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ORIGINAL RESEARCH ARTICLE

Small hive beetle (*Aethina tumida*) oviposition behaviour in sealed brood cells with notes on the removal of the cell contents by European honey bees (*Apis mellifera*).

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Summary

Small hive beetles (*Aethina tumida* Murray) can lay eggs cryptically through the cappings of sealed bee brood cells. However, honey bees (*Apis mellifera* L.) can detect this activity and respond by removing cell cappings and contents (hygienic behaviour). We were interested in identifying conditions that regulate this syndrome of stimulus and response. Beetle oviposition rate (proportion of cells in which beetles perforate the capping and oviposit) into sealed brood cells was shown to be unaffected by a range of beetle densities created experimentally in caged sections of sealed brood. Oviposition rate was, however, increased when beetles had access to a sealed brood cell not only at the capping, but also along at least one side if the cell was next to an empty cell. Beetle oviposition rates into sealed brood cells were unaffected by the presence of *Varroa destructor* mites in the cell within a range of 1-4 mites per cell. The expression of hygienic behaviour toward beetle-perforated brood cells ranged from 10.6 - 77.2 % across colonies, but was unaffected by the colony strength parameters of bee population and cm^2 brood.

Comportamiento de puesta del pequeño escarabajo de las colmenas (*Aethina tunida*) en celdas de cría operculadas con notas sobre la eliminación del contenido de las celdas por abejas europeas (*Apis mellifera*)

Resumen

El pequeño escarabajo de las colmenas (*Aethina tumida* Murray) puede poner huevos de forma críptica a través del opérculo de celdas con cría sellada. No obstante, las abejas (*Apis mellifera*) pueden detectar esta actividad y responder eliminando el opérculo de las celdas y su contenido (comportamiento higiénico). Nosotros estamos interesados en identificar las condiciones que regulan este síndrome de estímulo y respuesta. La tasa de puesta del escarabajo (proporción de celdas en las cuales los escarabajos perforan el opérculo y ponen huevos) en celdas de cría operculadas no se vio afectada por un rango de diferentes densidades de escarabajos creado artificialmente en cajas selladas de cría operculada. Sin embargo, la tasa de puesta aumentó cuando los escarabajos tuvieron acceso a celdas de cría operculadas no solo por el opérculo si no también por un lateral si la celda estaba próxima a una celda vacía. La tasa de puesta del escarabajo en celdas de cría operculadas no se vio afectada por la presencia de *Varroa destructor* en las celdas dentro del rango de 1-4 ácaros por celda. La expresión del comportamiento higiénico hacia las celdas de cría perforadas por los escarabajos varió de un 10,6 al 77,2% en las colonias, pero no se vio afectada por parámetros como la fuerza de la colonia, población de abejas y cm^2 de cría.

Keywords: *Aethina tumida*, hygienic behaviour, *Apis mellifera*, oviposition, Nitidulidae, small hive beetle

Introduction

Small hive beetles (*Aethina tumida* – hereafter referred to as "beetle") are endemic to sub-Saharan Africa (Hepburn and Radloff, 1998; Ellis and Munn, 2005; El-Niweiri *et al*., 2008) where they inhabit honey bee (*Apis mellifera*) colonies and are considered as occasional colony nuisances (Lundie, 1940). The beetle was found, however, in honey bee colonies in the U.S. in the mid-1990s and subsequently in Egypt, Australia, Canada, and Portugal (cf. Ellis and Munn, 2005; Hassan and Neumann, 2008; Neumann and Ellis, 2008). In its introduced range, the beetle can be a major colony pest although the circumstances leading to colony collapse due to beetles are not fully understood (Neumann and Elzen, 2004).

Small hive beetle adults are attracted to honey bee colonies as places to feed and reproduce (Lundie, 1940). Once there, female beetles oviposit in cracks and crevices around the colony (Lundie, 1940; Schmolke, 1974; Neumann and Elzen, 2004) as well as into sealed brood cells (Ellis *et al*., 2003). Beetle oviposition into concealed areas probably confers a reproductive benefit to the beetle. Indeed, investigators have shown that beetle eggs left unprotected in bee colonies are removed by the host bees within 24 hours (Neumann and Härtel, 2004; Spiewok and Neumann, 2006b). Consequently, it is important that cryptic beetle oviposition behaviour be studied further as this behaviour may lead to an accelerated increase in intra-colonial populations of larval beetles. This especially is true in instances where beetles oviposit in sealed brood cells because beetle larvae preferentially feed on bee pupae (Elzen *et al*., 2000), beetles can reproduce on brood (Ellis *et al*., 2002; Buchholz *et al*., 2008), and the presence of beetle larvae feeding on bee brood often indicates the decline of a colony.

Despite the suitability of sealed brood cells as beetle oviposition sites, female beetles do not oviposit in all cells in which they perforate the capping (Ellis *et al*., 2004; de Guzman *et al*., 2008), suggesting that decision making by female beetles is involved in the oviposition process. In an effort to understand what may influence this decision making process, we decided to investigate factors that may affect beetle oviposition into sealed brood cells. In particular, we wanted to determine the effects of beetle density, target cell type, and the presence or absence of other cell invaders in the target cell on beetle oviposition behaviour.

Regarding the first parameter, we hypothesized that beetle density, or the number of beetles per cm^2 of sealed brood, would affect the oviposition rate (the proportion of sealed brood cells in which beetles perforate the capping and oviposit) and the number of eggs that beetles oviposited per cell. We believed that high beetle densities would lead to increased competition for the limited oviposition sites, resulting in a corresponding increase in the beetle oviposition rate.

Second, we hypothesized that beetle oviposition behaviour would vary according to target cell type. Sealed brood cells bordering empty cells allow beetles to oviposit through the cell capping and through the cell wall, while sealed brood cells completely surrounded by other sealed brood cells provide beetles access only to the cell capping. Consequently, we believed that beetles would oviposit more into sealed brood cells bordering at least one empty cell because of the increased surface area through which beetles could oviposit.

Because varroa mites (*Varroa destructor* Anderson and Trueman) are ubiquitous in the USA, we decided to investigate the potential relationship between varroa presence in sealed brood cells and beetle choice to oviposit in the cells. Beetles and varroa have different life histories, so a reproductive interaction between the two does not seem intuitive. Consequently, we hypothesized that varroa presence in the target cell would not influence a beetle's decision to oviposit in that cell.

Finally, we decided to investigate the effects of beetle oviposition behaviour on the removal rate of the contents (beetle eggs and immature bee) from sealed brood cells in which beetles have perforated the cappings. Regarding this objective, we hypothesized that colony strength parameters (adult bee population, total cm^2 brood, and colony varroa populations) would correlate with bee removal of cell contents, working on the assumption that "stronger" colonies (more bees and brood and less varroa) would be better able to remove cell contents than "weaker" (less bees and brood and more varroa) ones. Collectively, these factors form the basis for the investigative work presented here.

Materials and Methods

We conducted the experiments on 32 colonies of mixed-race European honey bees at the University of Georgia's research apiary in Watkinsville, Georgia, USA, from September to October 2004. The colonies were housed in standard Langstroth hives and all colonies had previously been exposed to beetles and used as production colonies. We did not equalize colonies at the beginning of the study, so that we could maintain natural variation in colony strength parameters (adult bee population and cm^2 sealed brood) as this was one of our independent variables.

We assessed colony strength parameters (number of adult bees and total cm^2 brood) by averaging measurements taken for frames of adult bees and total brood one day prior to and one day following the study (with visual estimates per Skinner *et al*., 2001) and transforming the measurements with conversion formulas per Burgett and Burikam (1985). We determined colony varroa populations for the study period by averaging the number of varroa mites on a 24h varroa monitoring screen one day prior to and four days following the study.

To collect data on bee removal of cell contents, we followed the procedures of Ellis *et al*. (2003b; 2004) with some modifications. We removed a frame of sealed brood from each colony and placed 1 of 5 densities (0, 5, 10, 15, 20) of randomly collected, laboratory-reared adult beetles (cold-anesthetized in a vial surrounded by ice for 4 – 5 min) on a 10 cm^2 area of the comb in a metal push-in cage (10 x 10 x 2.5 cm). We did not place all beetle densities in a colony simultaneously because we conducted the experiment in the fall, when the colonies' queens were reducing their egg output. Consequently, colonies did not contain enough capped brood necessary to test all beetle densities simultaneously. Furthermore, we used randomly collected beetles produced in rearing programs (Mürrle and Neumann, 2004) rather than "sexed" beetles because sexing

beetles requires squeezing them with some force (Schmolke, 1974), with the effect of sexing beetles on their reproductive behaviour remaining unknown. The removal rate was calculated as: [the number of sealed brood cells (1) having beetle perforated cappings and (2) from which bees removed the cell contents] / [the total number of sealed brood cells having beetle perforated cappings] (Ellis *et al*., 2004).

The faces of the metal push-in cages we used to isolate beetles on patches of sealed brood were made of screen mesh (3 mesh per cm) to allow ventilation. We chose sections of comb containing approx. 60–90% sealed brood cells which were > 4 d from emerging so that no brood from the test area would emerge during the study. We replicated each beetle density once per colony (n = 32 colonies). We then replaced all test brood in the centre of the bee cluster in their respective colonies.

Twenty four hours later, we removed all cages and recollected the adult beetles from them. We then placed a transparent sheet of acetate over the target brood cells and marked all sealed brood cells containing beetle perforated cappings according to cell type (sealed brood cell with only the capping exposed or sealed brood cell with the capping and at least one side wall exposed). Similarly, we marked a maximum of 20 uninfested brood cells (no perforations in the cappings) from a control brood section (no beetles) each for sealed brood cells with only the capping exposed or with the capping and at least one side wall exposed. We replaced all test frames in the centre of the bee clusters in their respective colonies. After 24h, we examined the brood cells and counted marked cells which had been uncapped and the contents removed by the bees.

To determine oviposition rate, we confined 1 or 2 of the 4 beetle densities, 0 beetles not included, to 1 frame of sealed brood as before and replaced the frames in each of 16 colonies. Twenty four hours later, we removed the capping from all brood cells having a beetle perforated capping. For each cell in which beetles oviposited, we determined the number and location (top of pupa, side of pupa, or both) of beetle eggs within the cell, cell type (on capping exposed or capping and cell wall exposed), and the number of varroa mite foundresses. Oviposition rate was calculated as: [the number of sealed brood cells (1) having beetle perforated cappings and (2) containing beetle eggs] / [the total number of sealed brood cells having beetle perforated cappings] (Ellis *et al*., 2004).

Data analyses

We determined differences in beetle oviposition rates and the number of eggs that beetles oviposited per cell for the independent variables beetle density (0, 5, 10, 15, 20) and cell type using beetle density x cell type as the test error term in a factorial analysis of variance (ANOVA). For sealed brood cells with at least one cell wall exposed, we determined differences in the oviposition rate and the number of eggs that beetles oviposited per cell for the independent variables beetle density and egg oviposition location (side of pupa, top of pupa, or both) within each cell using beetle density x egg location as the test error term in a factorial ANOVA. When eggs were oviposited on both the top and side of the pupa in a perforated cell, we compared the number of eggs beetles oviposited in both locations using a one-way ANOVA. We used only sealed brood cells containing beetle eggs to determine the number of eggs beetles oviposited per cell in all analyses. We compared oviposition rate and the number of eggs oviposited per cell in cells with and without varroa and at five varroa populations per cell (0–4 varroa / cell) using a one-way ANOVA.

Using a one-way ANOVA, we analyzed the effect of colony (colonies 1–32) on the removal of brood cell contents recognizing a colony's removal rate at each beetle density (not including 0 beetles) as a replicate since a preliminary analysis showed the removal rate did not vary significantly between the different beetle densities ($P > 0.05$).

We compared the oviposition rate in sealed brood cells with beetle perforated cappings to the removal rate of the same for both cell types (only the capping exposed or the capping and at least one side wall exposed) and for the overall oviposition and removal rates using one-way ANOVAs. We analyzed the effects of colony strength parameters and varroa population on the removal of brood cell contents in cells with beetle perforated cappings using regression analyses testing for linear, quadratic, and cubic explanations.

We transformed all proportion data (removal rate and oviposition rate) before analyses using arcsin$\sqrt{}$proportion. For reporting in this manuscript we give the raw, untransformed means. We conducted all analyses using the statistical software packages Statistica (Statistica, 2001) and SAS (SAS, 1992), accepting differences at $\alpha \leq 0.05$. Where applicable, we used Tukey's test to compare means.

Results

Oviposition rate and number of eggs oviposited per cell

The number of beetles in test cages (5, 10, 15, or 20 beetles) did not affect the beetle oviposition rate ($F = 5.4$; df = 3,71; $P = 0.1$) or how many eggs they oviposited per cell ($F = 1.2$; df = 3,71; $P = 0.45$)(Table 1). The oviposition rate was significantly higher in sealed brood cells when the cells had at least one exposed side wall than if they did not ($F = 47.1$; df = 1,71; $P < 0.00$; Table 1). Despite an increased oviposition rate in sealed brood cells with an exposed cell wall, beetles did not oviposit more eggs into these cells ($F = 4.6$; df = 1,342; $P = 0.12$; Table 1).

The oviposition rate within sealed brood cells having at least one exposed wall and the number of eggs that beetles oviposited into these cells were unaffected by the number of beetles present in the test cages (oviposition rate: $F = 0.6$; df = 3,114; $P = 0.66$; number of eggs per cell: $F = 1.1$; df = 3,198; $P = 0.41$, Table 2). However, beetles oviposited more frequently through either the side wall or capping of cells than through both sites within a cell ($F = 23.4$; df = 2,114; $P < 0.00$; Table 2). Despite this, beetles oviposited more eggs per cell when ovipositing in both locations than in either the side or capping of the cell only ($F = 8.6$; df = 2,198; $P = 0.02$; Table 2). In instances where beetles oviposited at both locations within a brood cell having an exposed wall, they oviposited more eggs ($F = 24.9$; df = 1,64; $P < 0.05$) through the side of a cell (37.6 ± 4.9 (33) eggs) than through the capping (11.8 ± 1.6 (33) eggs) (here and throughout, data = mean ± s.e. (n)).

Table 1. The effects of beetle density and cell type on oviposition rate[a], the number of eggs beetles oviposited per cell (# eggs per cell), and the removal rate[b] of brood cells having beetle perforated cappings. Data are mean ± s.e. (n). Columnar data followed by the same letter, within beetle density and cell type, are not different at $\alpha \leq 0.05$. Tukey tests were used to compare means for oviposition rate and # of eggs per cell while LSMEANS tests were used to compare means for the removal rate.

beetle density	oviposition rate[a]	# eggs per cell	removal rate[b]
0	na	na	0.01 ± 0.002 (230)a
5	0.34 ± 0.08 (17)a	13.3 ± 1.4 (57)a	0.29 ± 0.04 (53)b
10	0.55 ± 0.08 (21)a	23.5 ± 2.9 (72)a	0.29 ± 0.04 (58)b
15	0.46 ± 0.07 (20)a	25.2 ± 2.9 (108)a	0.40 ± 0.04 (61)b
20	0.43 ± 0.07 (21)a	20.9 ± 2.1 (113)a	0.40 ± 0.04 (55)b
cell type			
border empty cell	0.57 ± 0.05 (42)a	25.0 ± 1.9 (214)a	0.44 ± 0.03 (117)b
surrounded by brood	0.32 ± 0.05 (37)b	16.1 ± 1.5 (136)a	0.24 ± 0.03 (110)a

[a]oviposition rate = [# of sealed brood cells (1) having beetle perforated cappings and (2) containing beetle eggs] / [total # of sealed brood cells having beetle perforated cappings]
[b]removal rate = [# of sealed brood cells (1) having beetle perforated cappings and (2) from which bees removed the cell contents] / [total # of sealed brood cells having beetle perforated cappings](Ellis *et al*. 2004).

Varroa presence in sealed brood cells (≥ 1 varroa / cell) did not affect the oviposition rate (F = 2.5; df = 1,69; P = 0.12) or number of eggs that beetles oviposited per cell (F = 1.3; df = 1,639; P = 0.26). The oviposition rate and number of eggs that beetles oviposited per cell in cells without varroa was 0.53 ± 0.05 (38) and 2.1 ± 1 (472) respectively while in cells with varroa, it was 0.41 ± 0.06 (33) and 10 ± 1.5 (169) respectively. The number of varroa in cells (from 0 to 4 varroa) did not affect the number of eggs beetles oviposited per cell (F = 1; df = 4,629; P = 0.39). Beetles oviposited 12.1 ± 1.0 (472), 10.1 ± 2.1 (93), 8.7 ± 1.7 (42), 4.7 ± 2.8 (18) and 6 ± 3.5 (9) eggs per cell in cells containing 0, 1, 2, 3, and 4 varroa respectively.

Removal of cell contents

The bee removal rate of cell contents from beetle perforated sealed brood cells varied significantly among colonies (F = 1.9; df = 31,86; P = 0.01). Removal rate among colonies ranged from 10.6% ± 4.7 (4) to 77.2% ± 11.2 (3). The variation between colonies in removal rates was not, however, explained by the total cm^2 brood or number of bees per colony as determined by linear, quadratic, and cubic analyses ($P > 0.05$). Interestingly, varroa mite-fall on monitoring screens correlated with colony removal of cell contents from beetle-perforated sealed brood cells (F = 2.2; df = 1,31; P = 0.04). A line with the equation $y = -0.002x + 0.66$ (R^2 = 0.14) suggests that higher colony populations of varroa correlated negatively with a colony's ability to remove cell contents from beetle-perforated brood cells.

The overall beetle oviposition and bee removal rates were significantly different from one another (F = 3.9; df = 1,304; P = 0.05). Female beetles oviposited 21.5 ± 1.3 (350) eggs per cell in 45.2% ± 3.7 (79) of all sealed brood cells in which they perforated the cell capping while bees removed contents from only 34.7% ± 2.0 (227) of cells in which beetles perforated the capping. This constitutes an overall removal rate of 76.8% of cells perforated and oviposited in by beetles (34.7/45.2 × 100). There were no differences between the oviposition rate and removal rate for beetle perforated brood cells having an exposed capping (F = 0.3; df = 1,145; P = 0.57) while there were for beetle perforated brood cells having an exposed capping and cell wall (F = 5.3; df = 1,157; P = 0.02; Table 1). Regarding the latter, the bee removal rate of cell contents was ~77% of the total beetle oviposition rate (Table 1: 0.44/0.57 × 100) for perforated sealed brood cells with an exposed wall. Finally, bees removed the cell contents of beetle perforated sealed brood cells having an exposed wall at a significantly higher rate than contents from beetle perforated sealed brood cells having only an exposed capping (F = 65.9; df = 1,432; $P < 0.00$; Table 1).

Table 2. The effects of beetle density and egg location on the oviposition rate[a] and the number of eggs beetles oviposited per cell (# eggs per cell) within sealed brood having at least one exposed wall. Data are mean ± s.e. (n). Columnar data followed by the same letter, within beetle density and egg location, are not different at $\alpha \leq 0.05$. Tukey tests were used to compare means.

beetle density	oviposition rate[a]	# eggs per cell
5	0.16 ± 0.05 (30)a	14 ± 1.9 (34)a
10	0.21 ± 0.05 (33)a	27.3 ± 4.8 (37)a
15	0.20 ± 0.05 (30)a	31.5 ± 4.1 (71)a
20	0.19 ± 0.04 (33)a	23 ± 2.5 (68)a
egg location		
side of prepupa/pupa	0.29 ± 0.05 (42)a	26.6 ± 3.4 (85)b
top of prepupa/pupa	0.21 ± 0.04 (42)a	15.1 ± 1.3 (92)b
both	0.07 ± 0.02 (42)b	49.4 ± 5.5 (33)a

[a]oviposition rate = [# of sealed brood cells (1) having beetle perforated cappings and (2) containing beetle eggs] / [total # of sealed brood cells having beetle perforated cappings](Ellis *et al*. 2004).

Discussion

Oviposition rate and number of eggs oviposited per cell

In general, beetle oviposition behaviour (oviposition rate and number eggs oviposited per cell) was unaffected by the beetle densities studied. We do not believe that this is because our choice of beetle densities was too low. In our study, we used 5, 10, 15, and 20 beetles per 100 cm^2 sealed brood. This equals 0.05, 0.10, 0.15, and 0.2 beetles per cm^2 sealed brood or about 175, 350, 525, and 700 beetles per colony in a typical European colony with 3500 cm^2 sealed brood. Beetle populations > 300 beetles/colony are considered to be above the economic threshold for beetles in colonies in the southeastern USA (J.D. Ellis, W.M. Hood, K.S. Delaplane; in prep). We had hypothesized that beetle density would correlate positively with the oviposition rate and number of eggs oviposited per cell due to increasing competition for limited oviposition sites. That we did not find this suggests other factors, perhaps brood characteristics (age, location, pheromones, etc.), affect beetle oviposition behaviour more than does beetle density. For example, semiochemicals produced by bee brood attract invading varroa mites (Nazzi and Milani, 2007) so it remains possible that beetle choice to oviposit into sealed brood cells is similarly mediated by chemicals, rather than by beetle density.

Consistent with our second hypothesis, we found that beetle oviposition rate did vary depending on whether the target brood cell had an exposed wall or not, with beetles exhibiting a preference for ovipositing in brood cells with at least one exposed wall. Two theories may explain this preference. First, beetles normally are less able to perforate and oviposit in cells through the cell capping (the only mode afforded by sealed brood cells surrounded by other sealed brood cells) probably because of bee defensiveness toward free-roaming beetles (Ellis, 2005). Instead, they are more likely to run from bee aggression into an empty cell and oviposit through the cell wall with fewer disturbances. Second, unprotected brood cells that have an exposed wall offer beetles an increased surface area through which to oviposit (cell capping and wall) thus increasing the chance that beetles will oviposit in this cell type.

The data also support our third hypothesis, that beetle decision to oviposit in a given sealed brood cell does not correlate with either the presence or absence or number of varroa mites already in the cell. Varroa mites reproduce in sealed brood cells, entering the brood cell shortly before it is capped (Martin, 2001) and bees can "sense" their presence and remove the cell contents (hygienic behaviour). Although it remains unclear what initiates bee hygienic responses to varroa in sealed brood cells, some hypothesize the presence of chemical cues that cause bees to uncap cells hosting varroa and remove the cell contents (Martin *et al*. 2002; Nazzi *et al*., 2004; Harris, 2007; Nazzi and Milani, 2007). Because beetles and varroa have different natural histories, precedent does not exist to believe that beetles can detect the presence of varroa in a cell because of chemical or other cues produced by the mite. Consequently, a beetle's decision to oviposit would be unaffected. Alternatively, it remains possible that beetles can detect varroa in a sealed brood cell, but that varroa presence simply does not affect the beetle decision to oviposit.

The data on beetle oviposition behaviour in sealed brood cells with an exposed wall are primarily descriptive and predictable. For example, beetles oviposited in such cells through one site (either the cell capping or the cell wall) more frequently than through both sites. Beetle oviposition through one site might discourage (although certainly not eliminate) further beetle oviposition through subsequent sites, possibly in response to limited food resources available for developing beetle larvae in a single brood cell. Indeed, when beetles did oviposit through both sites in a cell, the overall number of eggs oviposited per cell was nearly two times higher than when beetles oviposited only through a single site, certainly reducing the amount of food available per larva. Regardless, that beetles oviposited in both locations in only 7% of sealed brood cells in which they perforated the cell capping may indicate a reproductive decision by most beetles to not continue ovipositing in cells already containing other beetle eggs.

Removal of cell contents

Contrary to what we hypothesized, colony strength parameters (number of adult bees and total cm^2 brood,), did not affect a colony's ability to remove contents from beetle perforated brood cells. Despite this, some colonies removed cell contents from perforated brood cells better than others, a similar finding made for colony responses to other brood pests and pathogens (Boecking and Spivak, 1999). This suggests that European colonies express natural phenotypic variation with respect to the removal of contents from beetle perforated sealed brood cells and that this variation is unrelated to colony "strength." If true, one would expect some apparently strong colonies to suffer beetle depredation while some weak colonies survive (and vice versa), a finding anecdotally suggested in the southeastern U.S. De Guzman *et al*. (2008) found that the level of removal of beetle egg infested brood did not vary by host subspecies, with Russian (subspecies uncertain) and Italian (*A. m. ligustica*) honey bees removing such brood at the same rate. Consequently, more research into what mediates removal of beetle egg infested brood by bees must be conducted in order to understand what contributes to inter-colony variation in the phenotypic expression of this behaviour. Regardless, that phenotypic variation in the behaviour exists suggests it may be augmented via selective breeding programs (Ellis *et al*., 2004), thus making this trait a possible tool that can be used for beetle control purposes.

Interestingly, varroa populations within colonies correlated negatively to bee removal of cell contents from beetle-perforated brood cells. Why bees in colonies hosting large varroa populations had reduced hygienic responses to beetle eggs deposited in sealed brood cells remains unclear. It is possible that bees in these colonies were unable to handle beetle pressures due to already high varroa populations. These are the first data that suggest that colonies hosting large varroa populations are more vulnerable to beetle reproduction.

In our general observations, bees removed cell contents within 24 h from only ~77% of all brood cells perforated and oviposited in by beetles, thus allowing limited beetle reproduction. In an earlier study by our lab (Ellis *et al*., 2004) bees

removed cell contents after 48 h from a proportion of perforated brood cells equal to that of cells oviposited in by beetles (or nearly 100% removal). Yet in 24 h (the present study), bees failed to remove contents from ~23% of cells perforated and oviposited in by beetles. Beetle eggs can hatch within 24 hours (personal observation). Consequently, infested bee brood not removed in 24 h may be consumed by hatching beetle larvae, which in turn have time to hide from bees before the bees begin to remove the cell contents. This would ensure continuous and cryptic beetle reproduction when beetle densities are low, a suggestion supported by Spiewok and Neumann (2006a).

In conclusion, beetle density did not affect beetle oviposition behaviour, while cell type did. Beetle oviposition rates were significantly higher in sealed brood cells having an exposed wall and capping than in those with only an exposed capping. The presence or absence and number of varroa (1–4 mites / cell) per cell did not affect beetle oviposition behaviour into those cells. Bee removal of contents from beetle perforated sealed brood cells varied significantly by colony (10.6–77.2% colony removal range) but not by the colony strength parameters bee population and cm^2 brood. Total colony varroa population correlated negatively with bee ability to remove cell contents from beetle perforated cells. Finally, bees removed cell contents from only ~77% of all brood cells perforated and oviposited in by beetles.

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