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SHORT COMMUNICATION

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Prison construction and guarding behaviour by European honeybees is dependent on inmate small hive beetle density

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Abstract Increasing small hive beetle (*Aethina tumida* Murray) density changes prison construction and guarding behaviour in European honeybees (*Apis mellifera* L.). These changes include more guard bees per imprisoned beetle and the construction of more beetle prisons at the higher beetle density. Despite this, the number of beetles per prison (inmate density) did not change. Beetles solicited food more actively at the higher density and at night. In response, guard bees increased their aggressive behaviour towards beetle prisoners but did not feed beetles more at the higher density. Only 5% of all beetles were found among the combs at the low density but this percentage increased five-fold at the higher one. Successful comb infiltration (and thus reproduction) by beetles is a possible explanation for the significant damage beetles cause to European honeybee colonies in the USA.

Introduction

It has recently been reported that African honeybees (*Apis mellifera* L.) use physical prisons constructed of propolis to contain small hive beetles, *Aethina tumida* Murray (Neumann et al. 2001) within hives and that incarcerated beetles remain alive by mimicking hungry bees, thus eliciting a feeding response from the guard bees (Ellis et al. 2002a). Beetle imprisonment may be more advanced in African honeybees than in European ones (Solbrig 2001; Neumann et al. 2001), possibly because African honeybees and hive beetles are sympatric (Hepburn and Radloff 1998) while European honeybees and hive beetles are not.

The use of social encapsulation by African and European honeybees may limit beetle access to the combs. At low beetle populations, African and European bees encapsulate beetles in cracks and crevices throughout the hive away from the combs (Neumann et al. 2001; Ellis et al. 2003b). Because of this, beetles are unable to reach foodstuffs in the combs (including brood, honey, and pollen reserves), on which their reproductive potentials are high (Ellis et al. 2002b). If the encapsulation process fails, beetles may subsequently reach the combs and begin reproducing, leading to the damage frequently seen in European colonies (Elzen et al. 1999; Ellis et al. 2003a).

Despite similarities in fundamental social encapsulation behaviours at low beetle populations by African and European honeybees (Solbrig 2001; Ellis et al. 2003b), beetles only destroy European colonies. Because "infested" African colonies rarely host large populations of beetles while infested European colonies often do, the overall success or failure of beetle encapsulation by European honeybees may be dependent on intra-colonial beetle density. Here we report on the effects of increasing beetle density on prison construction and guarding behaviour of European honeybees.

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Methods

Experiments on honeybees of mixed-European origin (from Athens, Georgia, USA: at the time of the study, hive beetles had not yet been discovered in Athens) were conducted in Warren County, Georgia, USA (August–September 2001) where beetles had never previously been found. Three observation hives were used, each containing two frames of brood, one of honey, approximately 8,000 bees, and a laying queen. A transparent grid system, dividing each side of the colony into 160, 5×5 cm areas, was used to describe intra-colonial locations of beetles.

Twenty-five beetles were introduced into each hive and 15 days later, colonies were monitored twice daily at 09:00 and 20:00 hours (under red-light conditions) for 3 days. On the fourth day of observations, 25 more beetles were added to each colony and on days 5–7 the hives were monitored again. 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 5×5 cm 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. Because of this, neither beetles nor bees were counted twice in any observation, as guard bees and beetles do not readily move between prisons. The entire procedure lasted approximately 30 min per hive.

Intra-colonial distribution, behaviour, and number of imprisoned beetles, and number and behaviour of worker honeybee guards (guarding at prison entrances) were recorded. Beetle behaviour included resting or mating, and antennal or trophallactic contact with guard bees. Guard bee behaviour included biting, antennating, and trophallactically feeding beetles, and biting the area around beetle prisons (prison wall-working) (Solbrig 2001; Neumann et al. 2001; Ellis et al. 2003b).

Guard bee and beetle behaviours were analysed with a repeated-measures ANOVA design recognizing beetle density (25 or 50 beetles) and time (day or night) as main effects. Variables for which there were density level × time interactions were analysed by infestation level using dependent variable *t*-tests. Encapsulated beetle intra-colonial distribution was analysed by infestation level using χ^2 tests. Significant differences were accepted at the $\alpha \leq 0.05$ level and all analyses were conducted using Statistica (2001).

Results

We found that as beetle density increased, so did the number of beetle prisons (Table 1) (F=24.1; df=1, 16; P<0.001); yet the number of beetles per prison did not increase (Table 1) (F=0.6; df=1, 16; P>0.05). Further, the number of guard bees per prison was higher at the greater beetle density (Table 1) (F=4.4; df=1, 16; P=0.05) and during night at both the low ($|t|$=3.5; df=8; P<0.01) (day = 1.92±0.49, night = 2.63±0.36, n=9 for all data) and high ($|t|$=7.4; df=8; P<0.0001) (day = 2.67±0.31, night = 4.00±0.30, n=9 for all data) beetle densities. Similarly, there were more guard bees per imprisoned beetle by night (1.02±0.11, 18; mean±SE, n) than day (0.73±0.12, 18) (F=23.5; df=1, 16; P<0.001).

The beetles were significantly more active at the higher density (Table 1) (F=7.2; df=1, 16; P <0.05) and significantly more beetles made antennal contact with guard bees at the higher density (Table 1) (F=24.2; df=1, 16; P <0.001) and night (F=9.2; df=1, 16; P<0.01) (night = 0.21±0.03; day = 0.12±0.03, n=18 for all data). 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 either beetle density (F=0.1; df=1, 16; P>0.05) or time (F=2.9; df=1, 16; P>0.05). We found no density (F=1.0; df=1, 16; P>0.05) or time effects (F=0.1; df=1, 16; P>0.05) for the proportion of beetles mating.

Guard bees increased antennal contact with imprisoned beetles at the higher density (F=12.7; df=1, 16; P<0.01), but this did not lead to more guard bees feeding beetles (F=4.0; df=1, 16; P>0.05) (Table 1). Guard bees fed beetles more during night at the lower beetle density ($|t|$=3.3; df=8; P<0.05) (day = 0.01±0.01, night = 0.08±0.02, n=9 for all data) but not at the higher density ($|t|$=0.7; df=8; P>0.05) (day = 0.12±0.03, night = 0.10±0.03, n=9 for all data).

Table 1 Small hive beetle density effects on prison dynamics, beetle behaviour, and guard bee behaviour

	25 beetles	50 beetles
Prison dynamics		
Number of guard bees per encapsulated 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 ± 0.26b
Encapsulated beetle behaviour		
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 behaviour		
Biting at beetles	0.68 ± 0.06a	0.86 ± 0.03b
Antennal contact with encapsulated beetles	0.12 ± 0.03a	0.32 ± 0.04b
Feeding encapsulated beetles	0.05 ± 0.01a	0.11 ± 0.02a
Prison wall-working	0.32 ± 0.06a	0.04 ± 0.02b

For beetle and guard bee behaviour, data are the proportion of individuals observed doing the particular behaviour. Values are 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. Means were compared using ANOVAs

Table 2 Proportion of small hive beetles encapsulated in various intra-colonial locations at both beetle densities

Location	25 beetles	50 beetles
Top wall of hive	0.22±0.04	0.17±0.04
Bottom board of hive	0±0	0.02±0.02
Front wall of hive	0.32±0.05	0.42±0.05
Back wall of hive	0.41±0.03	0.14±0.02
Combs	0.05±0.02	0.25±0.06

Values are mean ± standard error; n=9 for all data

The proportion of guard bees biting at the beetles increased at the higher beetle density (F=4.9; df=1, 16; P<0.05) (Table 1). Interestingly, prison wall-working by guard bees significantly decreased with increasing beetle density (Table 1) (F=17.1; df=1, 16; P<0.001). More beetles were found among the combs at the higher rather than lower beetle density (χ^2=59.2; df=4; P<0.0001) (Table 22).

Discussion

Guard bees maintained constant numbers of beetles per prison despite a higher beetle density, suggesting an optimum beetle per prison density that is most efficiently guarded. When beetle density increased, guard bees responded by creating more prisons, not by encapsulating more beetles in existing prisons.

That the number of guard bees per prison increased at the higher beetle density is probably due to increased beetle activity at the higher beetle density. Conceivably the number of 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 honeybee nest where the combs are located.

Interestingly, 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 too could be a reason that beetles are problematic in European honeybee colonies. If trophallaxis is used by honeybees 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.

African honeybee subspecies are significantly more aggressive towards beetles than are European honeybees (Elzen et al. 2001). Yet, European bee aggressive behaviour increased at the higher beetle density, suggesting an attempt to control beetles in much the same way that African subspecies do (Neumann et al. 2001; Solbrig 2001).

Only 5% of all beetles were found among the combs at the lower beetle density, but this increased five-fold at the higher density, indicating a mass influx of beetles into the combs at the higher density (Table 2). Perhaps the bees could not control increasing beetle activity at the higher density. Further, because imprisoned beetles were not being fed more by guard bees at the higher beetle density (Table 1), they could have been driven to the combs in search of food.

We have shown that an increase in beetle density causes changes in prison construction and guarding behaviour of European honeybees. Most of the changes compromised the efficacy of social encapsulation, resulting in increased numbers of beetles reaching the combs. Comb infiltration by beetles may trigger their reproduction, ultimately leading to nest destruction in European colonies.

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