African meliponine bees (Hymenoptera: Apidae) maintained in man-made hives as potential hosts for the small hive beetle, Aethina tumida Murray (Coleoptera: Nitidulidae)

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Previous studies have shown that natural honeybee and bumble bee colonies are hosts of the small hive beetle (SHB) *Aethina tumida*, a pest of honeybee colonies in various regions of the world. Recent studies also reported the presence of SHBs in colonies of certain meliponine bee species. In this study, we investigated whether SHBs detect odors of African meliponine bees and their hive matrix components. We also compared the chemical profiles of the honeybee *Apis mellifera scutellata* and meliponine bee odors in order to identify common potential semiochemicals between the two bee species. We used dual-choice olfactometric assays to test the responses of adult male and female SHBs to intact colony odors from six meliponine bee species, namely *Hypotrigona gribodoi*, *Meliponula ferruginea* (black), *M. ferruginea* (reddish-brown), *Plebeina hildbrandti, M. bocandei* and *M. lendiliana* and their hive matrix components including pot honey, pot pollen, cerumen (involucrum) and propolis (batumen). We found that female SHBs responded more strongly to odors from intact colonies, pot honey and pollen from five out of the six species tested than male beetles. Chemical analysis identified several common components in colony odors emitted by both honeybees and a representative meliponine bee species, *M. ferruginea* (black). In particular, nine of these common components previously have been shown in honeybee volatiles to be semiochemicals for the SHB, suggesting that African meliponine bees can also serve as potential alternate hosts for the beetle. The implications of these results are discussed in the context of domesticating African meliponine bees in man-made hives for the pollination of crops.

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Abstract

Previous studies have shown that natural honeybee and bumble bee colonies are hosts of the small hive beetle (SHB) *Aethina tumida*, a pest of honeybee colonies in various regions of the world. Recent studies also reported the presence of SHBs in colonies of certain meliponine bee species. In this study, we investigated whether SHBs detect odors of African meliponine bees and their hive matrix components. We also compared the chemical profiles of the honeybee *Apis mellifera scutellata* and meliponine bee odors in order to identify common potential semiochemicals between the two bee species. We used dual-choice olfactometric assays to test the responses of adult male and female SHBs to intact colony odors from six meliponine bee species, namely *Hypotrigona gribodoi*, *Meliponula ferruginea* (black), *M. ferruginea* (reddish-brown), *Plebeina hildbrandti, M. bocandei* and *M. lendiliana* and their hive matrix components including pot honey, pot pollen, cerumen (involucrum) and propolis (batumen). We found that female SHBs responded more strongly to odors from intact colonies, pot honey and pollen from five out of the six species tested than male beetles. Chemical analysis identified several common components in colony odors emitted by both honeybees and a representative meliponine bee species, *M. ferruginea* (black). In particular, nine of these common components previously have been shown in honeybee volatiles to be semiochemicals for the SHB, suggesting that African meliponine bees can also serve as potential alternate hosts for the beetle. The implications of these results are discussed in the context of domesticating African meliponine bees in man-made hives for the pollination of crops.

Key words: *Aethina tumida*/small hive beetle/ stingless bees /honeybee /behaviour

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), with 6 of these species found in Kenya (Nkoba, 2012). Typically, a meliponine bee colony contains approximately 20,000 individuals, comprising of a single fertile queen, drones and workers. They pollinate approximately 90 crop species worldwide (Heard, 1999); Slaa *et al.,* 2006; Abramson, *et al*., 2007;). In Kenya, examples of some of the crops that are pollinated by meliponine bees include green pepper, cucumber, tomatoes and carrots. In Africa, meliponine bees are true generalists with regards to their nesting sites (Hubbell & Johnson, 1977; Roubik, 1990; Eltz *et al.,* 2002; Nkoba *et al.,* 2012). The majority of these bee species build their nests in either tree hollows, abandoned nests of other social insects (e,g ants, termites), which are both above- and below-ground environment (Wilson, 1971; Michener 1974; Roubik, 1990), thereby exhibiting a degree of plasticity in their nesting sites. Some species such as the *Trigona* and *Dactylurina* construct fully exposed aerial nests (Sakagami, 1982). One of the attributes of the majority of meliponine bee nests is their impeccable insulation of the entire hive (Michener 1974). Their foraged resources are mostly stored in pots, which are sealed with an involucrum sheet, comprising principally resin and wax. This makes the stingless bee colony an ideal candidate for domestication and use for pollination of crops.

In the face of global honeybee 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 in man–made hives for use in the pollination of crops and to provide ecosystem services (Nkoba *et al.,* 2014). This brings into question whether domestication of African meliponine bee species in fabricated hives would jeopardize meliponine bee health with regard to exposure to pathogens and pests such as the small hive beetle.

The small hive beetle (SHB), a parasite native to African honey bees on which it inflicts negligible damage, has in the past two decades become an invasive pest of European honey bees in the Americas, Australia, Asia and most recently Europe (Spiewok *et al*., 2007; Elzen *et al*., 1999; Neumann and Elzen, 2004; Mutinelli *et al.,* 2014; Neumann *et al*., 2016). It has also been found in the nests of bumble bees (Spiewok and Neumann, 2006) and most recently some meliponine bee species in various parts of the world including Kenya (Greco *et al.,* 2011;

Halcroft *et al.,* 2011; Neumann *et al.,* 2016; Nkoba, 2012), clearly demonstrating the capacity of the beetle to adversely affect a range of social bee colonies. Understanding the interaction between the SHB and African meliponine bees is key to developing tools for management of the SHB infestations in meliponine bee colonies, especially for those maintained in man-made hives.

The objective of this study was to a) examine the influence of odors released by different African meliponine bee species and their hive matrix components on responses of the SHB, and b) compare the chemical profiles of honeybees with those of meliponine bees in order to identify potential semiochemical signatures for these two social bee groups. We discuss our results in the context of the domestication of meliponine bees in man-made hives for pollination of crops.

Materials and Methods

Insects

Meliponine bee colonies

In July 2013, two colonies each of *Hypotrigona gribodoi*, *Meliponula ferruginea* (black), *M. ferruginea* (reddish brown), *M. bocandei, M. lendiliana* and *Plebeina hildebrandti* maintained in a meliponary in Kakamega (Nkoba *et al.,* 2012) in western Kenya (0º 30′N 34º 35′E) were transported to the meliponary of 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 experimental hive components (pot honey, pollen, batumen (propolis) and involucrum (cerumen)) used in all behavioral assays and chemical analysis.

Small hive beetles

Adult (10-14 days old) *Aethina tumida* populations used in this study, were maintained in the laboratory colony as described by Suazo *et al*. (2003) and Torto *et al.* (2010). Prior to each behavioral assay, beetles were starved of food and water for 24 hr and only individuals showing no signs of physical injury were used in the assays.

Odor sources

Odor sources from six meliponine bee species, namely *H. gribodoi*, *Meliponula ferruginea* (black), *M.ferruginea* (reddish-brown), *Plebeina hildbrandti, M. bocandei* and *M. lendiliana* colonies and their matrix components including pot honey, pot pollen, cerumen (involucrum) and propolis (batumen).

Dual choice olfactometer assays

The behavioral responses of adult male and female SHBs (10-14 days old) to stingless bee odors were studied using a dual choice olfactometer mounted on to a Perspex platform (19.5cm length and 9.5 cm width). The olfactometer consisted of a large Perspex Petri dish (9 cm in diameter) glued between two small Perspex Petri dishes (6 cm in diameter). The Petri-dishes had holes (1cm in diameter) drilled at the point of connection and the opposite ends of the smaller dishes which were connected to Teflon tubing to serve as entry/exit points for the SHB. A 1-cm wide hole drilled into the centre of the lid of the large dish connected the olfactometer to a vacuum pump (**Fig. 1**). The vacuum pump (parts assembled at the USDA/ARS, Gainesville, FL, USA) pushed and pulled charcoal-purified air through the olfactometer at 0.5L/min into two quick fit glass chambers (22.5cm length and 7.5 cm width). One chamber held the test odor (10g of each hive component), with the second chamber into which purified air only was passed to serve as the blank (control).

For experiments involving intact stingless bee colony odors, two holes were drilled on opposite sides of the hive to push and pull clean air in and out of the colony respectively. The hive entrances were left open to ensure normal colony activity and only those colonies with more than one entrance had all the other closed except one. The tips of Teflon tubes connected to the hives were plugged with clean screen mesh wire to prevent worker bees from clogging them with wax during volatile collection. Odors from each stingless bee colony were transferred via the Teflon tubes into the small Petri dishes of the olfactometer at 120 ml/min in each odor chamber in a room maintained at 26 ˚C and 70 % relative humidity. A red 25 W bulb placed 50 cm above the olfactometer evenly illuminated the experimental arena. Each starved beetle was used only once in the assays.

The behavioral responses of both sexes of the SHB to matrix component odors from each bee species were studied between 16:00 - 20:00 hr to coincide with optimal activity of the beetles (Suazo *et al.,* 2003). Twenty-five individuals of both sexes of the beetle were introduced into the olfactometer and the time spent to make a choice during a 10 min period was recorded. To minimize positional bias, positions of the treatments and blank olfactometer chambers were interchanged after five replicates. Intact colonies of the six species and their matrix components (pot honey, pot pollen, involucrum (cerumen) and batumen (propolis)) were tested for SHB responses.

Collection of volatiles

Volatiles were collected separately from one intact *M. ferruginea* (black) colony and a honey bee *Apis mellifera scutellata* colony 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). 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 the stingless bee colony, odour collection was done via one 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 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). Intact colony odours were collected on the adsorbent trap by pulling air from within the entire colony at 0.5 L/min for 6 hr. The adsorbed volatiles were eluted with 150 µl of dichloromethane (Sigma Aldrich, Munich, Germany) and stored at -80 °C prior to analysis. In order to obtain representative and profiles and to identify components that occur consistently, volatiles were collected from intact stingless bee and honeybee colonies 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 the intact colonies of *M. ferruginea* (black) 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 from the NIST 08 library and confirmed using commercially available authentic standards.

Chemicals

Authentic chemical standards (>95 % purity by GC) of Isopentyl acetate, 2- Heptanone, 2-Heptanol, α-pinene, Camphene, Decane, Octanal , Hexyl acetate, Limonene, Hexanoic acid, (*E*)-β-Ocimene, Octanol, (*E*)-Linalool oxide (furanoid), (*Z*)-Linalool oxide (furanoid), Methyl octanoate, 2-Nonanone, Methyl benzoate, Undecane, 2-Nonanol, Nonanal, Heptanoic acid, Phenyl ethyl alcohol, Dodecane, Decanal, Octanoic acid, Nonanoic acid, Tridecane, Tetradecane, (*E*)-β-Caryophyllene and α- Humulene were purchased from Sigma Aldrich (St. Louis, MO, USA).

Statistical Analysis

The time spent by each beetle in each odor zone was expressed as a proportion of the total recorded time and subjected to compositional transformation to homogenize the data variances. The transformed data were then subjected to: (1) t-tests separately comparing male and female responses to test odors versus control; (2) t-tests comparing male and female beetle responses to same test odor from each species; (3) two- way ANOVA comparing responses of both sexes to odors of the same hive component across all 6 species and (4) analysis of variance (ANOVA) separately comparing male and female responses to hive component odors from the same stingless bee species; The preference index of male and female beetles to each treatment was computed and used as a measure of attractiveness of hive components.

Results

Olfactometer assays

Intact colony odors

SHB responses to intact colony odors differed significantly between the two sexes (**Table 1**). Females significantly preferred colony odors of all the six species compared to the air controls; *H. gribodoi* ($t_{1, 48}$ = 12.70 P <0.001), *M. ferruginea* (black) ($t_{1, 48}$ =6.81, P <0.001), *M. ferruginea* (reddish-brown) ($t_{1, 48}$ = 7.86 P <0.001), *P. hildbrandti* ($t_{1, 48}$ = 8.42 P <0.001) *M. bocandei* ($t_{1, 48}$ = 11.348, P<0.001) and *M. lendiliana* ($t_{1, 48}$ = 7.86 P <0.001) respectively, whereas males significantly preferred odors of *M. ferruginea* (black) ($t_{1, 48}$ = 7.58, P<0.001). In pair-wise comparisons, female responses to colony odors from five out of the six species; *H. gribodoi* ($t_{1, 48}$ = 8.95, P<0.001), *M. ferruginea* (reddish-brown) ($t_{1, 48}$ = 7.82, P<0.001), *M. bocandei* ($t_{1, 48}$ = 8.97, P<0.001),*P. hildbrandti* ($t_{1, 48}$ = 7.04, P = 0.027) and *M. lendiliana* ($t_{1, 48}$ = 8.43, P < 0.001), were significantly greater than those recorded for males (**Fig. 2**).

Pot honey odors

In pair-wise comparisons, male SHBs were significantly attracted to pot honey odors compared to the control (air) in all but one stingless bee species *M. bocandei* ($t_{1, 48}$ = 1.54 P = 0.13) (**Fig. 2A**). Likewise, female beetles were significantly attracted to honey odors from four species with non-significant responses recorded for *M. ferruginea* (black) ($t_{1, 48}$ = 1.308 P = 0.19) (**Fig. 2A**) and *M. bocandei* ($t_{1, 48}$ = 1.607 P = 0.11) (**Fig. 2**). Responses of both sexes of the beetle to honey odors obtained from each bee species were not statistically different (**Fig. 2**).

Pollen odors

Male SHBs were significantly attracted to pollen odors obtained from colonies of *H. gribodoi* ($t_{1, 48}$ = 2.88 P = 0.006), *M. ferruginea* (black) ($t_{1, 48}$ =3.59, P <0.001) and *M. bocandei* ($t_{1, 48}$ = 2.72, P = 0.009). The preference for pollen odors obtained from colonies of *M. ferruginea* (reddish-brown) ($t_{1, 48}$ = -0.135, P = 0.893), *P. hildbrandti* ($t_{1, 48}$ = 0.44, P = 0.661) and *M. lendiliana* ($t_{1, 48}$ = -0.527, P = 0.601) (**Fig. 2**) were not significant. Unlike males, females significantly preferred pollen odors from all species compared to the air controls (Fig 2B). In pair-wise comparisons, female responses to pollen odors from *P. hildbrandti* ($t_{1, 48}$ = 2.28, P = 0.027) and *M. lendiliana* ($t_{1, 48}$ = 4.03, P < 0.001) colonies were significantly greater than those of males (**Fig. 2**).

231 ***Involucrum (cerumen) odors***

232 The odors from involucrum obtained from *M. ferruginea* (reddish-brown) ($t_{1, 48}$ = -3.96 P <
233 0.001) and *P. hildebrandti* ($t_{1, 48}$ = -3.55, P < 0.001) colonies elicited significant avoidance
234 response from males compared to controls (Fig. 2C). With the exception of involucrum odors
235 from *M. ferruginea* (black) ($t_{1, 48}$ = - 0.003, P = 0.998), similar odors from the other five
236 meliponine bee species differed significantly among females compared to the respective controls
237 (**Fig. 2**). In pair-wise comparisons between the sexes, females were significantly more attracted
238 than males to involucrum (cerumen) odors originating from *M. bocandei* ($t_{1, 48}$ = 2.17, P = 0.035)
239 and *M. lendiliana* colonies ($t_{1, 48}$ = 3.29, P = 0.002) (**Fig. 2**).
240
241 ***Batumen (propolis)***
242 In pair-wise comparisons between the sexes to batumen (propolis) odors, male SHBs were
243 significantly attracted only to propolis odors from *M. ferruginea* (black) ($t_{1, 48}$ = 2.29, P = 0.026)
244 and *P. hildebrandti* ($t_{1, 48}$ =2.59, P = 0.013) (**Fig. 2**) while females showed substantial attraction
245 for propolis of *M. ferruginea* (black) ($t_{1, 48}$ = 2.24, P = 0.03), *M. bocandei* ($t_{1, 48}$ = 6.28, P <
246 0.001) *P. hildebrandti* ($t_{1, 48}$ = 2.99, P = 0.004), and *M. lendiliana* ($t_{1, 48}$ = 1.98, P = 0.005)
247 colonies (**Fig. 2**). Females showed more significant attraction than males ($t_{1, 48}$ = 7.18, P < 0.001)
248 to propolis odors from *M. bocandei* (**Fig. 2**).
249
250 ***Analysis of volatiles***
251 Chemical analyses identified a total of 80 compounds from a diverse range of classes in the
252 volatiles released by intact colonies of both honeybees and the stingless bee species *M.*
253 *ferruginea* (black) (Fig. 3, Table 2). Of these, the identities of 30 compounds (8 terpenes, 4
254 esters, 4 hydrocarbons, 3 aldehydes, 4 fatty acids, 3 ketones and 4 alcohols) were confirmed
255 using commercially available synthetic standards, with the remaining 50 compounds identified
256 by comparison of their mass spectral data with library data only (**Table 2**). Of these
257 compounds, 29 were specific to honeybees, while 34 were associated with *M. ferruginea* (black),
258 with 17 compounds identified as common to both species (**Fig 3, Fig. 4**). Honeybee volatiles
259 were dominated by benzenoids such as benzyl alcohol, guaiacol, benzyl acetate, methyl
260 benzoate, methyl salicylate and ethyl acetophenone, whereas short chain fatty acids, for example,
261 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 stingless bee volatiles. The common components identified in the colony volatiles of both species included a wide range of chemical classes; esters, ketones, alcohols, terpenes, acids, alkanes and aldehydes. The honeybee alarm pheromones isopentyl acetate, 2-heptanone and 3-methyl-2-butenyl acetate, were identified as common to the volatiles of both species of bees, but the two compounds were detected in relatively lower levels in the stingless bee volatiles.

Discussion

This study investigated the behavioral responses of the small hive beetle (SHB) to odors from six African meliponine bee species, namely *Meliponula ferruginea* (black), *M. ferruginea* (reddish-brown), *M. bocandei*, *M. lendiliana, Plebeina hildbrandti* and *Hypotrigona gribodoi*, and found that both sexes of the beetle responded differently to the odors released from intact colonies of these meliponine bee species as well as their matrix components, including pot honey, pot pollen, cerumen and propolis. Previous work on stingless 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). Our findings provide the first behavioral evidence of SHB attraction to stingless bee species.

In general, females of the SHB responded more strongly to the different odors than males. These results suggest a number of reasons to account for these differences. Firstly, there could be sex variation in the sensitivity of the SHB to detect and process meliponine bee odors both at the peripheral and central nervous systems to successfully carry out biological processes such as feeding and reproduction. For instance, it would be advantageous for females to be more responsive to a wide range of stingless bee colony volatiles than males because an intact colony rich or limited with food resources such as pollen and honey would be essential for feeding by females to reach sexual maturity early and for pheromone production to attract the opposite sex for mating and egg development in mated females (Mustafa *et al*., 2015). On the other hand, since males were more responsive to only the colony odors of *M. ferruginea* (black) suggests that they may appear to be more selective in their nutritional needs than females. Secondly, whole colony matrix component quantity and concentration such as the number of stingless bees and

caste developmental stages, amounts of honey, pollen, cerumen and propolis present in a colony at the time of assays, would all contribute to the quality of the odor signal detected and behavioral response elicited in both sexes of the beetle. Our data appears to match these suggestions as shown by the strong responses of females to the different odor sources especially pollen odors compared to males across the six meliponine bee species studied. They are also in agreement with a previous study, which showed that female SHBs showed a stronger dose-dependent response than males in wind tunnel assays to odors of fresh pollen obtained from honeybee colonies (Suazo *et al.,* 2003). However, further studies are required to investigate these suggestions.

Interestingly, despite the strong responses to colony, pollen and honey odors, both sexes of the beetle responded rather weakly or even avoided odors of cerumen from certain meliponine species. Notably, odors that were emitted by cerumen obtained from the stingless bee species *Meliponula ferruginea* (black) and *Plebeina hildbrandti* were avoided by both sexes of the beetle, suggesting that they may contain repellents. Cerumen is a mixture of pure plant resins and bee secretions molded into protective sheaths in the brood and food chambers in most meliponine bees (Greco *et al*., 2009). It is known to possess anti-bacterial, anti-fungal and anti-predatory properties (Patricio *et al.,* 2002; Lehmberg *et al.,* 2008). Consistent with our observations for SHB responses to other matrix components, there were also notable sex variation responses to cerumen odors emitted, in particular by *M. ferruginea* (reddish-brown), *M. bocandei* and *M. lendiliana*. The basis for these differences is unknown, but it appears that it may be associated with the quality and quantity of the odor emitted by the whole hive matrix component. These results suggest that more detailed research is needed to investigate the pattern of responses of the SHB to colony and matrix component odors at different seasons.

A comparison of the odor profiles revealed a high and complex chemical diversity including esters, ketones, aldehydes, terpenes, benzenoids and hydrocarbons emitted by the intact colonies of honeybee and the stingless bee species *M. ferruginea*. Most of the compounds identified in the volatiles emitted by the two different colonies have previously been reported as components of floral volatiles (Knudsen *et al.*, 1993; Torto *et al.,* 2005, 2007b, 2007c; Strangler *et al.*, 2009). However, the level of qualitative similarity between the odor profiles was low (~20%). These results suggest that the nectar and pollen sources may be different for the two different bee

323 species in accordance with the fact that these two bee species show preference to certain plants
324 as pollen and nectar sources (Vit *et al.,* 2013). Although this study did not investigate the
325 volatiles emitted by the other five stingless bee species, we hypothesize that a similar chemical
326 diversity may be present in their odor profiles. Additional studies are needed to test this
327 hypothesis. Notably, a few of the compounds identified including isopentyl acetate, 2-heptanone,
328 octanal, hexyl acetate, nonanal, 2-nonanone, methyl benzoate and decanal are semiochemicals
329 for the SHB (Torto *et al.,* 2005). Of these semiochemicals, the honeybee alarm pheromones
330 isopentyl acetate and 2-heptanone, and the aldehydes octanal and nonanal, are also constituents
331 of the volatiles of *M. ferruginea* (black). Also, the compounds 2-heptanol, 2-heptanone, octanal,
332 hexanoic acid, nonanal, 2-nonanol, 2-nonanone, octanoic acid, decanal and decanoic acid to
333 name a few have been reported as components of the cephalic volatile bouquet of *Scaptotrigona*
334 *postica* queens (Engels *et al*.,1986) , with 2-heptanol as an alarm pheromone component of the
335 stingless bees *M. fasciata, M. interrupta triplaridis, and Trigona sylvestriana* (Engels *et al*.,
336 1986; Johnson *et al*., 1985; Smith and Roubik,1983).
337 Previous studies had shown that stingless bee colonies that were infested by the SHB were
338 predominantly from the *Trigona* and *Dactylurina* genera (Halcroft *et al.,* 2011; Neumann *et al*.,
339 2004). Thus, our results clearly show that, like previously shown in the host location of
340 honeybees by SHBs (Suazo *et al*., 2003; Torto *et al*., 2005; 2007a), olfaction also plays a major
341 role in the host location process of the beetle in locating stingless bee colonies. As such, we
342 suggest that the domestication of stingless bee species in fabricated man-made hives for
343 pollination services would require use of well-constructed hives, free of crevices and cracks,
344 which are known to facilitate easy entry by the SHB into man-made honeybee hives (Elzen *et al*.,
345 1999). We also suggest that, and an efficient maintenance schedule for these man-made hives
346 may be required to prevent infestations and expansion of the potential host range and dispersal
347 into new landscapes by the SHB.

348 In summary, we have shown that the olfactory responses of SHBs to stingless bee volatiles can
349 vary based on the species of the bee and its matrix components. We have also shown that sex of
350 the SHB can also determine its responses to these different odor sources, and that the volatile
351 profiles of both honeybees and stingless bees can be very complex and diverse, but a small
352 proportion of it is identical. Thus, our results suggest that the SHB has the potential to expand its

353 host range to include various species of meliponine bees, requiring that our quest to domesticate
354 stingless bees' species in man-made hives for future pollination of crops warrants further study.

355

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FIGURE CAPTIONS:

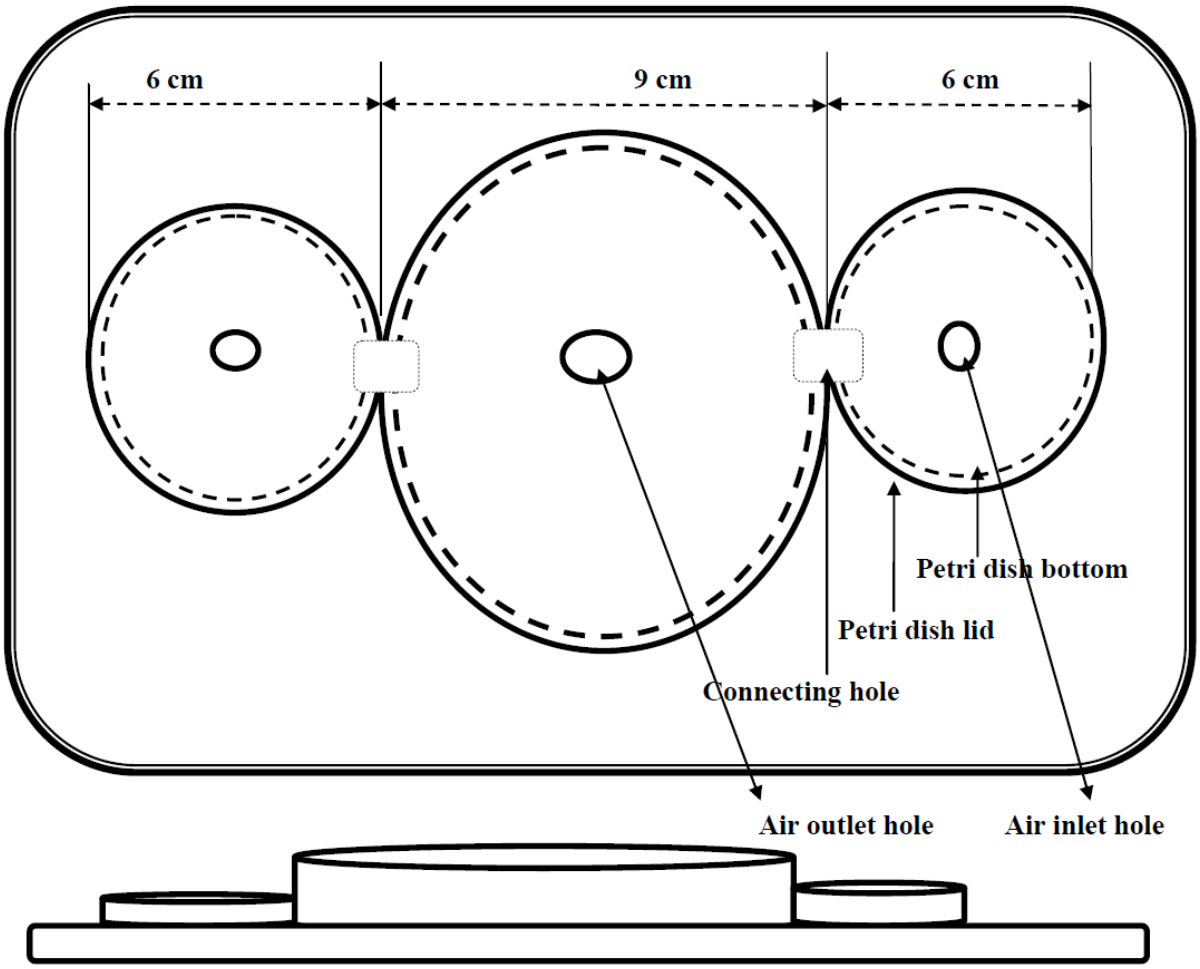

Figure 1: Olfactometer setup.

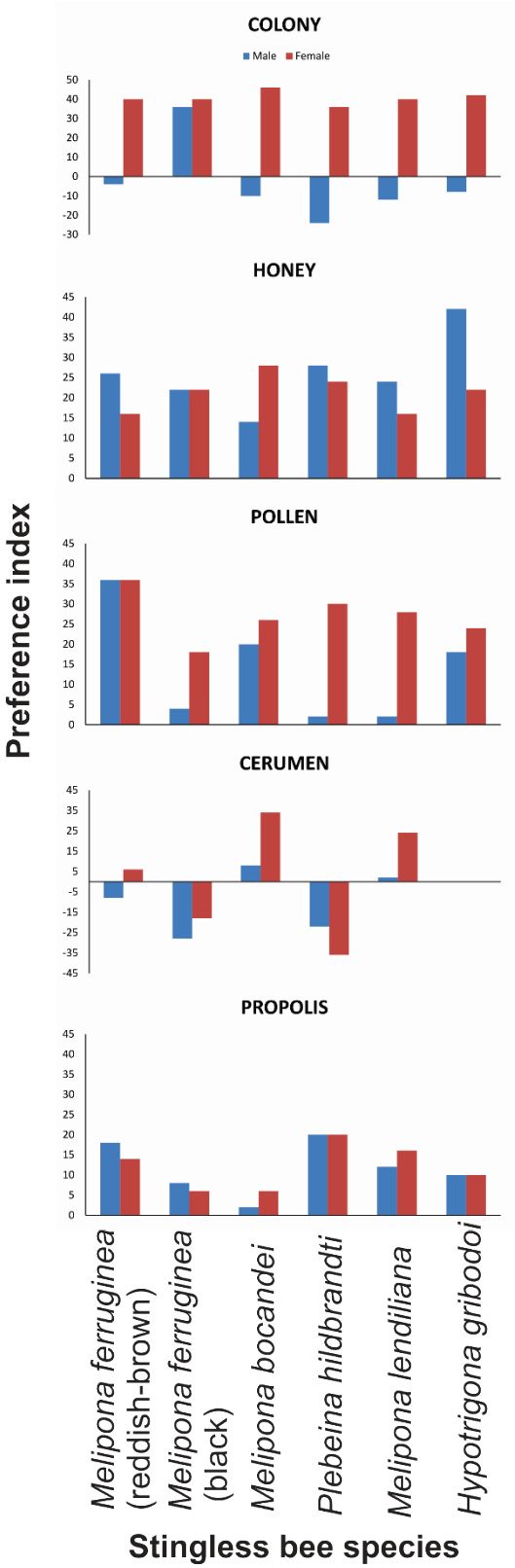

Figure 2: **Male and female SHBs responses to individual and whole hive components**.

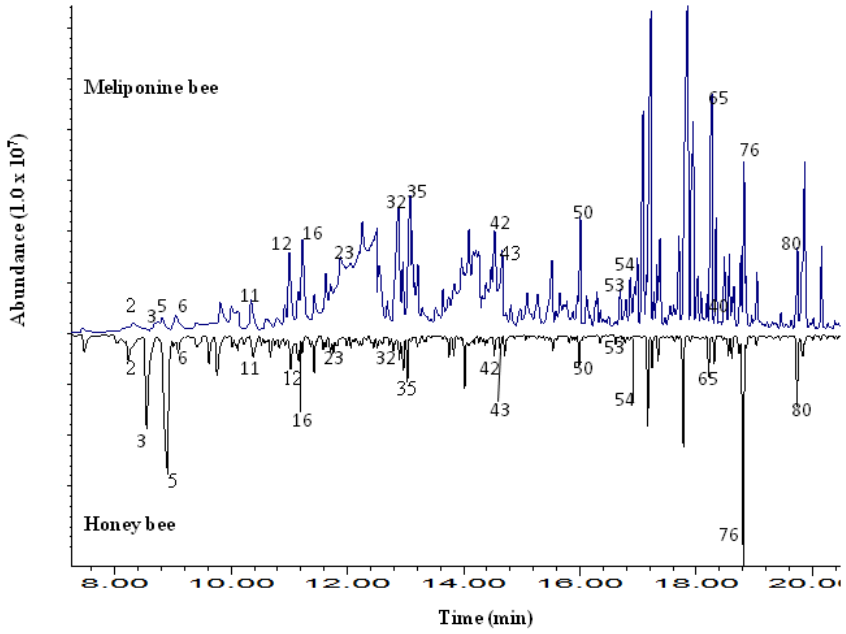

Fig 3: A representative chromatogram of chemical components of whole hive odors in Meliponine (*Meliponula ferruginea* (black) and Honeybees (*Apis mellifera scutellata*).

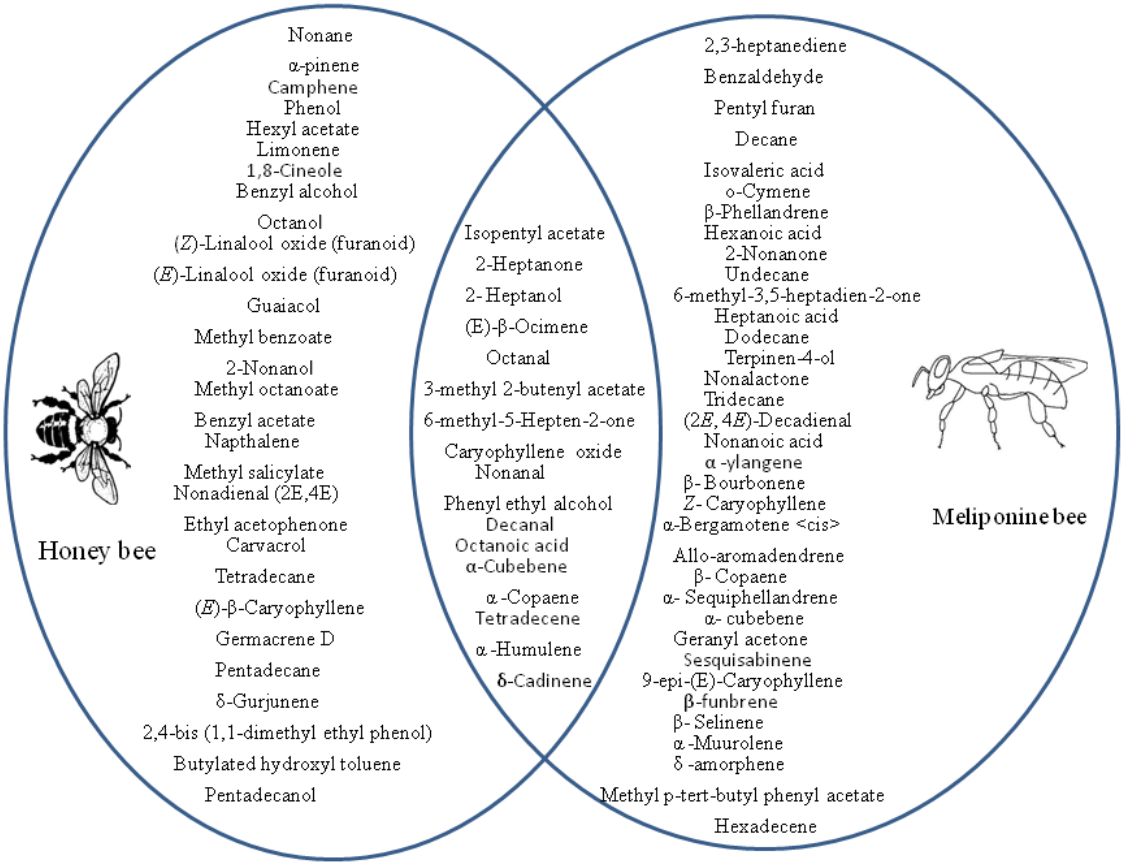

Fig 4: A representative diagram of unique and common chemical components of whole hive odors in Meliponine bees (*Meliponula ferruginea*) and Honeybees (*Apis mellifera scutellata*)

552 **TABLES**:

553

554 **Table 1: A summary of the ANOVA of *Aethina tumida* responses to odors of four hive**
555 **components from six Afro tropical stingless bee species**

	Pot honey	Pollen	Involucrum	Batumen	F value	df	P value
			Males				
Hypotrigona gribodoi	8.1328 ± 0.44A	2.5092 ± 0.50Bab	NA	2.0436 ± 0.64 C	11.099	2, 72	**<0.001**
Meliponula ferruginea (black)	2.5348 ± 0.50AB	1.2724 ± 0.28Bb	0.5384 ± 0.23BC	4.9376 ± 0.60AB	10.139	3, 96	**<0.001**
M. ferruginea (reddish-brown)	3.4796 ± 0.60	2.3136 ± 0.53ab	0.8472 ± 0.32	1.3664 ± 0.35	2.281	3, 96	0.084
P. hildebrandti	4.3068 ± 0.64A	2.5892 ± 0.54ABab	0.7756 ± 0.29B	3.0288 ± 0.58AB	3.624	3, 96	**0.016**
M. bocandei	3.89 ± 0.71	2.91 ± 0.61a	2.9016 ± 0.66	2.91 ± 0.35	0.726	3, 96	0.539
M. lendiliana	3.824 ± 0.36A	2.5308 ± 0.37ABab	1.3164 ± 0.32B	1.702 ± 0.33AB	3.521	3, 96	**0.018**
F value	1.546	2.849	1.581	1.111			
Df	5, 144	5, 144	4, 120	5, 144			
P value	0.179	0.017	0.184	0.357			
			Females				
Hypotrigona gribodoi	6.148 ± 0.84	3.1764 ± 0.62	NA	2.4916 ± 0.64b	2.009	2, 72	0.142
Meliponula ferruginea (black)	3.4108 ± 0.53	2.1684 ± 0.64	1.40166666666667 ± 0.40b	4.7596 ± 0.69Ab	2.663	3, 96	0.052
M. ferruginea (reddish-brown)	4.3688 ± 0.77A	3.37 ± 0.63AB	0.294 ± 0.08Bb	2.3712 ± 0.54ABb	3.962	3, 96	**0.011**
P. hildebrandti	3.5872 ± 0.65A	3.9808 ± 0.65A	0.3212 ± 0.13Bb	2.8476 ± 0.61Ab	7.648	3, 96	**<0.001**
M. bocandei	3.8792 ± 0.50B	4.1448 ± 0.68B	4.33 ± 0.36 Aba	6.2216 ± 0.29Aa	3.436	3, 96	**0.020**
M. lendiliana	4.3716 ± 0.35AB	4.6944 ± 0.35A	3.566 ± 0.35Aba	2.264 ± 0.36Bc	2.998	3, 96	**0.034**
F value	0.695	0.611	8.115	5.181			
Df	5, 144	5, 144	4, 120	5, 144			
P value	0.628	0.692	**<0.001**	**<0.001**			

556 Row means followed by the sample capital letter are not significantly different.

557 *Column means followed by the same small letter are not significantly different.

558 P-values in bold are indicate statistically different comparisons

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566 **Table 2**: **Chemicals identified from the volatiles released by intact** *Apis mellifera* **and**
567 *Meliponula ferruginea* **colonies.**

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Peak No.	Retention time (min)	Compound Name	Honey Bee	Meliponine Bee
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	+	+

‡Refers to compounds whose identities were confirmed with commercial synthetic standards

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