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Similar hive semio chemicals simulate the host potential of African meliponine bees (Hymenoptera: Apidae) to small hive beetle *Aethina tumida* Murray (Coleoptera: Nititulidae) infestations

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Abstract

Most eusocial bees especially honeybees and bumble bees are hosts of the small hive beetle (SHB) *Aethina tumida*, a noxious pest of domesticated and wild pollinator colonies in various parts of the world. During field surveys of meliponine bee nests in Kenya, we found small hive beetles in considerable number inside domesticated hives of the following meliponine bee species, *Meliponula ferruginea* (reddish-brown), *Melipona bocandei* and *Meliponula ferruginea (black)*. In this study, we aimed to investigate whether the presence of small hive beetles in these meliponine bee colonies was merely coincidental or that it was largely due to attraction to similar chemical volatiles present in their hives. We determined the volatiles in comparison with African honey bees, *Apis mellifera scutellata* through coupled gas chromatography/mass spectrometric (GC/MS) analysis. We found that irrespective of the meliponine bee species, a high similarity between the chemical profiles of colony odours released by the three meliponine bee species and that of the African savannah honeybee *Apis mellifera scutellata* were identified. These included a total of eighty compounds of which seventeen are shared components and five had previously been identified as semio chemicals from honeybee odours for the small hive beetle. Our results suggest a strong plasticity in the behaviour of the small hive beetle towards this potential host and that its presence in domesticated colonies of Afro-tropical meliponine bee species may be due to attraction to honeybee-like odours inclusive of already present semio chemicals released by these meliponine colonies. These results may have future implications in the context of domesticating African meliponine bees as alternative pollinators for agricultural crops.

Keywords: meliponine bees, small hive beetles, semio chemicals, honeybees, alternative pollinators

Introduction

African meliponine bees (Hymenoptera: Apidae) belong to the tribe *Meliponini* of which more than 19 species are native to Africa (Eardley *et al.*, 2004) [3], with 14 of these species found in Kenya (Nkoba, 2012) [25]. Typically, a meliponine bee colony contains < 20,000 individuals, comprising of a single fertile queen, drones and workers. Meliponine bees are considered important pollinators of most indigenous flora in both tropical and subtropical parts of the world. (Heard, 1999) [9]. they also pollinate > 90 crop species worldwide (Slaa *et al.*, 2006; Abramson, Wanderley, Wanderley, Silva, & Michaluk, 2007) [32, 1]. In Kenya, examples of some of these crops include bell pepper, cucumber, tomatoes and strawberries. Most meliponine bee species exhibit a degree of plasticity in their nesting sites preferences, building their nests in either tree hollows, abandoned nests of other social insects (ants, termites), which are both above and below-ground environments (Wilson, 1971; Michener 1974; Roubik, 1990) [45, 19, 29]. Some species such as the *Trigona* and *Dactylurina* construct fully exposed aerial nests (Sakagami, 1982) [30] and a key attribute of these nests is their impeccable insulation (Michener 1974) [19]. Their foraged resources are mostly stored in pots, which are sealed with an involucrum sheet, comprising principally of resin and wax. This makes meliponine bee species potential candidates for domestication and use for pollination of crops.

In the face of global managed honey bee population decline, there has been a renewed interest in the search for alternative pollinators, culminating into recent efforts in Kenya to domesticate African meliponine bee species for use in the pollination of crops and to provide ecosystem services (Nkoba *et al.*, 2014) [27]. This brings into question whether domestication of African meliponine bee species would jeopardize meliponine bee health due to exposure to noxious pests such as the small hive beetle (SHB).

The small hive beetle is endemic to sub-Saharan Africa, and a parasite native to African honeybees on which it inflicts negligible damage. In the past two decades, it has become an invasive pest of European honeybees in the Americas, Australia, Asia (Elzen *et al.*, 1999; Neumann and Elzen, 2004; Spiewok *et al.*, 2007; Mutinelli *et al.*, 2014; Neumann *et al.*, 2016) [5, 23, 35, 22, 24], and most recently Europe (Palmeri *et al.*, 2015). It has also been found in the nests of bumble bees, *Bombus impatiens* (Spiewok and Neumann, 2006) [34] and most recently Neo-tropical meliponine bee species of *Trigona* and *Dactylurina* genera in various parts of the world (Greco *et al.*, 2010; Halcroft *et al.*, 2011; Neumann *et al.*, 2016) [7, 8, 24]. However, for Afro-tropical meliponine bee species little is known about their interactions with small hive beetles because small hive beetles are free flyers with differing dispersal abilities and flight activity between male and female beetles, as they have the capacity to colonize a range of social bee colonies (Elzen *et al.*, 2000). Having a clear understanding of the interaction between the small hive beetle and African meliponine bees is key to determining host specificity of the beetle and to develop tools for their control and management in meliponine bee colonies, especially for species which are currently being domesticated in Kenya for pollination services.

One reason why studies on the interaction between meliponine bee species and small hive beetle is important is because of the high similarity in their biology with the honey bee, which makes it possible for the beetle to also reproduce in meliponine bee hives (Fig. 1). Also, since previous studies have shown that pollen, honey and adult workers from honeybee colonies are attractive to small hive beetles (Suazo *et al*., 2003) [38] similar hive matrix components of meliponine bees may also attract small hive beetles. Furthermore, if small hive beetles are attracted to cues derived from meliponine bee colonies, host finding could possibly occur in the field, thereby expanding the host range of potential hosts of this beetle. Here, we compared the chemical profile of the African savannah honey bee *Apis mellifera scutellata* using coupled gas chromatography/mass spectrometric (GC/MS) analysis with that of the three commonly domesticated meliponine bee species used for pollination purposes in Kenya in order to identify potential semio chemicals shared between these two social bee groups and other chemical compounds of known importance. We discuss our results within the context of the domestication of meliponine bees for pollination of crops.

Fig 1: A domesticated colony of *Meliponula ferruginea (black)* *Brood pots and pollen pots are indication of similar biology with the honey bee, *Apis mellifera*.

Materials and Methods

Meliponine bee colonies: We transferred a total comprising of 14 colonies (Fig 2a, b and c) of *Hypotrigona gribodoi*, *Meliponula ferruginea* (black), *M. ferruginea* (reddish brown), *M. bocandei*, *M. lendiliana* and *Plebeina hildebrandti* from a meliponary in Kakamega, western Kenya (0º 30′N 34º 35′E) to a meliponary at the International Centre of Insect Physiology and Ecology (*icipe*), Duduville campus (1º 17′S, 36º 49′E) in Nairobi. These colonies served as sources for the experiments (intact colony odors) used in the chemical analysis.

***Odor sources*:** Odors were derived from queen right meliponine bee species of *Meliponula ferruginea* (black), M*eliponula ferruginea* (reddish-brown) and *Melipona bocandei*. (Fig 2a, b and c).

Fig 2a: Internal nest architecture of *Meliponula ferruginea (black).*

Fig 2b: Internal nest architecture of *Melipona bocandei*

Fig 2c: Internal nest architecture of *Meliponula ferruginea (reddish brown).*

Collection of volatiles: Volatiles were collected separately from three different meliponine bee species and honey bee *A.m. scutellata* colonies in triplicates on pre-cleaned Super Q traps (30 mg, Alltech, Nicholasville, KY) using a mobile air delivery and vacuum pump system (parts assembled at the USDA/ARS-CMAVE, Gainesville, FL USA). A total of 12 honey bee colonies were maintained in standard Kenyan top bar hives at the Bee Health Unit, International Centre of Insect Physiology and Ecology (*icipe*), Duduville campus (1º 17′S, 36º 49′E). Prior to volatile collection, the colonies were examined for cracks, thereafter sealed with propolis from the same colony to minimize background chemical contamination. The honey bee hive entrance was reduced using beeswax and propolis to allow only two entry/exit points (1 cm high x 3 cm wide); one through which the adsorbent filter trap was inserted while the other served as passage for foragers and house bees. For each meliponine bee colony, odour collection was done via one manually drilled entry hole, while the hive entrance was left intact due to its small size and high number of entrance guards, increasing the likelihood of substantial colony disturbance and emission of alarm pheromones if used. Super Q traps were protected with clean wire mesh holders to prevent worker bees from clogging the tips with wax (Torto *et al*., 2007b) [41]. Colony odours were collected on the adsorbent trap by pulling air from within the entire colony at 0.5 L/min for 6 hours. The adsorbed volatiles were eluted with 150 µl of dichloromethane (Sigma Aldrich, Munich, Germany) and stored at -80 °C prior to analyses. In order to obtain representative profiles and to identify components that occur consistently, volatiles were collected from the three different meliponine bees and honeybee hives in triplicates.

Analysis of volatiles: Coupled gas chromatography/mass spectrometric (GC/MS) analysis was carried out on an Agilent Technologies 7890A gas chromatograph equipped with a capillary column HP-5 MS (30 m × 0.25mm ID ×0.25µm film thickness) and coupled to a 5795C mass spectrometer. An aliquot (1 µl) of the extracts of the volatiles obtained from three different intact colonies of *M. ferruginea* (black), *M. ferruginea* (reddish brown), *Melipona bocandei* and *A. m. scutellata*, was injected in the split less mode (Inlet temperature = 250 °C, Pressure = 6.83 psi), and helium was used as the carrier gas at 1.0 ml/min. The injector port was maintained at 280 °C. The oven temperature was then held at 35°C for 5 min, increased to 280 °C at 10 °C/min, and then held at 280 °C for 5.5 min. Mass spectra were recorded at 70 ev. Volatiles from the different intact colonies were identified by comparing their retention times and mass spectral data with those recorded from the NIST 08 spectral library and by co-injection with authentic standards. For compound quantification, peak areas were compared to an external standard corresponding to 5ng/µl of 2-heptanol.

Chemicals: Authentic chemical standards (>95 % purity by GC) of Isopentyl acetate, 2-Heptanone, 2-Heptanol, Octanal, α-Pinene, (*E*)-β-Ocimene, Limonene, Nonanal, Phenylethyl alcohol, Decanal, Octanoic acid, 3-methyl-2-butenyl acetate, Phenol, 6-methyl-5-hepten-2-one, Hexyl acetate, Hexanoic acid, (Z)-Linalool oxide, 2-Nonanone, Undecane, 2-Nonanol, Nonanal, Heptanoic acid, Naphthalene, Dodecane, Octanoic acid, Nonanoic acid, Tridecane, (Z)-Caryophyllene and α-Humulene were purchased from Sigma Aldrich (St. Louis, MO, USA).

Results

Analyses of volatiles

Chemical analyses identified a total of 80 compounds from a diverse range of classes in the volatiles released by intact colonies of honey bee, *Apis mellifera scutellata* and the three meliponine bee species. Of these, the identities of 30 compounds (8 terpenes, 4 esters, 4 hydrocarbons, 3 aldehydes, 4 fatty acids, 3 ketones and 4 alcohols) were confirmed using commercially available synthetic standards, with the remaining 50 compounds identified tentatively by comparison of their mass spectral data with library data only. Of these compounds, 29 were specific to honeybees, while 34 were uniquely associated with *M*eliponine bee species, with 17 compounds identified as common to both species (Fig. 3, Table 1). Honeybee volatiles were dominated by benzenoids such as benzyl alcohol, guaiacol, benzyl acetate, methyl benzoate, methyl salicylate, and ethyl acetophenone, whereas short chain fatty acids, for example, hexanoic acid, heptanoic acid and nonanoic acid and the sesquiterpenes β-bourbonene, (*Z*)-caryophyllene, (*Z*)-α-bergamotene, allo-aromadendrene, α-sequiphellandrene, sesquisabinene and 9-epi-(*E*)-caryophyllene dominated the volatiles of meliponine bee species. The common components identified in the colony volatiles of both honey bees and meliponine bees included a wide range of chemical classes; esters, ketones, alcohols, terpenes, acids, alkanes and aldehydes. They included the honeybee alarm pheromones isopentyl acetate and 2-heptanone which were between 2- and 2.5-fold more in the honeybee than the meliponine bee. Other shared compounds identified were tetradecene, caryophyllene oxide, α-cubebene, α-copaene, α-humulene, σ-cadinene, phenylethyl alcohol, octanoic acid, nonanal, 2-nonanone, 2-heptanol, 3-methyl-2-butenyl acetate, 6-methyl-5-hepten-2-one, (*E*)-β-ocimene, octanoic acid and decanal (Fig 3, Table 1) which were present in relatively similar levels in the volatile emissions of both species

Table 1: Chemicals identified from the volatiles released by intact *Apis mellifera* and *Meliponine bee* species.

Peak No.	Retention time (min)	Compound Name	Honey Bee	Melipona bocandei	M. ferruginea
1	7.43	2,3-heptanediene	-	+	+
2	8.53	Isopentyl acetate‡	+	+	+
3	8.91	2- Heptanone‡	+	+	+
4	9.00	Nonane	+	-	-
5	9.09	2-Heptanol‡	+	+	+
6	9.60	3-methyl-2-butenyl acetate	+	+	+
7	9.76	α-pinene‡	+	-	_
8	10.08	Camphene	+	-	_
9	10.37	Benzaldehyde	-	+	+
10	10.38	Phenol	+	-	_
11	10.93	6-methyl-5-Hepten-2-one	+	+	+
12	11.00	Pentyl furan	-	+	+
13	11.16	Decane	-	+	+
14	11.23	Octanal‡	+	+	+
15	11.37	Isovaleric acid	-	+	+

16	11.43	Hexyl acetate‡	+	-	-
17	11.62	*o*-Cymene	-	+	+
18	11.70	Limonene‡	+	-	_
19	11.71	β-Phellandrene	-	+	+
20	11.76	1,8- Cineole	+	-	-
21	11.82	Benzyl alcohol	+	-	-
22	11.88	Hexanoic acid‡	-	+	+
23	12.06	(*E*)-β-Ocimene‡	+	+	+
24	12.45	Octanol‡	+	-	-
25	12.51	(*Z*)-Linalool oxide (furanoid) ‡	+	-	-
26	12.77	(*E*)-Linalool oxide furanoid) ‡	+	-	-
27	12.79	Guaiacol	+	-	-
28	12.88	2-Nonanone‡	-	+	+
29	12.89	Methyl benzoate	+	-	-
30	12.95	Undecane	-	+	+
31	12.96	2-Nonanol‡	+	-	-
32	13.07	Nonanal‡	+	+	+
33	13.12	6-methyl-3,5-heptadien-2-one	-	+	+
34	13.19	Heptanoic acid‡	-	+	+
35	13.31	Phenyl ethyl alcohol‡	+	+	+
36	13.35	Methyl octanoate	+	-	-
37	14.02	Benzyl acetate	+	-	-
38	14.31	Terpinen-4-ol	-	+	+
39	14.36	Naphthalene	+	-	-
40	14.52	Methyl salicylate	+	-	-
41	14.54	Dodecane	-	+	+
42	14.63	Decanal‡	+	+	-
43	14.70	Octanoic acid‡	+	+	-
44	14.81	(2*E*, 4*E*)-Nonadienal	+	-	+
45	15.53	Ethyl acetophenone	+	-	-
46	15.72	Nonanoic acid‡	-	+	+
47	16.00	Tridecane‡	-	+	+
48	16.03	Carvacrol	+	-	-
49	16.29	(2*E*, 4*E*)-Decadienal	-	+	+
50	16.79	α-Cubebene	+	+	-
51	16.99	Nonalactone	-	+	+
52	17.08	α –ylangene	-	+	+
53	17.16	α-Copaene	+	+	+
54	17.24	Tetradecene	+	+	+
55	17.34	Tetradecane‡	+	-	-
56	17.32	β- Bourbonene	-	+	+
57	17.61	(*Z*)- Caryophyllene	-	+	+
58	17.70	α-Bergamotene <cis>	-	+	+
59	17.78	(*E*)-β-Caryophyllene‡	+	-	-
60	17.85	Allo-Aromadendrene	-	+	+
61	17.91	β- Copaene	-	+	+
62	18.02	α- Sequiphellandrene	-	+	+
63	18.08	Geranyl acetone	-	+	+
64	18.18	Sesquisabinene	-	+	+
65	18.21	α- Humulene‡	+	+	+
66	18.34	9-epi-(*E*)-Caryophyllene	-	+	+
67	18.55	Germacrene D	+	-	+
68	18.56	β-funbrene	-	+	+
69	18.61	Pentadecane	+	-	+
70	18.64	β- Selinene	-	+	+
71	18.74	δ-Gurjunene	+	-	+
72	18.76	α-Muurolene	-	+	+
73	18.81	2,4-bis (1,1-dimethylethylphenol)	+	-	-
74	18.82	Methyl *p*-tert-butyl phenyl acetate	-	+	+
75	18.86	Butylated hydroxyl toluene	+	-	-
76	18.95	δ-Cadinene	+	+	+
77	19.04	δ-Amorphene	-	+	+
78	19.73	Pentadecanol	+	-	-
79	19.74	Hexadecene	-	+	+
80	19.86	Caryophyllene oxide	+	+	+

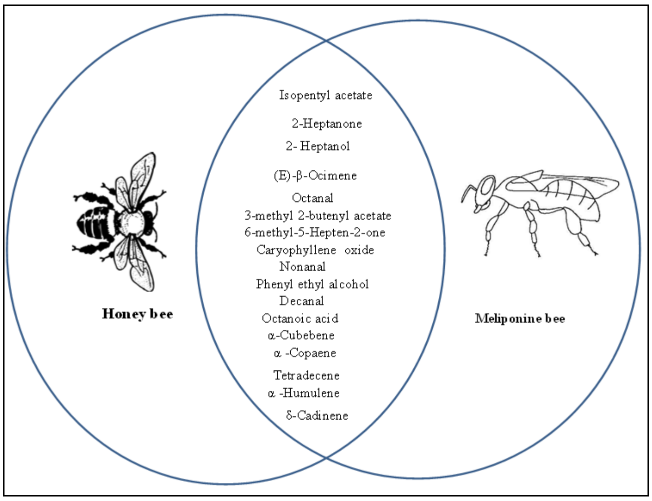

Fig 3: Shared chemical components identified from the volatiles released by intact *Apis mellifera* and *Meliponula ferruginea* colonies.

Discussion

Previous work on meliponine bees have mainly focused on pheromones within and between species (Jarau *et al*., 2003; Strangler *et al*., 2009; Cruz-Lopez *et al*., 2001; Engels *et al*.,1986; Johnson *et al*., 1983, 1985; Smith and Roubik,1983) [12, 37, 2, 4, 13, 31]. In this present study, we identified the chemical profiles of meliponine bee volatiles emitted from whole hives and compared them with the chemical profiles of *Apis mellifera scutellata* whole hives. Our findings reveal similar semio chemicals between meli ponine bees and honey bees which could predispose them to easy infestation by the small hive beetle and then ultimately hamper domestication efforts of meliponine bee species native to Africa. This finding is consistent with other investigations on the honey bee and bumble bee (Palmeri *et al*., 2015; Neumann *et al*., 2016) [24] indicating that bee species which share similar biology and chemical ecology are all suitable hosts for the small hive beetle.

Our findings revealed through comparison of the odor profiles, a high and complex chemical diversity including esters, ketones, aldehydes, terpenes, benzenoids and hydrocarbons emitted by the intact colonies of honeybee and the meliponine bee species *(*Table 1*)*. Most of the compounds identified in the volatiles emitted by the two different bee species have previously been reported as components of floral volatiles (Knudsen *et al*., 1993; Torto *et al*., 2005, 2007b, 2007c; Strangler *et al*., 2009) [16, 44]. However, the level of qualitative similarity between the odor profiles was low (~20%). These results suggest that both nectar and pollen sources may be different for the two different bee species, as previously reported (Vit *et al*., 2013) [39]. Notably, a few of the compounds identified in the volatiles of meliponine bees including isopentyl acetate, 2-heptanone, 2-nonanol, octanoic acid and 2-heptanol are semiochemicals for the small hive beetle which had previously been identified in honey bee volatiles (Torto *et al*., 2005) [44]. Also, the compounds 2-heptanol, 2-heptanone, octanal, hexanoic acid, nonanal, 2-nonanol, 2-nonanone, octanoic acid, decanal and decanoic acid to name a few have been reported as components of the cephalic volatile bouquet of *Scaptotrigona postica* queens (Engels *et al*.,1986) [4], with 2-heptanol as an alarm pheromone component of meliponine bee species such as *Melipona fasciata, Melipona interrupta triplaridis, and Trigona sylvestriana* (Engels *et al*., 1986; Johnson *et al*., 1985; Smith and Roubik,1983) [4, 14, 31]. These further points out that the composition of odor cues of meliponine bee species may serve as a predictor for its attractiveness for these free-flying small hive beetles. Previous studies had shown that meliponine bee colonies that were infested by the small hive beetle were predominantly from the *Trigona* and *Dactylurina* genera (Halcroft *et al.,* 2011; Neumann *et al*., 2004) [8, 23]. Thus, our results suggest that as previously shown in the host location of honeybees by the small hive beetle (Suazo *et al*., 2003; Torto *et al*., 2005; 2007a, Bobadoye *et al*., 2018) [38, 44] that olfaction plays a strong role in the location of these meliponine bee colonies by the beetle as they both share a great number of volatile compounds that facilitate host location.

Finally, it is important to consider the several challenges that may occur when bees are domesticated, key among them being their health due to exposure to pests and pathogens. Consequently, we suggest that the domestication of meliponine bee species for pollination services would require use of well-constructed hives, free of crevices and cracks, which are known to facilitate easy entry by the SHB (Elzen *et al*., 1999) [5]. We also recommend that modifications be made to currently used hives for domesticating meliponine bee species to prevent infestations and expansion of the potential host range and dispersal into new landscapes by the small hive beetle.

In summary, we are highlighting the fact that olfaction plays a role in the attraction of Small hive beetles to meliponine bees and that the small hive beetle has the potential to expand its host range to include various other species of meliponine bees. We have also shown that the similarity in the chemical composition of the volatiles of honey bees and meliponine bees which includes five identified semiochemicals (Isopentyl acetate, 2-nonanol, 2-heptanone and octanoic acid) for the small hive beetle can facilitate this host range expansion. These results could further guide approaches into developing hive designs to effectively domesticate meliponine bees' species for future pollination of crops.

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