CHAPTER III

SOCIAL DEFENSE OF THE DWARF HONEYBEE (*Apis florea*) RELEASED BY THE WEAVER ANT (*Oecophylla smaragdina*)

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Summary

The dwarf honeybee, *Apis florea* is widespread throughout Asia, where it builds its open single comb in bushes, shrubs and trees. In this arboreal habitat, one of the dominant insectivorous predators is weaver ant, *Oecophylla smaragdina* and the main mechanism of *A. florea* to protect its nest against ants and other crawling arthropods are "barriers" of sticky material (sticky bands) which the bees build around the branches and all structures which connect the comb to the outside. We studied whether the presentation of an *O. smaragdina* ant on the comb releases a specific behavioral response of the bees. After the exposure of a living *O. smaragdina* worker, held by a forceps on the top of the *Apis florea* comb, the number of bees at the sticky band zone increased and remained on higher level for 2 hours compared to control experiments (presentation of an empty forceps, *Tenebrio molitor* larva or another arboreal ant species, *Crematogaster rogenhoferi*). Further, more sticky material was deposited by the bees after exposure of weaver ant. This behavior seems to be a specific reaction of *A. florea* to its most important predator *O. smaragdina*.

3.1 Introduction

Apis florea is widespread throughout Asia, where it builds its single comb around small branches of bushes and shrubs. The exposed position of the comb with the honey storage, bees and brood attracts a wide range of different intruding and predatory species and the colony must be prepared to defend at any point of the surface of the nest. So it is not surprising that *A. florea* posses a wide array of specific social defence mechanisms among which shimmering (Butler, 1954) and hissing are most conspicuous. Shimmering behaviour is released by optical stimuli and for example a butterfly - apparently attracted by the odour of honey - releases "shimmering behaviour" (a specific movement) of a few bees hanging in the curtain and otherwise does not interfere with colony activities. Hissing behaviour is released by mechanical stimuli or by "piping" of forager bees disturbed on their return to the nest (Sen Sarma *et. al.*, 2002). Hissing alerts the whole colony and results in steep decrease of foraging activities. The hissing sound per se may repel mammals and even large Asian bears (Koeniger and Fuchs, 1973) or initiate a full stinging defence of the colony (Koeniger and Fuchs, 1975).

A different permanent threat in the arboreal habitat of *A. florea* is posed by the weaver ant *O. smaragdina,* which ranks among the most important predators of *A. florea* (Seeley, 1983). In direct fighting, the *A. florea* workers, because of their diminutive size, are no match for the large weaver ants. Colonies rely instead on sticky bands of plant resins plastered around the branch carrying the nest (fig. 3.1). Thus the comb with the brood, the honey storage and the bees is sealed off completely by sticky barriers on all structures connecting to other parts of the canopy. In a survey in northern Thailand Seeley and Seeley (1982) examined 76 *A. florea* colonies. Only 28 of them had sticky bands, but 24 of those were under ant attack. This indicated a strong correlation between sticky bands and the presence of ants. Experimental data on the question how the presence of *O. smaragdina* ants is related to *Apis florea*'s construction of sticky bands are not yet available.

In our experiments we provoked *A. florea* colonies by exposing a weaver ant on the top of the colony and observed the reaction in regard to the sticky band. In comparison to the weaver ant the reaction after presentation of an empty forceps, a

Tenebrio molitor larva and another arboreal ant species (*Crematogaster rogenhoferi*) was recorded. Our aim was to demonstrate whether the presence of a weaver ant inside the barriers of the sticky bands causes an immediate and specific social response of *A. florea* colony.

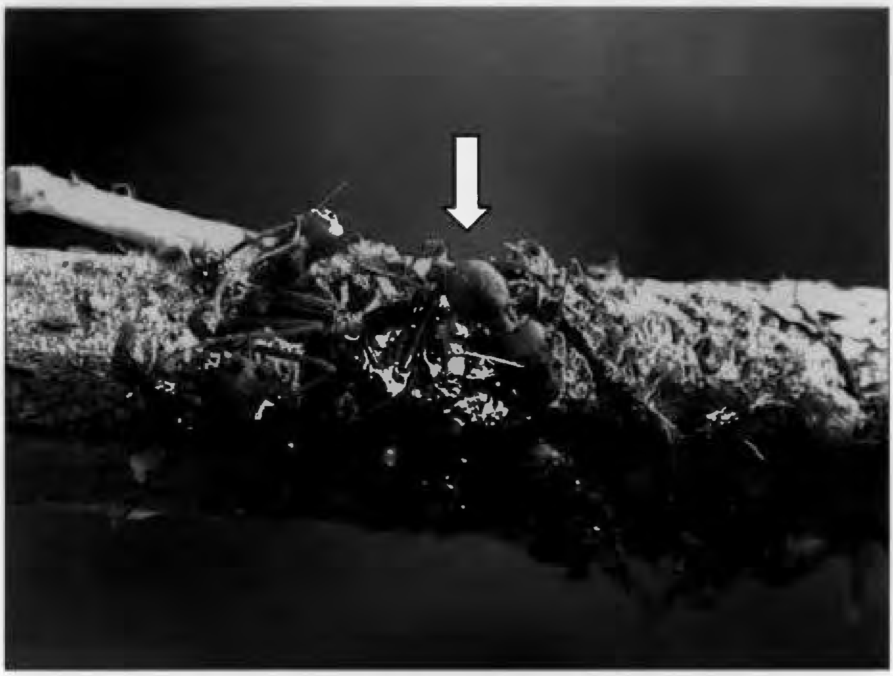

Figure 3.1 The weaver ant worker stuck on the sticky band of *A. florea*. Photo by O. Duangphakdee.

3.2 Materials and methods

3.2.1. The bees

Observation and experiments were conducted at the campus of Chulalongkorn University (Bangkok) in March to August, 2003 and in January to April 2004. Eighteen colonies of *A. florea* were collected in a coconut estate near Maeklong, Samut Songkram Province. The *A. florea* nests were detected in height of 3m to 5m. Climbing up smoothly during day time the colony was gently smoked and afterwards sprayed with water. Surrounding leaves and twigs were carefully removed. Finally both sides of the nest branch were smoothly removed by sharp garden cutter. The colony was hung at one end of the nest branch and was brought down avoiding accelerations and jerks. Both ends of the nesting branch were tightly fixed in a wooden box for transportation to Bangkok. There, the colonies were hung on small trees in front of entomology laboratory (in CU campus) maintaining the vertical position of the comb. The bees started foraging pollen and nectar at the new location. Additionally, we offered 50 g candy (icing sugar mixed with honey) daily on the top of the comb.

3.2.2.The ants

O. smaragdina was also collected at Maeklong. We selected a fairly large (40 cm x 30 cm) leave nest in a mango tree and carefully clipped the surrounding twigs leaving only the branch with the nest. After the ants had calmed down and the main force of them had returned into the nest again (15 min) we cut the nesting branch and swiftly put the nest into a transport box which was closed immediately. In front of entomology laboratory we fixed the *O. smaragdina* nest to a small potted mango tree. The pot of this tree was kept in container filled with detergent water to prevent ants from escaping. Within 2 days the ants had built a new nest and had transferred their larvae into it. The old abandoned nest was removed. The ants were fed with 10 g. of *T. molitor* (Coleoptera: Tenebrionidae) larvae, 5 g. of tinned mackerel fish and 10 ml of honey syrup per day.

3.2.3 Presentation of different objects to the *Apis florea* colony

An *O. smaragdina* forager was collected from the feeding dish of the ant nest. A *C. rogenhoferi* worker was caught from a foraging path at a nearby tree. We held the ant by a forceps at its petiolus and put it for 1 minute at the center of the top of the *A. florea* comb (fig 3.2). Further, a *T. molitor* larva of about 1.5 cm length was presented to the colony at a similar position also for 1 min. As control, we took an empty forceps and left it also for 1 minute on the *florea* comb.

Figure 3.2 The weaver ant worker was held by the forceps and presented on the top of *A. florea*'s colony. Photo by O. Duangphakdee.

3.2.4 Number of bees in sticky band zone

As parameter for the colony reaction we counted the bees present in the sticky band zone, which was the surface of the nest branch extending from where the branch protruded from the comb to a distance of 4 cm. We counted the number of bees each colony for 5 minutes before the presentation of the ant, directly after (within 2 minutes), 1 hour after and 2 hours later. Each count was 5 times repeated at each of the sticky bands. We took the mean of the counts for further analysis. Our observation position was 50 cm away and was maintained constant for every colony.

3.2.5 Deposition of material in sticky band zone

A double layer of plastic bands (Prihimo, made in Japan) of known weight were fixed in the sticky zone (fig. 3.3). After 2 hours they were carefully removed. Because of the inner layer's contamination by some sticky materials that was present before the experiment, only the outer layers were used to calculate the weight of deposited materials from increase in the weight of the band, determined by an 0.001 mg accuracy balance (Sartorius CP2245).

Figure 3.3 The double layers of plastic band were fixed on the sticky band zone. Photo by O. Duangphakdee.

3.3 Results

3.3.1. Behavior of bees towards different objects presented on the upper side of the comb

The reaction of the bees towards the *O. smaragdina* worker held in the forceps happened without delay. The bees shied away from the ant and formed– head towards the ant - a multi layered wall around it,. The distance to the ant was 4-7 cm. At the same time, many bees from the comb rushed on the upper side joining the group of defending workers. After half a minute some worker bees launched counter attacks against the ant. The attacking bee seized the antenna or a leg of the ant by mandibles and tried to fly off with it. Since the ant was held by the forceps the bee did not succeed carrying the ant away. In some cases at the end of the 1 min exposure period more bees joined the attack and stinging behavior occurred. Regularly, when we removed the ant, a few bees were attached by their mandibles to the ant so tightly that they were removed together with the ant from the comb. At the end, after we had released the ant from the forceps at the ant arena the bees let the ant go and flew back to the colony which was about 2 m away.

The reaction of the bees to a worker of *C. rogenhoferi* or a larva of *T. molitor* were limited to the group of bees which came into direct contact or which happened to be within a narrow radius. The bees turned the head toward the ant or mealworm immediately. Shortly later, 3 to 5 seconds after presentation, a few bees attacked by biting and stinging. In comparison to the behavior released by a weaver ant, the reaction was different. While at least 20-50 bees responded to *O. smaragdina* but not more than 5 - 15 bees attacked *C. rogenhoferi* or *Tenebrio* larva. In addition, we did not notice bees from the comb rushing up and joining the defense. In summary, the reaction of the *A. florea* colony to presentation of *C. rogenhoferi* or *Tenebrio* remained locally restricted while the response released by an *O. smaragdina* worker spread over the whole colony or at least a large part of it.

An "empty" forceps did not cause increased the activity level on the comb. The bees moved towards to forceps and started "head-pushing" behavior (Sen Sarma *et al.* 2000). In a few experiments one or two bees bit the tip of the forceps with the mandibles. The relative low level of activity caused by the empty forceps was strikingly different from the "excitement" and fast movements released by an *O. smaragdina* worker.

3.3.2. Number of bees at the sticky band zone

Under our experimental conditions *O. smaragdina* workers had no access to the *A. florea* colonies and we did not observe much activity at sticky band zones. In several scans not even a single bee was detected. In average we found between 1 to 3 bees. The bees remained motionless facing outside for a few minutes until they turned around and disappeared in curtain covering the comb. Directly after presenting a *O. smaragdina* worker on the comb the relative increase of the number of bees at the sticky band zone was significant (Wilcoxon, $p < 0.0005$; fig 3.4). Even after 2 hours the relative increase was still significant (Wilcoxon, $p < 0.001$). However the number of bees decreased significantly between 1 min and 1 hr after the ant stimulus was presented (Wilcoxon, $p < 0.002$). The difference between 1 h and 2 h was not significant (Wilcoxon, $p < 0.122$) (fig. 3.4).

In control colonies, after presentation of a *Crematogaster* worker, a *Tenebrio* larva or an empty forceps on the comb, we did not observe an increase of bees in the sticky band zone (Fig. 3.5). Apparently, the increase of bees was a specific reaction to *O. smaragdina*.

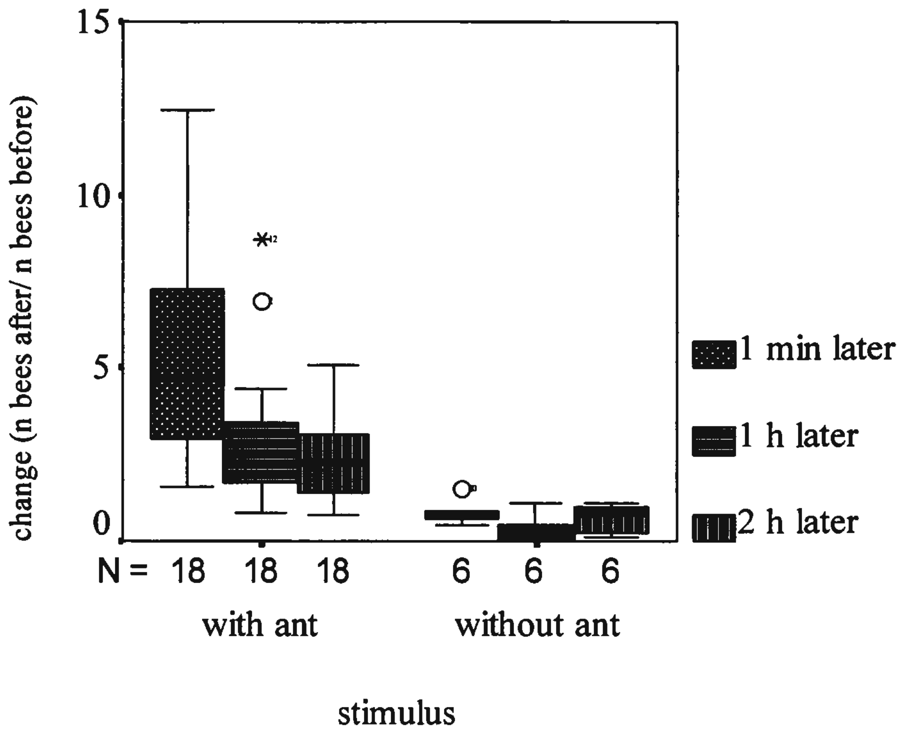

Figure 3.4 Increase of bees counted at the sticky band zone after presentation of weaver ant worker.

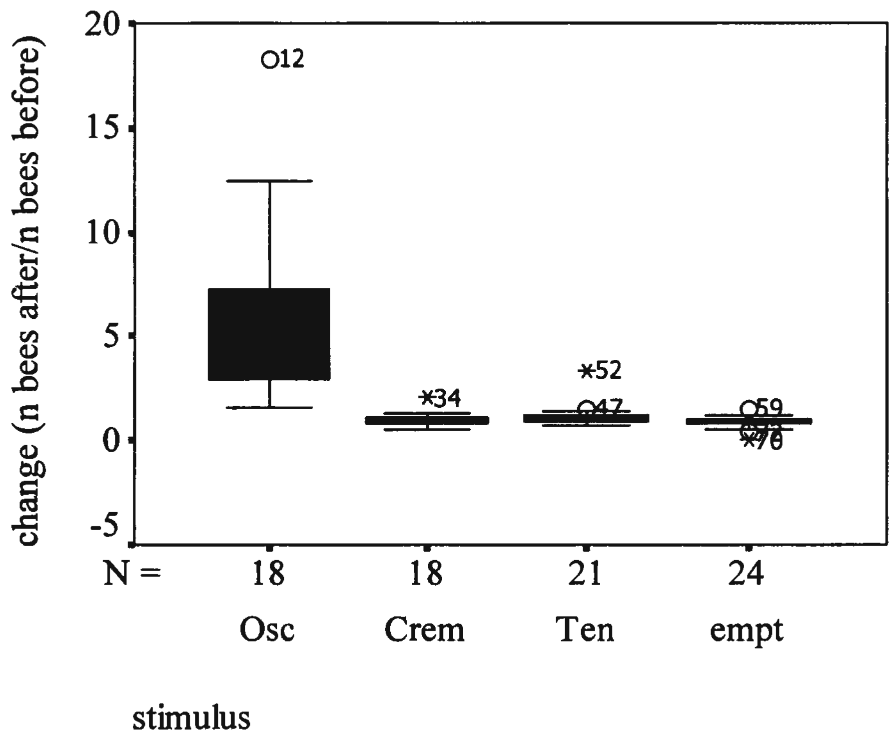

Figure 3.5 Increase of bees counted at the sticky band zone after presentation of different stumili: Osc = *O. smaragdina*, Crem = *C. rogenhoferi*, Ten = *T. molitor* larva, empt = empty forceps.

3.3.3. Behavior at sticky band after presentation of *O. smaragdina*

Ant presentation on the comb resulted first in reinforcement of the guard bees at interior side of the sticky bands. A larger group of bees (n = 12 – 25) settled densely packed head towards the sticky band in a 2 or 3 fold layer. Looking along the branch over the sticky band towards the comb we saw two or three rows of worker bee heads, comparable to bricks in a wall. A small number of bees went beyond these guard bees and started to "work" on the sticky band. These bees permanently scraped the surface of the branch by their mandibles. The antennae were constantly moving back and forth. The tips of both antennae touched the surface and/or the mandibles and then swung back. The movements looked similar to the behavior of *A. mellifera* workers sealing wooden surfaces inside the hive with propolis (Meyer, 1954). The work on the sticky band went on for several minutes (3 to 10 min). Then the bee moved on to the comb. In a few cases (when we succeeded to keep track of it) it returned within 1 or 2 minutes and carried on working at the sticky band.

We did not see bees carrying material in the pollen baskets to the sticky band. Further, we did not notice that any larger particles were brought by the mandibles. However, we could not watch the head of these bees constantly during their excursions on the combs. As soon as the bee reached the nest it dived into the layer of bees. So we can not exclude that the bees did collect some smaller parts of material from the comb and fixed this to the sticky band.

3.3.4. Depositions in sticky band zone

In average the weight of depositions on the plastic band after ant exposure was 2.7 mg with a standard deviation of $\pm$2.73. In the control colonies (without ant presentation) we measured a mean of 1.4 mg $\pm$ 0.91 mg (fig. 3.6). The difference was highly significant (Mann-Whitney; $p = 0.0005$) while there was no difference within the control group. So the presentation of an *O. smaragdina* worker on the colony resulted not only increasing numbers of bees but also in a larger amount of depositions.

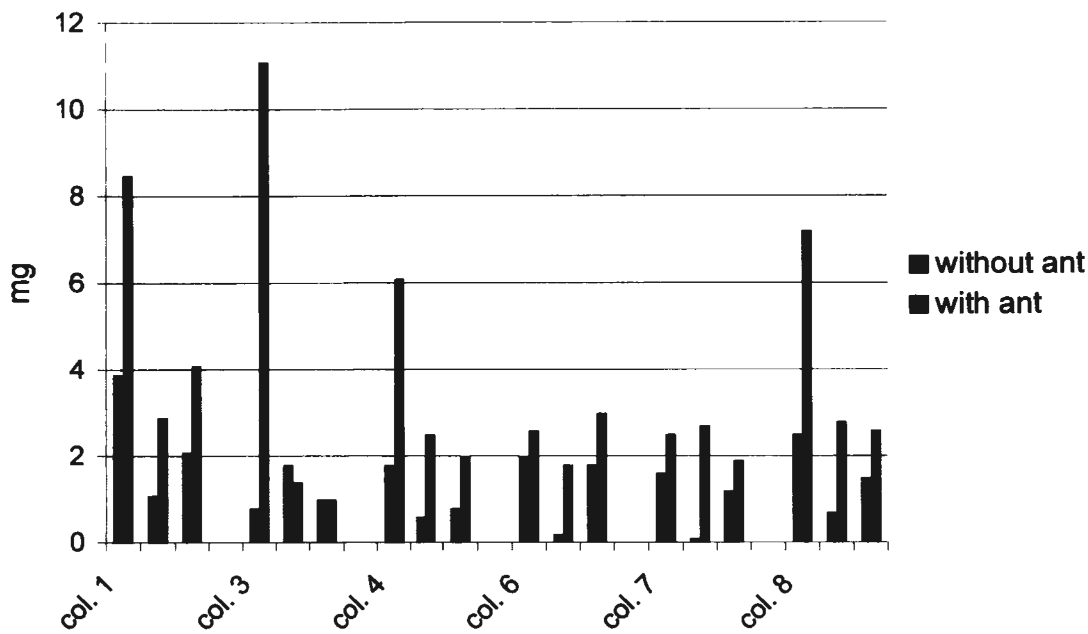

Figure 3.6 Deposition of material in sticky band zone.

3. 4 Discussion

The use of sticky or repellent barriers to protect colonies against ants is wide spread among different taxonomic groups of social insects. In several species of social wasps (Polistinae and Stenogastrinae) the pedicel, by which the nest is fixed to substrate, is regularly impregnated by repellent pheromones from sternal glands. The nest entrance of many species of Meliponinae is protruded by a tube built out of plant resins and sticky materials (Wilson, 1971). So the "invention" of barriers against crawling arthropods has happened several times during evolution independently from each other.

Generally, the nest branch is the only surface connection of the *A. florea* comb to the "outside world" and it disembogues just under the platform into the comb. This platform serves as location of the bee dances and functions as information center of the foragers (Lindauer, 1956; Koeniger *et al.*, 1982; Seeley, 1985; Dyer, 1985). At the platform, the bees must deal with mainly two different kind of interferences. Old leaves or debris from the higher portion of the vegetation will fall on the platform and must be removed. Recently a specific "head pushing" behavior of *A. florea* to remove debris was described (Sen Sarma *et al.*, 2000). We observed this behavior when we put the empty forceps on the platform. So bees "classified" the forceps alone as "debris". Afterwards we did not notice any increase of activity at the sticky bands. The second kind of interferences which must be expected at the upper side of comb comes from crawling arthropods or other intruders which succeed to surpass the sticky band. Many of those might endanger the existence of the colony and need to be expelled without delay and at all costs. When we presented the forceps with the *O. smaragdina* worker we saw the latter kind of defensive behavior. The direct reaction of the bees to the ant was aimed to expel the intruder. Though the reaction to *C. rogenhoferi* or *T. molitor* involved less bees their intention to remove the presented insects was obvious. Presentaion of *C. rogenhoferi* or *T. molitor* did not result in increasing numbers of bees working at the sticky bands. Interestingly, however the significant long term effect was restricted to the presentation of *O. smaragdina* and besidesan increase of activity also within 2 hours the amount of material deposited at the sticky band was larger compared to presentation of the forceps. The "purpose" of this behavior is clear. Increasing the number of guard bees and enforcing the sticky band were means of precaution and would enable the colony to repel further intruders before they could enter the comb.

The weaver ant, *O. smaragdina* is a dominant species. Colonies defend their huge territories against competing ant species (Hölldobler and Wilson, 1990). Their effective recruitment system enables weaver ants to fast communal foraging and to target even a large prey (Hölldobler, 1983). Therefore the existence and survival of an *A. florea* colony within the territory of *O. smaragdina* depends on an effective protective system. A main requirement of such defense against *O. smaragdina* is to prevent any foraging success of a weaver ant scout. As we have witnessed several times, an odor trail of a successful scout ant can recruit larger numbers of ants which will overpower defending bees and cause absconding of the colony (Wongsiri, 1989). At the end of such raids, the honey storage, the brood and quite a number of worker bees will lose to the weaver ants.

The reinforcement of the sticky barriers seems to be a specific behavioral response of *A. florea* to its most prominent predator *O. smaragdina*. A comparable phenomenon termed enemy specification (Wilson, 1975) is known to operate between several ant species. These species recognize their most dangerous adversary species and fight against them without delay by specific mass attacks (Hölldobler, 1983).

The question of how *A. florea* recognizes the weaver ant remains open. The experiments, however, with *C. rogenhoferi* and larvae of *Tenebrio* point to cues or semiochemicals specific to *O. smaragdina*.