

Research article

Behavioral defense strategies of the stingless bee, *Austroplebeia australis*, against the small hive beetle, *Aethina tumida*

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Abstract

Small hive beetle, *Aethina tumida* Murray, is a parasite of social bee colonies and has become an invasive species, raising concern of the potential threat to native pollinators in its new ranges. Here we report the defensive behavior strategies used by workers of the Australian stingless bee, *Austroplebeia australis* Friese, against the small hive beetle. A non-destructive method was used to observe in-hive behavior and interactions between bees and different life stages of small hive beetle (egg, larva and adult). A number of different individual and group defensive behaviors were recorded. Up to 97% of small hive beetle eggs were destroyed within 90 min of introduction, with a significant increase in temporal rate of destruction between the first and subsequent introductions. A similar result was recorded for three-day-old small hive beetle larvae, with an increased removal rate from 62.5% to 92.5% between the first and second introductions. Of 32 adult beetles introduced directly into the 4 colonies, 59% were ejected, with the remainder being entombed alive in hives within 6 h. Efficiency of ejection also significantly increased between the first and third introductions. Our observations suggest that *A. australis* colonies, despite no previous exposure to this exotic parasite, have well-developed hive defences that are likely to minimise entry and survival of small hive beetles.

Keywords: *Austroplebeia australis* / *Aethina tumida* / defense strategies / stingless bee / resin.

Introduction

As global trade and travel increases, biological invasions become more frequent (Mooney and Cleland, 2001; Levine and D'Antonio, 2003; Cassey et al., 2005). Introduced parasites may switch hosts, thereby posing new threats to native species and raising concerns about conservation. Due to the lack of co-evolution between host and parasite, these new hosts do not possess any specific defense behaviors against the new pest, having to rely entirely on generalist means (Poitrineau et al., 2003), which may or may not provide them sufficient resistance.

The small hive beetle, *Aethina tumida* Murray (Coleoptera: Nitidulidae), appears to be such an invasive parasite. It is native to sub-Saharan Africa where it is a parasite and scavenger of honeybee colonies, *Apis mellifera scutellata* Linnaeus (Hymenoptera: Apidae) (Lundie, 1940; Neumann and Elzen, 2004). In both North America and Australia, the beetle has become well established (Neumann and Elzen, 2004; Spiewok et al., 2007; Neumann and Ellis, 2008). The spread of small hive beetle has been facilitated by managed and feral populations of European honeybee subspecies, which were themselves introduced to the Americas and Australia (Moritz et al., 2005). European honeybee subspecies seem to be more susceptible than African ones to small hive beetles, as European colonies collapse more often from beetle infestations (USA: Elzen et al., 1999, 2000; Australia: Annand 2008), thereby considerably enhancing beetle reproduction.

A number of recent studies suggest that the small hive beetle may be able to exploit colonies of other social bees as alternative hosts, especially in the absence of its original host. It has been found to infest commercial colonies of the bumblebee, *Bombus impatiens* Cresson (Hymenoptera: Apidae), in North America in the field (Spiewok and Neumann, 2006) and in green houses (Hoffmann et al., 2008). Moreover, the beetle can successfully reproduce in laboratory *B. impatiens* colonies (Ambrose et al., 2000; Stanghellini et al., 2000) as well as in managed hives of Australian stingless bees (*Austroplebeia* and *Trigona*) (R. Luttrell, personal communication April 2006.). Since small hive beetles have also been reported to naturally infest colonies of stingless bees (*Dactylurina staudingerii*) in West Africa (Mutsaers, 2006), it is not surprising that small hive beetles may also use them in their new ranges. Indeed, colonies of Australian stingless bees, genera *Trigona* (Jurine 1807) and *Austroplebeia* (Moure 1961), provide honey, pollen, wax, brood and adult bees, all the preferred foods of small hive beetle (Ellis et al., 2002; Spiewok et al., 2007). While the removal of feral honeybee competitors may be considered advantageous for populations of native bees (Paini, 2004), the removal of the primary host of the small hive beetle could result in a host switch which might subsequently pose a threat to the native bee populations within its new range (Neumann et al., 2006).

Anecdotal reports of small hive beetle invasion of weakened stingless bee colonies (A. Dollin, Australian

Native Bee Research Centre, personal communication 2005) prompted studies to elucidate the ability of Australian stingless bees to defend themselves against exotic hive invaders. Greco et al. (2010) reported that *Trigona carbonaria* Smith (Hymenoptera: Apidae) was capable of effectively defending its hive against invading adult small hive beetles through workers “mummifying” the beetles with hard resinous material before they were able to cause any damage. While Australian *Trigona* spp. are known to store large quantities of resin and batumen in their nests, which they utilize as part of their defensive strategies (Klumpp, 2007; Dollin, 2010a), *Austroplebeia* species collect and store comparatively small amounts of resin, which is mixed with wax to produce cerumen for nest structures (Michener, 2007). We therefore hypothesized that colonies of *Austroplebeia* may be less able to defend themselves against small hive beetle invasion. In this study, we investigate the defensive behavior of colonies of *A. australis*, the most commonly cultivated species in this genus.

Materials and methods

Insect species in this study

Austroplebeia australis lives in social colonies of five to ten thousand workers (personal observation MH) that build a clustered brood structure surrounded by pollen and honey pots. Crevices and nest breaches are closed with cerumen; however, excess resin deposits are minimal, especially compared to those of *Trigona* (Dollin, 2010a). Worker bees measure approximately 4 to 4.5 mm in length and possess a vestigial sting (Michener, 2007).

In its native range, the small hive beetle causes little or no damage to the well-adapted native African honeybee colonies, unless the colony is weak, diseased or has absconded (Neumann et al., 2001; Neumann and Härtel, 2004), but in the more docile races of European honeybee, small hive beetle infestation can be catastrophic. Adults fly to the hive entrance and walk into the hive. Bees find it difficult to grasp or sting the small, hard bodies of the beetle (Lundie, 1940; Schmolke, 1974; Neumann et al., 2001). The small 5-7 mm beetles are able to access cracks and crevices within the hive where larger honeybees cannot reach (Schmolke, 1974; Neumann et al., 2001). The beetles mate and later the female lays approximately 13 eggs per day (Mostafa and Williams, 2002). These hatch and the larvae commence feeding on the hive resources, defecating throughout the hive as they feed. The combination of contaminated honey and faecal matter causes the hive content to ferment and melt down (Lundie, 1940; Neumann and Elzen, 2004; Spiewok and Neumann, 2006). Usually the colony of bees will abscond, leaving the beetles to completely take over the hive. Eventually the hive mass, along with the mature beetle larvae, oozes out of the hive entrance, allowing the larvae to burrow into the soil to pupate (Neumann and Elzen, 2004).

Experimental setup

Experiments were conducted in 2006, from late autumn (May) to early spring (November), at the University of Western Sydney's (UWS) Hawkesbury campus, Richmond, New South Wales, Australia (33°36'S, 150°45'E) using four *A. australis* colonies obtained from a commercial stingless bee producer (R. Zabel, Australian Stingless Native Bees, Gatton) in Queensland (27°33'S, 152°17'E). Each colony was well established in an Original Australian Trigona Hive box (OATH, Dollin and Heard, 1999) and was housed in controlled temperature (CT) rooms maintained at $28 \pm 2^\circ\text{C}$, as *A. australis* is naturally found in the warmer regions of northern NSW and Queensland, north of 32°S (Dollin, 2010b).

All colonies had access to external foraging via a clear vinyl tubing (1 m x 10 mm diam.) through the external wall of the CT room. To minimise the chances of colony destruction and to facilitate observation, small hive beetles were not introduced directly into the hive box. Instead, observation platforms (Halcroft et al., 2007) were utilized to optimize observations of beetle and bee behavior under undisturbed conditions. An observation platform, manufactured with two sealed access holes, was installed between the entrance tube and each hive box. A piece of 3 mm metal mesh was cut to fit the tubing at the entrance of the hive box to prevent adult small hive beetles entering the box but still allowing the bees normal access. Investigations did not commence until one week after the platforms had been fitted to hives, after confirmation that the colonies had accepted them as indicated by the construction of entrance fortifications and beginnings of hive structures. Previous investigations showed that *A. australis* colonies accept the platforms as part of their nest and built typical entrance fortification and pollen and honey pots within these annexes (Halcroft et al., 2007).

Introduction of small hive beetles into *A. australis* nests

Adult small hive beetles were collected from naturally infested *A. mellifera* hives at the UWS apiary to start a laboratory culture following routine rearing protocols (Mürle and Neumann, 2004) and to obtain defined age cohorts of the different beetle life stages. The aim was to test the response of *A. australis* workers to different life stages (egg, early instar larvae and adults), as all of these can be found inside hives once a successful invasion of adult beetles has occurred. Thus different small hive beetle life stages were repeatedly introduced into the OPs and the responses of the worker bees to their introduction were recorded. In addition, adult beetles were introduced into the hive via the external entrance and observations were made of *A. australis* worker behavior.

Introduction of small hive beetle eggs

Groups of 30 small hive beetle eggs were separated from clusters of laboratory-reared eggs (Mürle and Neumann, 2004), and each group was transferred onto a 10 mm x 25 mm piece of colored plastic sheet. These were introduced into each observation platform ($n = 4$) via

the access holes. Bee activity was observed and egg numbers were monitored and recorded at intervals of 30, 60 and 90 min. Observation platforms were checked after 24 h, and the plastic sheets with any remaining eggs, were carefully removed via the access holes using long forceps. This process was repeated twice more the following day, with one hour between each experiment.

Introduction of small hive beetle larvae

Ten, three-day-old, 8 mm x 1 mm, laboratory-reared small hive beetle larvae were randomly selected and placed into a 30 mm long (10 mm diam.), closed-ended silicone tube, which was then inserted into an access hole of each hive observation platform (n = 4). Removal of larvae by the bees was monitored, and the time of each removal was recorded over a 15 minute period. Each observation platform was subsequently checked after 2 h to confirm that no larvae remained in the structure. This procedure was repeated once more for each hive within a 24 h period. As a control, three pieces of latex shaving, each measuring approximately 8 mm x 1 mm (of similar dimensions to a three-day-old small hive beetle larva), were placed inside one of the observation platforms, and the behavior of the bees was monitored for 2 h.

Introduction of adult small hive beetle

Observation platforms: Three laboratory-reared adult small hive beetles were introduced into each of the four platforms. Beetle movements and worker bee behavior were monitored once each hour, for a period of 6 h. The final locations of beetles within the observation platforms were then noted. All platforms were inspected 24 h after small hive beetle introduction; the lids were taken off and any remaining small hive beetle were located, photographed and removed. This procedure was repeated three days later, but the the number of adult beetles introduced was increased to 5 for each observation platform, and the platforms were left undisturbed for 60 h before being examined.

External entrances: Adult small hive beetles were collected from naturally infested *A. mellifera* hives at the UWS apiary. On 2 November 2006 (week 1), individual beetles were introduced to the external entrance tube of 4 *A. australis* hives and the time taken for guard bees to eject the beetle was recorded (first treatment). If the beetle successfully broke through the colony defenses, a period of 5 min was allowed to elapse before the next beetle was introduced. If the beetle was ejected by the guard bees, the time was noted and the next beetle was introduced immediately. The experiment was repeated twice (second and third treatments), resulting in a total of 15 adult small hive beetles being introduced into the entrance of each of

the four hives within one day. Approximately 60 h after the final introduction, the observation platforms were opened and the adult small hive beetles were located, counted and photographed. This procedure was repeated on 28 November 2006 (week 2) using the same hives.

Data analyses

Data for egg and larvae removal were analyzed by Kaplan-Meier survival analysis using pairwise comparisons in SPSS 17 (IBM Company, Chicago, Illinois). Data for adult small hive beetle ejection from the hive entrances were analyzed by ANOVA and means were compared by multiple comparisons and Dunnett's T-test in SPSS 17 (IBM Company, Chicago, Illinois).

Results

Behaviors of *A. australis* workers recorded following introduction of small hive beetles into colonies were categorized into various defensive strategies (Table 1). Some behaviors were specifically associated with introduction of specific small hive beetle life stages, whereas other behaviors occurred more generally (Table 1).

Introduction of small hive beetle eggs

When discovered by bee workers, small hive beetle eggs were inspected for several seconds and then challenged. Some eggs were immediately consumed by workers, while others were daubed with resin. Approximately 50% of eggs were consumed or daubed with resin within the first 30 min, and this increased to 97% within 90 min of introduction (Figure 1). Simultaneously, other workers carried out nest fortification. The efficiency of egg destruction increased significantly between the first and second treatments ($\chi^2 = 16.697$, $p < 0.001$) and between the first and third treatments ($\chi^2 = 17.798$, $p < 0.001$). There was no significant difference in the rate of egg destruction between the second and third treatments ($\chi^2 = 1.754$, $p = 0.185$).

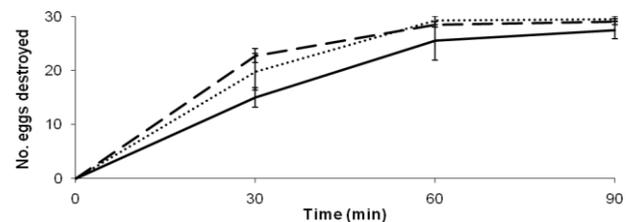


Figure 1: Number of destroyed small hive beetle eggs (consumed, removed or daubed) over the observation period (N = 4 colonies, 120 eggs, 3 introduction periods). Means and their standard errors are shown (solid line = 1st introduction, dotted line = 2nd introduction, dashed Line = 3rd introduction).

Table 1: Defense behaviors of individual *A. australis* worker bees against different small hive beetle life stages

Behavior	Small hive beetle life stage	Description
Inspection	Eggs, larvae, adults, latex	Inquisitive, methodical inspection of 'intruder'. Antennation with no further exploration. Calm, unhurried behavior
Challenge	Eggs, larvae	Inquisitive, methodical inspection of 'intruder'. Antennation and manipulation using forelegs. Some, but not all, inspecting workers armed with soft, sticky resin, carried in mandibles. Calm, unhurried behavior.
Nest fortification	Eggs, larvae, adults	Numerous workers patrolling nest. Inspection for possible breaches. Adding more cerumen or working existing cerumen. Workers carrying a piece of cerumen in their mandibles. Carried out by numerous workers. Methodical, unhurried behavior.
Attack	Adults, larvae	Grappling with and biting 'intruder'. Aggressive, hurried behavior.
Riding	Adults	Climbing on top of 'intruder', clinging to the body. Aggressive, hurried, frenzied behavior.
Resin daubing	Adults	Collecting soft, sticky resin globules from storage areas throughout hive. Resin globules carried in mandibles and in corbiculae. Depositing resin on body of 'intruder'. Aggressive, agitated behavior.
<i>En masse</i> attack	Adults	Individuals working in consort to immobilize or incapacitate 'intruder'. Attacking vulnerable under-side of small hive beetle. Aggressive, frenzied behavior. Many workers becoming frenzied.
Herding	Adults	Large numbers of workers attacking and surrounding 'intruder'. Worker group moving around the nest (OP in this case) pushing 'intruder' into corners, against nest structures or out of nest entrance. Aggressive, frenzied behavior. Many workers becoming frenzied.
Entombment	Adults	Working in consort; one bee depositing soft, sticky resin on body of 'intruder'; then another bee plastering a piece of cerumen onto the sticky surface. Behavior repeated by numerous bees, until 'intruder' covered in resin and cerumen. 'Intruder' becomes immobilized. Aggressive, frenzied behavior. Whole colony becoming frenzied.
Wing fanning	Adults and larvae	Individuals lining the length of entrance tube, facing towards the outside, and fanning wings. Numbers ranging from < 5 - > 20.

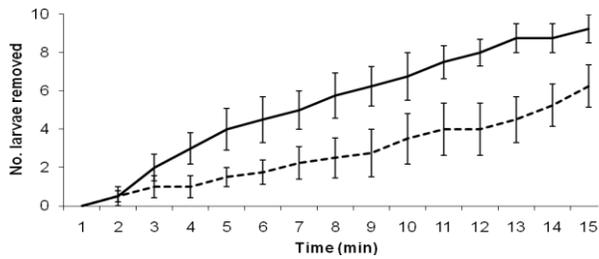


Figure 2: Number of removed small hive beetle larvae over a 15 min period in two treatments (introduction times). Means and their standard errors are shown (dashed line = 1st introduction, solid line = 2nd introduction)

Introduction of small hive beetle larvae

The latex shavings introduced into the observation platform as an inert intruder treatment were inspected briefly by several workers, but did not elicit any defense response. They were removed by the bees from the platform within 24 h.

Individual bees were observed to carry three-day-old small hive beetle larvae through the entry tube to the outside. Nest fortification was observed in all hives.

Removal of small hive beetle larvae increased from 62.5% to 92.5% between the first and second treatments (Figure 2). Pairwise comparisons of the four hives showed this increase to be highly significant ($\chi^2 = 8.680$, $p = 0.003$).

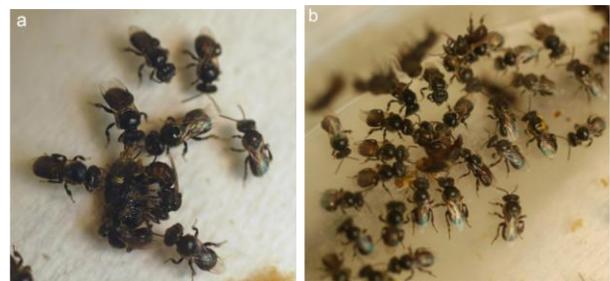
Introduction of adult small hive beetles

Observation platforms: Nest fortification behavior commenced within 1 min of adult small hive beetles being introduced into the observation platform. Worker bees attacked the intruders, with several workers attempting to ride the beetles. Riding bees were quickly dislodged as the beetles scurried under sections of hive structure. The number of attacking worker bees increased, and resin was daubed onto the beetles' bodies. Ejected beetles were often (about 60%) daubed with sticky globules of resin (Figure 3a). Assaulted beetles attempted to escape through the entrance tube with guard bees continuing their harassment. Bees were occasionally (2-3 times) observed riding beetles and biting them as they exited the hive (Figure 3b). The number of guard bees at the entrance increased from one or two prior to beetle introduction, to eight to ten after beetle introduction, and their numbers also increased along the entrance tube.



Figure 3: (a) Ejected adult small hive beetle covered in sticky globules of resin. (b) *A. australis* worker 'riding' adult beetle out of hive entrance.

Adult beetles remaining within the observation platform were attacked by increasing numbers of worker bees and were observed showing the turtle defense posture (Neumann et al., 2001). The defensive behavior of the colony proceeded through the stages of attack and riding, to growing numbers of bees attacking *en masse* (Figures 4a, b) and included resin daubing (Figures 4c). Workers harvested soft resin from small "ammunition deposits" located on the ceiling and floor of the platform (Figure 4d). Herding, resin daubing and entombment were carried out by up to 45 bees at a time. Cerumen used in the entombment was harvested from the nest structures within the platform. As a beetle was daubed with layers of resin and cerumen (Figure 5a), it was herded against nest structures (Figure 5b). This enabled the bees to build layers of cerumen around the beetle, incorporating it into the existing structure and entombing the beetle alive (Figure 5c). Once the beetle was immobilised, the number of bees involved in the attack declined, with only four or five bees continuing the entombment. Bee attendants frequently checked the entombed beetle for several hours after it had been immobilised.



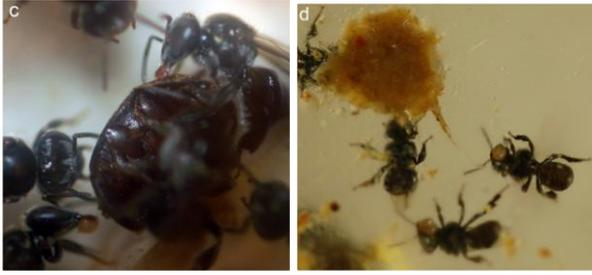


Figure 4: (a) Initial attack by small number (9) of *A. australis* worker bees against adult small hive beetle intruder. (b) *En masse* attack by large number (37) of *A. australis* worker bees against adult small hive beetle intruder. (c) Resin daubing by *A. australis* worker onto adult small hive beetle. Note turtling posture of beetle. (d) *A. australis* workers collecting stick resinous ammunition from small stores throughout the hive.

The behaviors of the colony changed during the experimental period. Activity escalated from challenge through to *en masse* attack and herding, and concluded with entombment. Nest fortification was initiated soon after beetle introduction and continued throughout the ‘invasion’ period. Wing fanning commenced as defense activities escalated. Occasional nest mate conflicts were noted during periods of frenzied activity. The colony was observed to be busy but calm 24 h after the initial small hive beetle introduction.



Figure 5: (a) *A. australis* workers building layers of resin and cerumen on the body of an adult small hive beetle. (b) Adult small hive beetle being herded against hive structure by *A. australis* workers. (c) Adult small hive beetle entombed alive within the hive structure. (d) An *A. australis* guard armed with sticky resin, held in its corbiculae

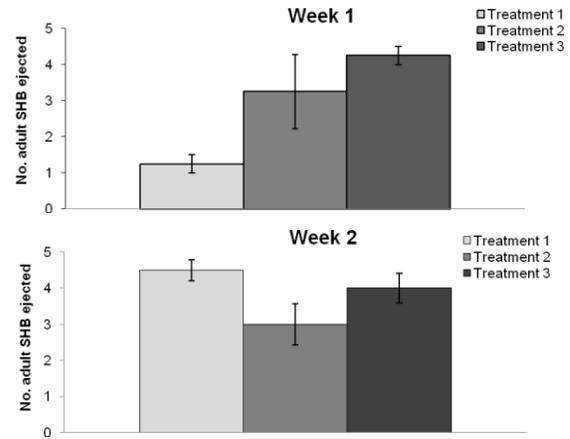


Figure 6: Number of adult small hive beetles (SHB) ejected from the hives for each treatment (introduction time) for weeks one and two. Bars represent standard errors of the means.

The majority of entombed beetles remained alive for the first 24 h, but all were dead after 60 h. Removal of beetle body parts by worker bees commenced between 24 and 60 h. Of the 32 adult small hive beetles introduced directly into the observation platforms, 59% were ejected and the remaining 41% were entombed within 6 h. The ejection times ranged from 5 to 240 min and immobilization times from 45 to 350 min.

Hive entrances: As each beetle was introduced to the entrance, guard bees immediately attacked and rode the intruder, which assumed the turtle defense posture of adult small hive beetle. Subsequent beetle introductions resulted a four-fold increase in numbers of guard bees visible at the entrance and 2 – 3 were armed with resin (Figure 5d). Increased guard numbers coincided with increased beetle ejection rates.

In week 1, the ejection rate of introduced adult small hive beetles during the first treatment was 25%. However, over the next two release periods the ejection rate increased significantly ($F_{2, 9} = 5.895$, $p = 0.023$), to 85% by the third treatment (Figure 6). There were no significant differences in ejection rates between the first and second ($p = 0.320$) or second and third ($p = 0.738$) treatments. In week 2 there was no significant difference between any of the treatments ($F_{2, 21} = 1.724$, $p = 0.203$).

All adult small hive beetles introduced at the hive entrances were either removed or entombed. During week 1, 58% were ejected from the hive entrances. This increased to 77% in week 2. The remaining adult small hive beetles were entombed within the hive structure of the observation platform.

Discussion

These studies suggest that healthy *A. australis* colonies are able to effectively defend themselves against small hive beetle invasion. We observed a range of behavioral responses by worker bees, which appear to form part of a communal colony defense strategy. The introduction of different life stages of small hive beetle into the hives often resulted in different defensive behaviors.

This phenomenon may be associated with the apparent level of threat perceived by the worker bees. While *A. australis* has not co-evolved with small hive beetle, Australian stingless bees have many other natural predators, such as ants, wasps, assassin bugs, spiders and lizards (Klumpp 2007); and nest parasites, such as beetles (*Brachyepelus auritus*, *B. basalis*, *Carpophilus planatus*) (Lea 1910,1912), *Brachyepelus planus*, *B. meyricki* (Rayment 1935); flies (*Ceriana ornate*) and wasps (*Braconid* spp.) (Klumpp 2007). It is likely that the defense strategies we observed have been developed against the native beetle species listed above.

Austroplebeia australis defensive behavior against eggs and larvae of small hive beetle appears to be similar to that of *Apis mellifera* (Neumann and Härtel, 2004), including consumption of eggs (Neumann et al., in review). While the inspection and challenge of the eggs appeared unhurried, the daubing of resin suggests that the bees perceive them as a threat. Workers of both *A. mellifera* (Neumann and Härtel, 2004) and *A. australis* remove larvae by picking them up and carrying them out of the hive entrance. In the current study, the removal of small hive beetle larvae by workers in one of the four hives was hampered by conflicts between the workers. As previously reported for *A. mellifera* (Neumann and Härtel, 2004), bees occasionally fought over a larva and were observed in a ‘tug-of-war’ with larvae or attempting to steal larvae from each other. This erratic behavior declined during the course of the first treatment. During the second treatment, the bees generally worked more independently and larval removal was efficient and timely.

The defensive behavior of *A. australis* toward the adult small hive beetle was different from that reported for *A. mellifera* (Neumann et al., 2001; Ellis et al., 2003) or *Bombus impatiens* (Hoffmann et al., 2008). Honeybees have difficulty lifting or stinging adult beetles due to the beetles’ turtle defensive posture (Lundie, 1940; Neumann et al., 2001), and the beetle is also able to hide in cracks and crevices within hives, because it is much smaller than *A. mellifera* workers (Neumann and Elzen, 2004). Those colonies that are able to defend best against the adult small hive beetle practice social encapsulation, where they confine beetles in prisons constructed of propolis (Neumann et al., 2001; Ellis et al., 2003). This behavior, however, does not remove the potential threat, and may merely postpone it.

Unlike *A. mellifera*, stingless bees do not rely on a stinger to defend their colonies against hive intruders. Although the small hive beetle turtle defense behavior (Neumann et al., 2001) is a very effective defense against worker bee attack, it does not protect against *en masse* attacks and bombardment with resinous ammunition. In addition, the relatively small size of Australian stingless bees (approximately 4 mm long) enables them to access small hive beetles hiding in nest cracks and crevices. In the current study, the *A. australis* colonies confined and entombed the beetles, similar to the behavior of *T. carbonaria* (Greco et al., 2010), resulting in incapacitation

rather than “social encapsulation”. This behavior resulted in 100% mortality of small hive beetles introduced into the observation platforms and at the hive entrances (n = 62), with dismemberment and partial removal of several of the entombed beetles within 60 h of their introduction.

Of the few known cases of small hive beetle invasion of Australian stingless bee hives, most colonies were either severely weakened by extreme temperatures (> 42°C, M. Duncan, January 2006; A. Dollin, April 2006, personal communication), physically damaged through tree felling (R. Luttrell, April 2006; C. Alonso, February 2007, personal communication) or had been recently split for hive propagation (A. Dollin, April 2006, personal communication; MH, personal observation 2007). Colonies affected by extremely high temperatures suffer from ‘slumping’ of stores and large numbers of bees smother and drown. In stressed honey bee colonies, beetle invasion may go unchallenged (Lundie, 1940; Hepburn and Radloff, 1998; Neumann and Elzen, 2004). In the event of severe physical hive damage, bees may be unable to reseal breaches quickly enough to prevent adult small hive beetle entry. Colonies that have been successfully resealed soon after being damaged or split have been reported to effectively remove both larvae and adults of small hive beetle from the hive (A. Dollin, April 2006; C. Alonso; R. Luttrell, February 2007; A. Beil 2009, personal communication). We invariably observed worker bees inspecting edges and seals of the observation platforms and connection tubing while carrying resin globules. It appears that the practice of nest fortification enables the preservation of nest integrity, which is one of the most important defense strategies used by this species and probably, other stingless bee species.

Although *A. australis* colonies did not immobilise intruders as swiftly as *T. carbonaria* (cf. 10 min, Greco et al. 2010), this study showed that they are capable of defending their hives against invasion by small hive beetle and probably, similar predators. The current studies showed that in cases where the initial lines of defense were breached due to reduced points of attachment on the beetle, *A. australis* can effectively recruit in-hive worker defenders and change their behavior to ensure that hive invaders are incapacitated through entombment. Wing fanning by workers within the observation platforms and hive entrance tubes was observed prior to and during *en masse* attack, suggesting that this activity may be associated with the dispersal of alarm pheromones (Michener, 1974) and worker recruitment for nest defense.

Throughout the experimental period, worker bees were observed communicating through wing fanning, antennation and trophallaxis. Repeated introductions of small hive beetles over the six week experimental period resulted in increasing efficacy of defensive strategies within the *A. australis* colonies. This suggests that the colonies in our studies may have learned from their initial exposure to hive intruders and were able to improve their defensive behavior, resulting in increased removal rates of all introduced small hive beetle life stages. As with honeybee

colonies, stingless bee colonies can also be regarded as super-organisms with an enormous capacity to learn through complex communication and feed back (Tautz, 2008). Here we have shown that strong colonies of *A. australis* housed within intact nest structures, are unlikely to succumb to small hive beetle attack. However, field studies would be required to properly assess the impact of small hive beetle on natural populations of Australian stingless bee populations.

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