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The alternative Pharaoh Approach:

Stingless bees mummify beetle parasites alive

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Abstract

Workers from social insect colonies use different defence strategies to combat invaders. Nevertheless, some parasitic species are able to bypass colony defences. In particular, some beetle nest invaders cannot be killed or removed by workers of social bees, thus creating the need for alternative social defence strategies to ensure colony survival. Here we show, using Diagnostic Radioentomology, that stingless bee workers (*Trigona carbonaria*) immediately mummify invading adult small hive beetles (*Aethina tumida*) alive by coating them with a mixture of resin, wax and mud, thereby preventing severe damage to the colony. In sharp contrast to the responses of honeybee and bumblebee colonies, the rapid live mummification strategy of *T. carbonaria* effectively prevents beetle advancements and removes their ability to reproduce. The convergent evolution of mummification in stingless bees and encapsulation in honeybees is another striking example of evolution between insect societies and their parasites.

Keywords Stingless bee, Diagnostic Radioentomology, beetles, parasites

Introduction

Social insects live in colonies exploited by parasites which feed on stored food or brood (Schmid-Hempel 1998), generating the need for efficient defence mechanisms. Parasitizing beetles pose particular difficulties for social insects because their exoskeletons protect them from direct primary defence strategies such as biting or stinging. The small hive beetle (SHB), *Aethina tumida* (Coleoptera: Nitidulidae), is such a parasite. It scavenges honeybee (*Apis mellifera*) colonies endemic to sub-Saharan Africa (Lundie1940; El-Niweiri et al. 2008; Neumann and Ellis 2008) and has become an invasive species with established populations in North America and Australia (Neumann and Ellis 2008). SHB invade nests to feed on brood, stored food or dead bees (Lundie 1940; Schmolke 1974; Neumann and Elzen 2004; Spiewok and Neumann 2006a) often causing complete destruction of nests, especially from their feeding larval stages (Neumann and Elzen 2004). Although adult SHB are vigorously attacked by workers (Elzen et al. 2001), they usually bypass the bees' nest entrance defences and remain difficult to kill or eject due to their hard exoskeleton and defence behaviours (Neumann et al. 2001, Neumann and Elzen 2004). Cape honeybees, *A. m. capensis*, display alternative defence mechanisms by encapsulating small hive beetles in tombs made from resin (Neumann et al. 2001, Ellis et al. 2003b). Despite the lack of co-evolution between host and parasite, European honeybees also encapsulate small hive beetles (Ellis et al.2003b) suggesting that encapsulation appears to be part of the general alternative defence of honeybee colonies.

Recent evidence suggests that SHB also parasitize colonies of bumblebees (*Bombus impatiens*, Spiewok and Neumann 2006b, Hoffmann et al. 2008) and stingless bees (*Trigona carbonaria*; Anne Dollin, personal observations). Analogous to propolis usage by honeybees, stingless bees use batumen to seal nest cavities (Michener 1961). Therefore, we hypothesised that stingless bees may show an analogue to honeybee social encapsulation of SHB, by restricting parasite advancement and reproduction. We tested this hypothesis by observing experimental infestations of *T. carbonaria* using Diagnostic Radioentomology (DR).

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Materials and methods

Laboratory reared (Muerrle and Neumann 2004) adult SHB with $BaSO_4$-marked elytra, were introduced to entrances of five *T. carbonaria* hives, N=10 beetles per hive, via transparent plastic observation tubes (Hoffmann et al. 2008). Beetle-bee interactions at hive entrances were visually observed (5 min). To non-invasively monitor movements of beetles that managed to bypass guards, hives were CT scanned at 5 min intervals for 90 min in a human body scanner (GE HiSpeed 64 Slice, General Electric Company), (Greco et al. 2006). Beetle distributions in hives were assessed using BeeView 3D rendering software (Disect Systems Ltd; Suffolk, UK). Two dimensional images were created to enable precise measurements of vector distances of SHB from hive entrances with on-screen linear callipers (Greco et al. 2005) and 3D images were generated to provide visual spatial representation of SHB with respect to hive structures. One hive was randomly selected after scanning and snap frozen with liquid nitrogen for visual screening to compare SHB positions with respect to scanned images. We applied a linear mixed model in a block design, with "colonies" as 5 random blocks and "time" as a fixed factor with 10 observations of distances for each time point, to assess any "colony" or "time" effects (it was not possible to fit a 'repeated measurements' model because beetles were not identified as individuals):

$Y_{ijk} = \mu + C_i + t_j + \varepsilon_{ijk}$ where,

Y_{ijk} was k^{th} distance measured in colony i at time j

μ was global mean

C_i was effect of colony I, I = 1,2,…,5 (random)

t_j was effect of time j, j = 0, 5, 10 (fixed)

ε_{ijk} was the random error of the kth observation in colony i at time j, k = 1,2,…,10

$C_i \sim N(0, \sigma^2)$

$\varepsilon_{ijk} \sim N(0, \sigma^2)$.

We also tested a model of diffusion (random walk) to assess whether SHB were prevented from normal random walk distributions. If we assume the beetles to move randomly and free of attacks then the model of "random walk" can be helpful for modelling the distribution of measured

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distances at a given time. This is equivalent to the process of diffusion of a solute in a solvent and can be checked statistically by a Chi-square goodness-of-fit test with the normal distribution of the measured distances. For all statistical analyses we used SYSTAT 12 software (Cranes Software International Ltd.).

Results

Upon introduction of SHB, visual observations confirmed that workers from all hives immediately attacked and coated all introduced beetles with batumen. The attacks by workers (Fig. 1a) caused most beetles to remain motionless in the turtle defence posture (Neumann et al, 2001). When not attacked, beetles were observed moving further into the hive. However, most *T. carbonaria* bees continuously attacked, thereby keeping the SHB in the turtle defence posture until mummified (Fig. 1b). Although 3 beetles (mummified on the spot, < 5 mm from hive entrance), did not progress 47 did (Table. 1). In one hive, two SHB reached a distance of 170 mm from the entrance, just below the brood (Fig. 2a). All SHB advancements ceased within 10 min of introduction (Fig. 2b). After 90 min, mean distance from hive entrances was 64 ± 51 mm (maximum distance = 170 mm, minimum distance = 2 mm, N = 50 measurements). The linear mixed model showed that there was a significant "time" and "colony" effect on beetle distribution, $p < 0.05$ and < 0.001 respectively. Beetles travelled furthest between time 0 and 5 min and travelled least in colony 1 and furthest in colony 4. At "time" 0 to 5 min the model for diffusion or "random walk" was accepted, $Chi^2 = 4.12$, $p = 0.53$ (5 min), df = 5, showing that beetles were able to disperse between 0 and 5 min. At "time" 10 min the model for diffusion or "random walk" was rejected, $Chi^2 = 24.11$, $p < 0.001$, showing that beetles were unable to disperse freely between 5 and 10 min. Dissection of the snap frozen hive confirmed positions and batumen coatings which corresponded to previously scanned images (Fig. 2c & 2d).

Discussion

Our data clearly show that *T. carbonaria* workers efficiently prevented SHB advancements and subsequent reproduction within 10 min by coating them with batumen directly on their bodies. Here we adopt the term mummification because honeybees do not coat live beetles directly with propolis but instead confine them in prisons (Neumann et al., 2001, Ellis et al. 2003). Mummification by stingless bees appears to be more effective than social encapsulation by honeybees because in sharp contrast to *Apis mellifera* (Neumann ret al. 2001), all introduced SHB were affected.

A number of native nitidulid species has been reported in *Trigona* nests (*Brachypeplus auritus*, *B. basalis* Lea 1910; *Carpophilus planatus* Lea 1912, *B. planus* and *B. meyricki* Rayment 1935). Although these endemic beetles do not normally harm the colony (personal observations AD), mummification may have evolved to prevent them and other successful invaders from reproducing in the nest. Social encapsulation of live intruders in wax or propolis confinements has been described from *Apis mellifera* (Neumann et al., 2001). Likewise, *Bombus* and stingless bees have been reported to coat intruders with resin has been reported (Kerr and Lello 1962; Michener 1974; Nates and Cepeda 1983; Roubik 1989; Betz and Koelsch 2004; Lars et al. 2008). However, to our knowledge, this is the first report of mummification of live nest intruders in colonies of social bees.

When SHB beetles adopt the turtle defence posture most honeybee workers eventually leave the beetles, which then scurry into hiding or progress further into the colony (Neumann et al, 2001). In contrast, *T. carbonaria* workers continuously attack, thereby keeping the SHB in the turtle defence posture, which enables other workers to mummify live beetles with batumen. Indeed, our data suggest that many beetles are immobilised between 5 and 10 min thus unable to move unhindered according to a model assuming random walk. It appears that the combination of continuous attacks and quick recruitment of mummifying workers underlies this efficient alternative defence mechanism of *T. carbonaria*. There have however, been reports of heat-stressed *T. carbonaria* colonies being destroyed by small hive beetles (MG, personal observations), suggesting that this invasive species may still pose some threat to native pollinators.

In conclusion, a single stingless bee worker is not able to eject or kill beetle parasites unaided. Only a team with individuals performing specific tasks (e.g. wrestling or gluing) can overcome parasite advancements. Live encapsulation of SHB by stingless bees has probably evolved as an alternative defence mechanism to prevent successful reproduction of nest parasites. It is evidently effective, because beetles are quickly immobilised, preventing successful reproduction. This seems especially important in light of the high reproductive potential of SHB. The convergent evolution of live mummification of nest parasites in stingless bees and social encapsulation in honeybees is another striking example of evolution between insect societies and their parasites.

References

Betz O, Koelsch G (2004). The role of adhesion in prey capture and predator defence in arthropods. Arthropod Structure & Development 33: 3–30

Breed, MD (2003). Nestmate Recognition Assays as a Tool for Population and Ecological Studies in Eusocial Insects: A Review. Journal of the Kansas Entomological Society. 76: 539-550

Breed MD, Guzman-Novoa E, Hunt GJ (2004) Defensive behaviour of honey bees: Organization, Genetics, and Comparisons with Other Bees. Annual Review of Entomology 49:271–98

Ellis JD, Pirk C, Hepburn HR, Kastberger G, Elzen PJ (2002) Small hive beetles survive in honeybee prisons by behavioural mimicry. Naturwissenschaften 89: 326-328

Ellis JD, Hepburn HR, Ellis AM, Elzen PJ (2003) Social encapsulation of the small hive beetle (*Aethina tumida Murray*) by European honeybees (*Apis mellifera* L.). Insectes Sociaux 50:286–291

Elzen PJ, Baxter JR, Westervelt D, Randall C, Delaplane KS, Cutts L, Wilson WT (1999) Field control and biology studies of a new pest species, *Aethina tumida* Murray (Coleoptera, Nitidulidae) attacking European honey bees in the Western hemisphere, Apidologie 30:361–366

Elzen PJ, Baxter JR, Neumann P, Solbrig A, Pirk CWW, Hepburn HR, Westervelt D, Randall C (2001) Behaviour of African and European subspecies of *Apis mellifera* toward the small hive beetle, *Aethina tumida*. Journal of Apicultural Research 40:40-41

Greco MK, Spooner-Hart R, Holford P (2005). A new technique for monitoring *Trigona carbonaria* nest contents, brood and activity using X-ray computerised tomography. Journal of Apicultural Research 44:97-100

Greco MK, Bell M, Spooner-Hart R, Holford P (2006) X-ray computerized tomography as a new method for monitoring *Amegilla holmesi* nest structure, nesting behaviour and adult female activity. Entomologia Experimtalis et Applicata 120:71-76

Hoffmann D, Pettis JS, Neumann P (2008) Potential host shift of the small hive beetle (*Aethina tumida*) to bumblebee colonies (*Bombus impatiens*). Insectes Soc 55:153–162

Kerr, WE and Lello, E, (1962). Sting glands in stingless bees. Journal of the New York Entomological Society 70, pp. 190–214.

Lea AM (1910) Australian and Tasmanian Coleoptera inhabiting or resorting to the nests of ants, bees and termites. Proc R Soc Victoria [NS] 23: 116-230.

Lea AM (1912) Australian and Tasmanian Coleoptera inhabiting or resorting to the nests of ants, bees and termites. Proc R Soc Victoria [NS] 25 Suppl: 31-78.

Lehmberg L, Dworschak K, Bluethgen N (2008) Defensive behaviour and chemical deterrence against ants in the stingless bee genus *Trigona* (Apidae, Meliponini). Journal of Apicultural Research 47:17-21

Lundie AE (1940) The small hive beetle *Aethina tumida*, Science Bulletin 220, Department of. Agriculture and Forestry, Government Printer, Pretoria.

Michener CD (1961) Observations on the nests and behaviour of *Trigona* in Australia and New Guinea (Hymenoptera: Apidae). American Museum of Novitates 2026 (08-05-1961): 1-45

Michener CD (1974) The social behaviour of the bees; A comparative study. Harvard University Press, pp. 404.

Muerrle TM, Neumann P (2004) Mass production of small hive beetles (*Aethina tumida* Murray, Coleoptera: Nitidulidae). Journal of Apicultural Research 43:144-145

Nates G and Cepeda O, (1983) Comportamiento defensivo en algunas especies de meliponínos. Boletín del Departamento de la Biologia de la Universidad Nacional de Colombia, Bogotá **1**, pp. 65−81.

Neumann P, Pirk CWW, Hepburn HR, Solbrig AJ, Ratnieks FLW, Elzen PJ, Baxter JR (2001) Social encapsulation of beetle parasites by Cape honeybee colonies (*Apis mellifera capensis* Esch.). Naturwissenschaften 88: 214-216

Neumann P, Elzen PJ (2004) The biology of the small hive beetle (*Aethina tumida* Murray, Coleoptera: Nitidulidae): Gaps in our knowledge of an invasive species. Apidologie 35: 229-247

Neumann P, Ritter W (2004) A scientific note on the association of *Cychramus luteus* (Coleoptera: Nitidulidae) with honeybee (*Apis mellifera*) colonies. Apidologie 35: 665-666

Neumann P, Ellis JD (2008) The small hive beetle (*Aethina tumida* Murray, Coleoptera: Nitidulidae): distribution, biology and control of an invasive species. Journal of Apicultural Research 47:181−183

Rayment T (1935) A cluster of bees. The Endeavour Press, Sydney.

Schmid-Hempel, P. 1998 Parasites in social insects. Princeton University Press.

Schmolke MD (1974) A study of *Aethina tumida*: the small hive beetle, Project Report, University of Rhodesia

Spiewok S, Neumann P (2006a) Cryptic low-level reproduction of small hive beetles in honeybee colonies. Journal of Apicultural Research 45:47-48

Spiewok S, Neumann P (2006b) Infestation of commercial bumblebee (*Bombus impatiens*) field colonies by small hive beetles (*Aethina tumida*). Ecological Entomology 31:623-628

Spiewok S, Pettis JS, Duncan M, Spooner-Hart R, Westervelt D, Neumann P (2007) Small hive beetle, *Aethina tumida*, populations I: Infestation levels of honeybee colonies, apiaries and regions. Apidologie 38:595–605

Table 1. Mean and maximum vector distances in mm travelled by SHB from hive entrances over a 90 min period (N= 150). Beetles did not advance further into the hives after 10 min. Three beetles were mummified at hive entrances (*).

HIVE	BEETLE 1		BEETLE 2		BEETLE 3		BEETLE 4		BEETLE 5	
	mean	max	mean	max	mean	max	mean	max	mean	max
1	*5.0	11	10.2	15	12.5	21	14.2	24	18.0	27
2	*4.3	6	9.0	10	12.2	16	20.7	28	44.7	68
3	6.3	11	12.7	24	22.7	32	29.0	35	36.0	53
4	7.0	16	41.7	80	49.0	89	75.0	120	83.7	135
5	*4.0	5	10.7	17	16.3	31	47.7	60	50.3	65

HIVE	BEETLE 6		BEETLE 7		BEETLE 8		BEETLE 9		BEETLE 10	
	mean	max	mean	max	mean	max	mean	max	mean	max
1	21.3	31	30.0	37	49.7	60	60.0	73	69.0	78
2	53.7	84	64.7	88	68.3	93	79.3	95	93.0	96
3	58.0	76	73.7	92	79.3	96	91.3	100	107.0	113
4	92.7	138	108.3	142	116.7	157	132.3	170	157.7	170
5	55.0	68	64.3	79	75.7	97	92.7	112	111.7	115

Fig 1. A *T. carbonaria* worker mummifies a live small hive beetle by coating it with batumen on the beetle's elytra and legs (a) and visual confirmation of a mummified beetle on the floor of a *T. carbonaria* hive (b).
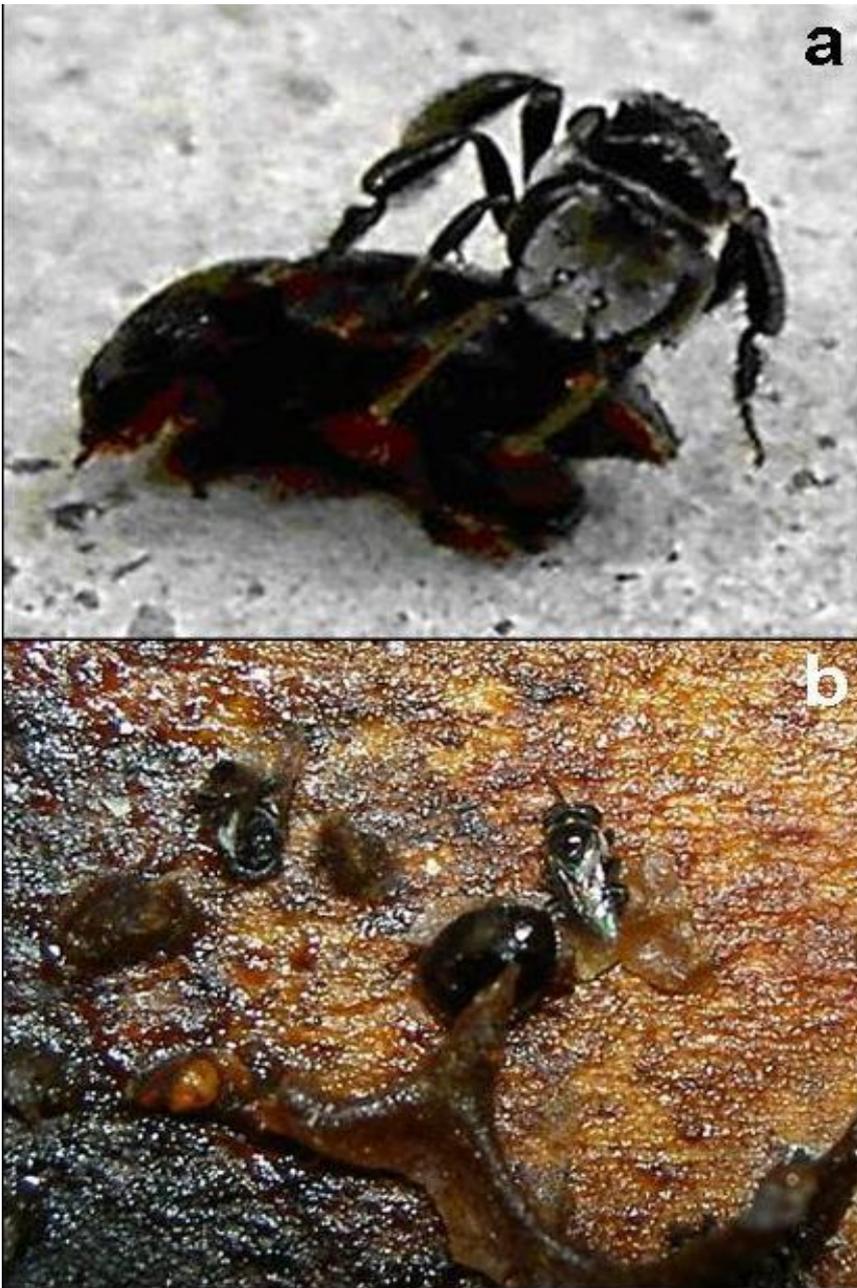
Fig 2. Live mummification of adult small hive beetles in *T. carbonaria* hives: (a) 3D CT image of *T. carbonaria* brood (single arrow) and two small hive beetles below brood (double arrows); (b) 2D CT image of small hive beetles (short arrows) in entrance of *T. carbonaria* hive demonstrating no change in position after 10 min (c) 2D CT image, taken 90min after introduction to the hive, of an

adult small hive beetle which has been mummified by guards of *T. carbonaria* and (d) visual confirmation of the beetle's position and evidence of the batumen coating, applied by guard bees, which prevented further movements by the beetle.