype by social environment interactions; AHB were more persistent in colonies with more AHB than EHB, but EHB were not responsive to changes in genotypic constitution of the colony (58, 74).

Increased movement of guards often precedes a defensive response near the colony entrance. Guard bees often extrude their sting while standing in the nest entrance, attracting other bees, which may exhibit guarding behavior, or fly out to sting. The number of guards in one study was correlative with the number of stings in a colony defensive assay, and prior removal of guards decreased the number of stings in assays conducted 4 h later (7).

Pursuing and Stinging

Nonguard defenders (soldiers) comprise another group of bees involved in colony defense (23, 45). Soldiers pursue and sting and may be differentiated from both guards and foragers of the same age by having less wing wear. However, the

evidence so far is insufficient to conclude that pursuers and stingers are a separate group of specialized defenders. Breed (23) estimated that about 10% of an EHB colony population might be involved in a maximal defensive response. This low percentage of bees involved in defense could allow for soldier specialization. However, in AHB colonies at least 50% or more of the colony population may sting an intruder under certain circumstances (47, 118). This high proportion of stingers suggests no soldier specialization, but rather that when necessary, most bees of flying age may defend the colony, at least for highly defensive genotypes of bees. Additionally, in defensive trials, foragers of AHB (recognized by their pollen loads) may also sting (106). Therefore, the term "soldiers" is appropriate to designate bees that attack (pursue or sting), although not in the sense of task specialization, at least in AHB.

Contrary to popular belief, worker *A. mellifera* that have stung may not die immediately; instead they sometimes continue pursuit and fly around the intruder's face (45). Most pursuers do not sting but harass the intruder to intimidate it, which effectively deters most vertebrate predators. Cunard & Breed (45) found that in EHB 25% of the pursuers ended by stinging, and once they had stung, many continued pursuing and harassing the intruders after they had lost their sting. This behavior constitutes a residual value for defense, because after a bee loses its sting, she dies within a few hours or days.

GENETICS

Honey bee defensive responses are modulated by environmental conditions such as high humidity, heat, and nectar availability (62, 123). A large portion of the defensive phenotype can be attributed to genetic factors; many investigators have calculated high heritabilities for some defensive behavior traits [reviewed in (41, 129)]. Genetic influences on defensive behavior are evident from frequent observations that certain lines and races of bees are more "aggressive" than other strains (115). The highly defensive behavior of AHB in the Americas represents an extreme example of strain differences. In most cases, observation of defensive behavior relies on colony-level traits such as the number of stings in a target, numbers of pursuers, or number of bees that emerge from the colony entrance in response to alarm pheromone.

Classical Genetics

Genetic effects are also known for individual behaviors related to colony defense. Guards in one study were a nonrandom sample of subfamilies, indicating genetic effects on guarding behavior (109). Studies have analyzed whether certain traits related to defensive behavior show dominant or additive patterns of inheritance by crossing defensive and gentle stocks of bees to obtain hybrids (F1) and, in some cases, backcrosses. Genetic dominance for a higher stinging response not only may make it more difficult to breed gentle bees, but also may confer a selective

advantage to the bees in areas with high predation, as dominance results in more highly defensive colonies in the population.

Tests of different EHB stocks and between EHB and AHB in Latin America have yielded mixed results, sometimes indicating additivity and sometimes dominance [reviewed in (41, 129)]. Recent studies not far from the northern edge of AHB expansion indicate dominance of the AHB phenotype for number of stings in colony-level assays (46, 59, 60). A larger study involving 70 colonies with either F1 or backcross workers also showed dominance for greater number of stings in assays in Mexico. The degree of dominance for number of stings was estimated at 84.3, 200.8, and 145.8% for hybrid, backcross EHB, and backcross AHB colonies, respectively (57). These estimates are based on the assumption that interactions between individuals in the colony are additive, but in fact AHB and EHB have an altered likelihood of stinging if they are cofostered (60). In addition, high-defensive genotypes appear to recruit low-defensive genotypes to sting (58).

EHB and AHB are separable using multivariate morphometrics (115). Wing size, a tool that distinguishes EHB from AHB, is phenotypically but not genetically correlated with stinging response (40, 56, 73, 107). Although the traits for body size and defensiveness are not genetically linked, selection for larger wings in AHB areas has proven useful as one tool in a successful breeding program for decreasing stinging responses. Choosing larger bees indirectly selects for an overall reduction in the proportion of AHB alleles in the genome (62).

QTL Analyses

The development of DNA markers amplified in polymerase chain reactions provided an opportunity to make a genetic map of the bee genome (75) and to map genes that influence defensive behavior. Five putative quantitative trait loci (QTLs) that influence honey bee defensive behavior were mapped on the basis of colony-level assays (73). To accomplish this, drones of an F1 queen (an AHB/EHB hybrid) were each used in single-drone inseminations of super-sister queens from the EHB parent. (In Hymenoptera, which have a haplodiploid sex-determining mechanism, super-sisters have the same mother and father and share an average of 75% of their alleles by direct descent.) In this mating scheme, most of the genetic variance between colonies derived from the genotype of the drone fathers of the colonies and were segregated for AHB- and EHB-derived alleles. The resulting genetic map was based on segregation of marker alleles in the haploid drone fathers of experimental colonies, and the number of stings in colony assays was related to the inheritance of specific marker alleles from the AHB parent of the F1 queen. Only one of the five QTLs (*sting-1*) was actually significant at $p < 0.05$ after controlling for the experiment-wise error rate by taking into account the 350+ markers involved in this test for effects on the stinging phenotype. In general, AHB-derived marker alleles at the QTLs were associated with higher number of stings. Although most of the QTLs acted independently, *sting-4* and *sting-1* behaved epistatically (Figure 2). A large effect of the colony's paternal genotype at *sting-1* on the number of stings occurred only if the drone father also had the AHB-derived allele at *sting-4*.

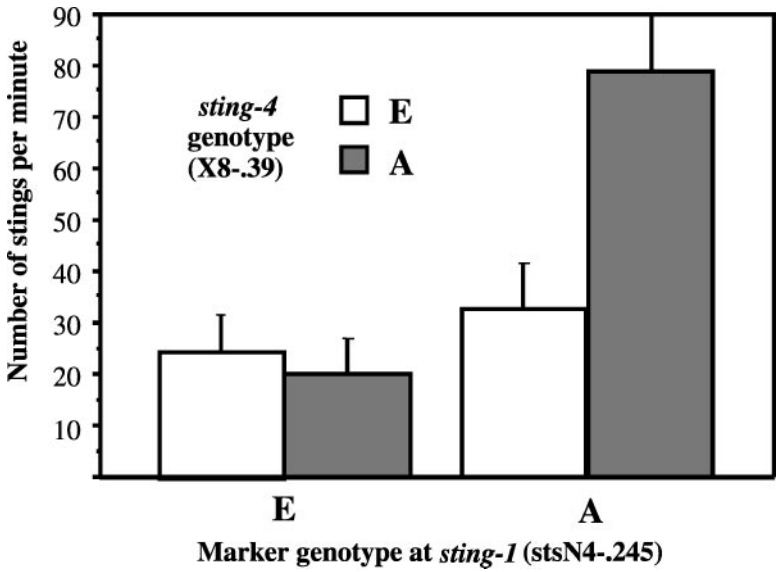

Figure 2 Epistasis between *sting-4* and *sting-1*. Colonies registered high numbers of stings in the assay only if their drone fathers had the AHB parental allele for both *sting-1* and *sting-4* as determined by nearby marker genotypes—from unpublished data from 162 colonies (73). The *x* axis shows the marker genotype at *sting-1*; *sting-4* genotype is indicated by the color of the bars.

Ratings of the same colonies based on practical beekeeper observations showed that markers linked to these QTLs also correlated with traits such as tendency to fly up off the combs during colony manipulation and tendency to sting the beekeeper's hands. For example, the marker allele inherited from the AHB parent of the F1 queen linked to *sting-2* was associated with tendencies to fly up and sting (Kruskal-Wallis tests; $p < 0.0001$ and $p < 0.005$, respectively).

Identifying QTLs opens up the possibility of determining how specific genes influence individual behavior. If an F1 queen is mated to a single EHB drone, the behaviors of her progeny can be related to whether they inherited an AHB or EHB marker allele linked to specific QTLs. In two sets of crosses the marker genotypes near *sting-1* were associated with the task of guarding or being among the first to sting, thus confirming the effects of *sting-1* on defensive behavior (57). Similar studies with highly defensive EHB showed that samples of backcross bees that guarded for at least two days were biased toward the defensive-parental allele for markers near *sting-1*, *sting-2*, and *sting-3*, suggesting that the same genes influence defensive behavior in EHB stocks (8).

Some random DNA markers exhibit large frequency differences between AHB and EHB populations (93, 130). However, at this time, markers cannot be used as diagnostic tools to determine if honey bees carry undesirable alleles for genes

influencing defensive behavior. Before this is possible, we need to have more precise determination of the gene location and to obtain markers that tightly flank the gene (or are within the gene itself). Otherwise, random crossover events and the presence of multiple alleles will confound the analyses. Sequencing of the honey bee genome in 2003 may make it possible to identify some of the genes that natural selection has acted upon to create different defensive behavior phenotypes.

Interactions Among Components of Defensive Behavior

Many components of honey bee defensive behavior are correlated, suggesting that common genetic and behavioral mechanisms underlie, influence, and regulate distinct features and the intensity of colony defense (56). Attributes of guarding correlate well with other defensive measures. Breed and colleagues (29, 30) found that the number of days that each individual worker guards (persistence) and colony response to alarm pheromone are highly correlated ($r = 0.88$). When investigators controlled colony environmental effects by cofostering bees in the same hive, the correlation between individual guarding persistence and the alarm response of the source colonies remained (29). This correlation also extends to behavioral differences between AHB and EHB, between which the comparisons of both guarding persistence and colony defensive responses are much more extreme. AHB are more persistent in colonies containing high proportions of bees of AHB genotype, but EHB are not responsive to reinforcement of guarding behavior (74). Guzmán-Novoa et al. (58) found a correlation between individual guarding and defensive components such as response to alarm pheromone, pursuing, and stinging. In another recent study, Arechavaleta-Velasco & Hunt (7) found that colonies with more guarding activity stung more in defensive trials.

With respect to stinging, in EHB/AHB colonies constructed by cofostering the two genotypes, 81% of the bees that stung during the first 10 sec of stinging tests were AHB, but from 10 to 30 sec, AHB and EHB were equally likely to sting. When tested in their own colonies, two of the three source EHB genotypes did not sting and did not pursue in any of the eight trials conducted, whereas all three AHB genotypes did. These results suggest behavioral interactions among bees of differing genotype; the more defensive type of bees may affect the response thresholds of less defensive bee genotypes, recruiting them to sting (58). A previous study had already shown genotypic effects in propensity to sting between cofostered EHB and hybrid (AHB/EHB) honey bees. Cofostered EHB were more likely to sting in colonies containing hybrids than in their natal nest (60).

A response-threshold model in which bees vary in their responsiveness to stimuli can be invoked to explain these interactions among genotypes (110). In this model, response variation is at least partially due to genetic differences; some workers are more likely than others to respond to low stimuli. For example, Guzmán-Novoa et al. (58) showed that the most defensive bees initiate the defensive attack when cofostered in mixed-genotype colonies. More sensitive bees might react to stimuli (odors, movement, and vibration) at lower levels than less defensive bees and may

also be more able to produce alarm pheromone in higher quantities to recruit other (less sensitive) bees to attack. In some contexts, genotypes with a greater tendency to engage in defensive responses may inhibit other workers from responding (as in guarding the nest entrance), and in other defensive contexts (as in mass stinging of a vertebrate) the rapid and intense response of bees of one genotype may recruit bees of the other genotype.

CONCLUSIONS

What factors unify bees in their colony defense mechanisms? Their main weapons (biting and stinging) certainly drive some level of commonality of defensive reaction among bees as diverse as megachilids and *Apis*. The use of olfactory nestmate recognition as a way of identifying threats is also nearly universal among bees. Defensive behavior is part of the scheme of division of labor in bee colonies; this is the mechanism by which the appropriate amount of labor is allocated to defense. However, so little is known about analogies and homologies in division of labor across the diversity of social bees that we cannot draw generalizations about how evolution has driven the placement of defensive labor in bees' work schemes.

An extremely important feature that divides most of the primitively eusocial bees from the highly eusocial bees is the storage of food materials in the nest. The halictids and allodapines face relatively more pressure from social parasites and less from robbers simply because there is little in their nests to steal. A few *Bombus* spp., most meliponines, and all *Apis* spp. store food resources or have sufficient brood in their nests to attract a broad range of thieves and predators; this broadens the range of possible threats to include vertebrates, and consequently the defensive mechanisms of a few *Bombus* spp., some meliponines, and all *Apis* spp. are inclined to discourage relatively (compared to the bees) large animals.

Not surprisingly, defensive responses of bees are as diverse as the threats they face. Many of the most interesting defensive adaptations, such as prisons for small hive beetles, are cleverly threat specific. An anecdotal approach is tempting, in which the "best" adaptations are cataloged without regard to developing an evolutionary understanding of the potentialities and limitations that affect colony defense in bees. Even when we narrow our consideration to the honey bees, defensive behavior is a complex trait. The intensity of the defensive reaction (guarding, pursuing, or stinging) depends on many factors. It is influenced by genes and by numerous environmental effects and interactions between individuals and between environmental and genetic effects. Appropriate dissection of defensive behavior should include measurement of the main components of a defensive response under different environmental conditions and with different genotypes of honey bees. Ultimately, bee defensive behavior will be best understood in the contexts that structure our review: division of labor, communication, enemy identification, and genetics.

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