MS BERNADETTE WITTWER (Orcid ID : 0000-0002-5531-2939)

PROFESSOR MARK ELGAR (Orcid ID : 0000-0003-0861-6064)

Article type : Research Paper

Non-volatile chemicals provide a nest defence mechanism for stingless bees *Tetragonula carbonaria* (Apidae, Meliponini)

Shijie Wang*, Bernadette Wittwer†, Tim A. Heard‡, Jason Q. D. Goodger† Mark A. Elgar†

* Environment and Plant Protection Institute, Chinese Academy of Tropical Agricultural Sciences, Haikou, Hainan 571101, People's Republic of China

† School of BioSciences, University of Melbourne, Victoria 3010, Australia

‡ School of Life and Environmental Sciences, University of Sydney, NSW 2006, Australia

Keywords: propolis; *Corymbia* resin; ant deterrence; nest defence; social insects

Words: 4302 (includes everything in this document)

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the [Version of Record](#). Please cite this article as [doi: 10.1111/eth.12768](#)

This article is protected by copyright. All rights reserved

24

25

26

27

28 Correspondence:

29 Mark Elgar, School of BioSciences, University of Melbourne,

30 Melbourne, Victoria 3010, Australia

31 E-mail: m.elgar@unimelb.edu.au

32 Abstract

33 Social insects construct nests that protect their brood and food resources from both the physical environment and
34 natural enemies. Stingless bees use plant-derived resins, mixed with wax to form propolis, in the construction of their
35 nests, and these products can be effective sources of defence against natural enemies, including ants. However, it is
36 not known whether this defence, in the form of deterring or repelling workers, derives from the physical properties
37 or chemical compounds of these products. The nest entrance of *Tetragonula carbonaria* is constructed with propolis
38 and *Corymbia* resins, and we ask whether non-volatile chemicals present in these products act as a defence against
39 ants. Our field experiments revealed that workers of *Iridomyrmex mayri* Forel were deterred from crossing a
40 chemical barrier comprising non-volatile, non-polar (hexane) extracts of propolis and *Corymbia* tree resin. However,
41 polar (ethanol) extracts did not have this effect. These data are the first to demonstrate that the chemical
42 components alone of entrance propolis and resins can provide a nest-defence function against ants.

43 Introduction

44 Social insects face a range of natural enemies, including conspecifics, predators, parasites, pathogens and
45 parasitoids that benefit from gaining access to the vulnerable larval stages and food stores that are
46 concentrated within the nest (Hermann 1984). There is an extraordinary diversity of defence mechanisms that
47 have evolved apparently in response to these threats, and they broadly relate to the architecture of the nest,
48 and the morphological and behavioural characteristics of the defending workers. Thus, social insect nests are
49 typically characterized by physically impenetrable walls, with very few entrances that are vigorously defended
50 by individual workers (Hermann 1984). Included in the armoury of defending workers are enlarged mandibles
51 for biting; morphological features that block the nest entrance; chemical defences that include stings; or the
52 sheer numbers of defending individuals (Hermann 1984; Michener 2007; Shorter & Rueppell 2012).

Our understanding of the defence mechanisms of social bees is dominated by studies of *Apis*, with their ‘suicidal’ sting arguably being the best-known defence mechanism in social insects (Breed et al. 2004). Although stingless bees (Meliponini) lack the capacity to sting their enemies, they nevertheless have numerous means of defence, including biting, producing unpleasant odours, and using sticky materials to immobilise enemies (Wille 1984). Some species of stingless bees are highly eusocial, forming complex nests that contain both food materials and brood (Vit et al. 2013; Heard 2016; Leonhardt 2017). Furthermore, their nests are vulnerable to a range of natural enemies, including wasps, flies, beetles, mites and ants that consume the honey and juvenile stages, attack the adults, and often destroy the internal structure of the nest (Roubik 1989). Species of smaller, mass-recruiting ants may exert a significant predatory selection pressure on these bees, as they can gain access through existing entrance holes and overwhelm the nest by deploying large numbers of attacking workers (see also Roubik 2006).

The building materials used to construct the nests of some meliponine bees can include substances that appear to be particularly effective against predators (see also Roubik 2006). These materials include *Corymbia* resins and propolis (Simone-Finstrom & Spivak 2010), a mixture of resinous materials and bee secretions including beeswax (Tomás-Barberán et al. 1993; Bankova & Popova 2007; Massaro et al. 2011), salivary enzymes and lipids (Popova et al. 2014). Resin and propolis are used to construct specific architectural features in the nest, such as honeypots and pillars (Roubik 2006), and may minimize pathogen infection by preserving a sterile environment (Patricio et al. 2002; Sawaya et al. 2004; Bankova & Popova 2007; Lehmberg et al. 2008; Massaro et al. 2011; Massaro et al. 2015). Importantly, bees also immobilize intruders by actively applying the sticky resin to their cuticle, effectively mummifying them (Pasteels et al. 1983; Neumann et al. 2001; Ellis et al. 2003; Roubik 2006; Greco et al. 2010; Halcroft et al. 2011; Leonhardt 2017). While field experiments showed that the resinous material acts as an ant deterrent (Duangphakdee et al. 2009), it is not clear whether this was due to the physical nature of the resin, the component chemicals, or a combination of both.

The nest entrance of the highly eusocial Australian stingless bee *Tetragonula carbonaria* (Apidae, Meliponini) is a simple hole that is connected to the inner nest by an entrance tube (Wille 1983). The entrance, made of propolis and directly exposed to the natural environment, is the only access to and from the beehive, and is defended by guards (Wittwer & Elgar 2018). The nest entrance propolis of *T. carbonaria* is dark brown and hard, and quite different from the softer propolis used to construct brood cells and storage pots. Interestingly, workers of *T. carbonaria* extract relatively higher amounts of resin from nearby species of *Corymbia* (Myrtaceae), compared with the sympatric stingless bee *Austroplebeia australis*, suggesting *T. carbonaria* has a greater need for

these products (Leonhardt et al. 2014). Here we use field experiments to ask whether the chemical properties of *T. carbonaria* nest entrance propolis and its derivative *Corymbia* resin can deter ants (*sensu* Bentley & Day 1989; Zhang et al. 2012; Rojas et al. 2017). Specifically, we derived polar (ethanol) and non-polar (hexane) extracts from resin and propolis, to create chemical ‘barriers’ around artificial food baits. We then observed whether these ‘barriers’ deter workers of *Iridomyrmex mayri* Forel, one of many species of small, mass-recruiting ants that are sympatric with *T. carbonaria* and attack individual workers of this species (T.A.H., personal observations).

Materials and Methods

Sample Collection and Preparation

We collected entrance propolis from two, managed colonies of *T. carbonaria* (E 153°0’7”, S 27°29’14” and E 153°1’55”, S 26°41’53”) that were housed in wooden nest boxes, and resin that had seeped from the wounds of *Corymbia* trees from SE Queensland, Australia. The samples were stored in glass containers at 4°C until extract preparation. Entrance propolis and resin extracts for the behavioural experiments were obtained by placing pieces of each into separate containers with either the polar solvent ethanol (10 %, w/v) or the non-polar solvent hexane (100%), where they remained in the dark for 24 h at room temperature. Each extract comprised multiple sources of either entrance propolis or resin extract, thereby simplifying the sampling design of the behavioural assays. Samples from these four extracts were used for the behaviour assays.

Behaviour bioassays

The field experiment investigated, in a fully crossed design, the response of workers of *I. mayri* to the extract source (entrance propolis, *Corymbia* resin or solvent control) and the type of solvent (ethanol or hexane) used to obtain the extract. Clean filter papers (Whatman No. 3, $\varphi=12.5$ cm) were delineated by concentric rings into three sections: a ‘centre’ with a radius of 2 cm; a circular, 2 cm wide ‘barrier’; and the ‘outer’ part of the filter paper. For each treatment, around 120 drops of extract (approximately 300 mg of entrance propolis or resin) or solvent only (control) were evenly applied by glass pipette to the ‘barrier’ ring of the filter paper. The solvent and potential volatile chemicals were allowed to evaporate for 48 hours, after which the filter paper was placed in a sealed container.

The trials were conducted over three days in Toohey Forest, Griffith University Nathan Campus (E 153°3’32”, S -27°-32’-59”) during November 2015. For each trial, the treated filter paper was lodged near frequented foraging trails, and roughly 0.5 g of tinned tuna (in brine) was placed in the centre of the ‘central’

section of the filter paper. Each trial lasted for 20 minutes, and we noted, every five minutes, the number of ants that were in the 'centre', 'barrier' and 'outer' sections of the filter paper. We minimised any 'learning' effects of the workers by replicating each treatment across 5 colonies that were dispersed in different localities within Toohey Forest, and the assignment of treatments to each location was randomized for each day. The experiment comprised 4 trials per treatment, yielding 120 trials in total. Filter papers were used once only, and the behaviour data were collected blind to the treatment. We preferred this experimental design to 'choice' experiments because it is difficult, in the field, to distinguish the preference of workers over their response to established foraging trails.

Statistical analyses

We used separate mixed-effects models to investigate the sources of variation in the number of ants that were in the barrier section and the central section of the filter paper, with solvent (hexane or ethanol), treatment (control, entrance propolis, and *Corymbia* resin), ant sampling time (5, 10, 15 and 20 min) as fixed effects, number of ants on the outer section of the paper as a covariate, and colony identity as a random effect, with the variance partitioned using restricted maximum likelihood (REML). We sequentially removed non-significant ($P > 0.1$) interaction terms and report the reduced model. We used Tukey HSD post-hoc tests to examine differences between treatments. All analyses were performed in JMP (Version 10.0 for Mac OS X, SAS Institute, North Carolina).

Results

The movement of workers of *I. mayri* across the 'barrier' to access the tuna bait was influenced by the chemicals extracted from the entrance propolis and from the *Corymbia* resin. The reduced mixed models revealed that worker access to the bait depended upon significant source by solvent interaction terms (Table 1). Accordingly, we conducted separate analyses for each of the extraction methods, and these models revealed that ants were deterred by the hexane extracts of both entrance propolis and *Corymbia* resin (Tables 2 and 3). Fewer workers reached the bait in the centre section if the barrier had been treated with either entrance propolis or *Corymbia* resin (Fig 1a), and there was a similar pattern for the number of workers observed on the barrier section (Fig 1c). However, chemicals from entrance propolis and *Corymbia* resin that had been extracted by ethanol did not affect the movement of workers across the barrier (Fig 1d) and, curiously, the number of workers feeding at the bait was significantly higher for the entrance propolis treatment (Fig. 1c).

Irrespective of the solvent used to extract the chemicals, the number of workers of *I. mayri* on the barrier

section was influenced by both the number on the outer section and the duration of the trial (Table 3).

However, the number of ants that crossed into the central section was influenced by the number on the outer section but, not the duration of the trial (Table 2).

Discussion

This study demonstrates that chemicals, most likely non-volatile, present in the *Corymbia* resin and the nest entrance propolis manufactured by workers of *T. carbonaria* deter the movement of workers of *I. mayri* ants. Indeed, the non-polar (hexane) extracts from the entrance propolis and *Corymbia* resin deterred workers of *I. mayri*, despite the tuna bait controls (hexane only) attracting increasing numbers of other workers, suggesting the chemicals found in these products can offer significant nest protection against ants. Interestingly, the ants were not deterred by polar (ethanol) extracts, and may even be attracted to certain polar chemicals (e.g. sugars and glycosides) extracted from the entrance propolis by ethanol.

Our experiments add to an increasing body of evidence revealing that diverse organisms use chemical deterrents to protect themselves or their brood from attack by ants. Orb-weaving spiders deposit a pyrrolidine alkaloid (2-pyrrolidinone) on the silk of their webs (Zhang et al. 2012; Henneken et al. 2017), which inhibits worker ants from moving onto the web (Zhang et al. 2012). Plants similarly use chemical deterrents to protect their nectar investment: volatile mono- and sesquiterpenes within floral scents can act as ant repellents (Junker et al. 2011), while the pollen of other plants is an effective close-range ant deterrent, with increasing effectiveness in species with greater volumes of nectar (Ballantyne & Willmer 2012). The cuticular chemistry of four species of *Tetragonula* and one of *Lepidotrigona* stingless bees have ant-deterrent properties (Lehmberg et al. 2008), and the entrance propolis of several species of stingless bees deters the passage of highly aggressive weaver ants *Oecophylla smaragdina* (Duangphakdee et al. 2009). The latter experiments used the raw material and thus could not determine whether it was due to the physical and/or chemical properties of the propolis. Our field experiments, using chemical extracts, confirm that for *T. carbonaria*, the non-volatile, non-polar chemicals present in the propolis are sufficient to act as an ant deterrent (*sensu* Bentley & Day 1989; Zhang et al. 2012; Rojas et al. 2017).

Why ants are deterred by the chemical properties of the propolis are not known. There may be intrinsic features of the chemicals that are dangerous or toxic to ants, and they avoid it accordingly. The passage of some ants across the barrier and their subsequent survival suggests that this explanation is unlikely.

Alternatively, the ants may associate the chemical odour with the dangerous mechanical properties of the propolis, as workers of *T. carbonaria*, like many other stingless bees, commonly use the sticky substance to

immobilize intruders (Leonhardt 2017). Interestingly, the production of resin by *Corymbia* may facilitate their seed dispersal by workers of *T. carbonaria* (Wallace & Trueman 1995).

Our experiments revealed that the ants were deterred by non-polar, non-volatile compounds extracted from both tree resin and bee propolis. The chemical composition of stingless bee propolis varies according to the available plant sources (Leonhardt et al 2009), suggesting some propolis constituents are sequestered from plants. Moreover, both stingless bee propolis and *Corymbia* resin contain a number of shared chemical compounds including sesqui- and di-terpenoids, phloroglucinols and flavonoids (Lehmberg et al. 2008; Massaro et al. 2014, 2015; Duangphakdee et al. 2009; Gershenzon & Dudareva 2007; Leonhardt et al. 2011; Wollenweber et al. 2000; Lamberton 1964; Milborrow et al. 1987). In particular, the resinous terpenoids can act as ant repellents (Pasteels et al. 1983). We suggest stingless bees are actively collecting resin from *Corymbia* trees and adding it to the entrance propolis in order to deter ants from entering their nests.

Acknowledgements

We thank Sara Leonhardt for kindly helping us obtain samples of entrance propolis, and Qike Wang for assistance with the experiments. This project was supported by the National Natural Science Foundation of China (31402145), the Natural Science Foundation of Hainan Province (20153082), the China Scholarship Council (201403260006) and ‘948’ Project (2014-Z14). The authors declare no conflicts of interest.

Literature cited

- Ballantyne, G. & Willmer, P. 2012: Nectar theft and floral ant-repellence: a link between nectar volume and ant-repellent traits? *PLoS One* **7**, e43869.
- Bankova, V. & Popova, M. 2007: Propolis of stingless bees: a promising source of biologically active compounds. *Pharmacognosy Reviews* **1**, 88–92.
- Bentley, M. D. & Day, J. D. (1989). Chemical ecology and behavioural aspects of mosquito oviposition. *Ann. Rev. Entomol.* **34**, 401–421.
- Breed, M. D., Guzmán-Novoa, E. & Hunt, G. J. 2004: Defensive behavior of honey bees: organization, genetics and comparisons with other bees. *Ann. Rev. Entomol.* **49**, 271–298.
- Duangphakdee, O., Koeniger, N., Deowanish, S., Hepburn, H. R. & Wongsiri, S. 2009: Ant repellent resins of honeybees and stingless bees. *Insectes Soc.* **56**, 333–339.
- Ellis, J. D., Hepburn, H. R., Ellis, A. M. & Elzen, P. J. 2003: Social encapsulation of the small hive beetle (*Aethina tumida* Murray) by European honeybees (*Apis mellifera* L.). *Insectes Soc.* **50**, 286–291.

200 Gershenzon, J. & Dudareva, N. 2007: The function of terpene natural products in the natural world. *Nature Chem. Biol.* **3**, 408–414.

201 Greco, M. K., Hoffmann, D., Dollin, A., Duncan, M., Spooner-Hart, R. & Neumann, P. 2010: The alternative Pharaoh approach:
202 stingless bees mummify beetle parasites alive. *Naturwissenschaften* **97**, 319–323.

203 Halcroft, M., Spooner-Hart, R. & Neumann, P. 2011: Behavioral defence strategies of the stingless bee, *Austroplebeia australis*,
204 against the small hive beetle, *Aethina tumida*. *Insectes Soc.* **58**, 245–253.

205 Heard, T. A. 2016: *The Australian Native Bee Book*. Sugarbag, West End, Brisbane, Australia.

206 Henneken, J., Goodger, J. Q. D., Jones, T. M. & Elgar, M. A. 2017: Variation in the web-based chemical cues of *Argiope keyserlingi*. *J.*
207 *Insect Physiol.* **101**, 15–21.

208 Hermann, H. R. 1984: Defensive mechanisms: general considerations. In: Defensive Mechanisms in Social Insects. (Hermann, H. R.,
209 ed). Praeger, New York. pp. 1–31.

210 Junker, R. R., Gershenzon, J. & Unsicker, S. B. 2011: Floral odour bouquet loses its ant repellent properties after inhibition of
211 terpene biosynthesis. *J. Chem. Ecol.* **37**, 1323–1331.

212 Lamberton, J. 1964: The occurrence of 5-hydroxy-7, 4'-dimethoxy-6-methylflavone in Eucalyptus waxes. *Aust. J. Chem.* **17**,
213 692–696.

214 Lehmberg, L., Dworschak, K. & Blüthgen, N. 2008: Defensive behavior and chemical deterrence against ants in the stingless bee
215 genus *Trigona* (Apidae, Meliponini). *J. Apicultural Res.* **47**, 17–21.

216 Leonhardt, S. D. 2017: Chemical ecology of stingless bees. *J. Chem. Ecol.* **43**, 385–402.

217 Leonhardt, S. D., Blüthgen, N. & Schmitt, T. 2009: Smelling like resin: terpenoids account for species-specific cuticular profiles in
218 Southeast-Asian stingless bees. *Insectes Soc.* **56**, 157–170.

219 Leonhardt, S. D., Heard, T. A. & Wallace, H. 2014: Differences in the resource intake of two sympatric Australian stingless bee
220 species. *Apidologie* **45**, 514–527.

221 Leonhardt, S. D., Wallace, H. M. & Schmitt, T. 2011: The cuticular profiles of Australian stingless bees are shaped by resin of the
222 eucalypt tree *Corymbia torelliana*. *Austral Ecol.* **36**, 537–543.

223 Massaro, F. C., Brooks, P. R., Wallace, H. M. & Russell, F. D. 2011: Cerumen of Australian stingless bees (*Tetragonula carbonaria*):
224 gas chromatography–mass spectrometry fingerprints and potential anti-inflammatory properties. *Naturwissenschaften* **98**,
225 329–337.

226 Massaro C.F., Katouli, M., T. Grkovic, T., Vu, H., Quinn, R.B., Heard, T.A., Carvalho, C., Manley-Harris, M., Wallace, H.M., & Brooks, P.
227 2014: Anti-staphylococcal activity of C-methyl flavanones from propolis of Australian stingless bees (*Tetragonula carbonaria*)
228 and fruit resins of *Corymbia torelliana* (Myrtaceae). *Fitoterapia* **95**, 247–257.

229 Massaro, C. F., Smyth, T. J., Smyth, W. F., Heard, T. A., Leonhardt, S. D., Katouli, M., Wallace, H. M. & Brooks, P. 2015: Phloroglucinols
 230 from anti-microbial deposit-resins of Australian stingless bees (*Tetragonula carbonaria*). *Phytotherapy Res.* **29**, 48–58.

231 Michener, C. D. 2007: *The Bees of the World*, 2nd Ed. John Hopkins University Press, Baltimore.

232 Milborrow, B. V., Kennedy, J. M. & Dollin, A. 1987: Composition of wax made by the Australian stingless bee *Trigona australis*. *Aust.*
 233 *J. Biol. Sci.* **40**, 15–26.

234 Neumann, P., Pirk, C., Hepburn, H., Solbrig, A., Ratnieks, F., Elzen, P. & Baxter, J. 2001: Social encapsulation of beetle parasites by
 235 Cape honeybee colonies (*Apis mellifera capensis* Esch.). *Naturwissenschaften* **88**, 214–216.

236 Pasteels, J. M., Grégoire, J. C. & Rowell-Rahier, M. 1983: The chemical ecology of defence in arthropods. *Ann. Rev. Entomol.* **28**,
 237 263–289.

238 Patricio, E. F. L. R. A., Cruz-López, L., Maile, R., Tentschert, J., Jones, G. R. & Morgan, E. D. 2002: The propolis of stingless bees:
 239 terpenes from the tibia of three *Frieseomelitta* species. *J. Insect Physiol.* **48**, 249–254.

240 Popova, M., Reyes, M., Le Conte, Y. & Bankova, V. 2014: Propolis chemical composition and honeybee resistance against *Varroa*
 241 *destructor*. *Nat. Prod. Res.* **28**, 788–794.

242 Rojas B, Burdfield-Steel E, Pakkanen H, Suisto K, Maczka M, Schulz S, & Mappes J. 2017: How to fight multiple enemies:
 243 target-specific chemical defences in an aposematic moth. *Proc. R. Soc. B* **284**, 20171424.

244 Roubik, D. W. 1989: *Ecology and Natural History of Tropical Bees*. Cambridge University Press, New York.

245 Roubik, D. W. 2006: Stingless bee nesting biology. *Apidologie* **37**, 124–143.

246 Sawaya, A. C. H. F., Tomazela, D. M., Cunha, I. B., Bankova, V. S., Marcucci, M. C., Custodio, A. R. & Eberlin, M. N. 2004: Electrospray
 247 ionization mass spectrometry fingerprinting of propolis. *Analyst* **129**, 739–744.

248 Shorter, J. R. & Rueppell, O. 2012: A review on self-destructive defence behaviors in social insects. *Insectes Soc.* **59**, 1–10.

249 Simone-Finstrom, M., Spivak, M. 2010: Propolis and bee health: the natural history and significance of resin use by honey bees.
 250 *Apidologie* **41**, 295–311.

251 Tomás-Barberán, F. A., García-Viguera, C., Vit-Olivier, P., Ferreres, F. & Tomás-Lorente, F. 1993: Phytochemical evidence for the
 252 botanical origin of tropical propolis from Venezuela. *Phytochemistry* **34**, 191–196.

253 Vit, P., Pedro, S. R. M. & Roubik, D. 2013: *Pot-honey: a Legacy of Stingless Bees*. Springer, New York.

254 Wallace, H. M. & Trueman, S. J. 1995: Dispersal of *Eucalyptus torelliana* seeds by the resin-collecting stingless bee, *Trigona*
 255 *carbonaria*. *Oecologia* **104**:12–16.

256 Wille, A. 1983: Biology of the stingless bees. *Ann. Rev. Entomol.* **28**, 41–64.

Wittwer B. & Elgar, M.A. 2018: Cryptic castes, nest odour and colony defence in a social insect *Tetragonula carbonaria* (Apidae: Meliponini). *Ethology* xx: xxx–xxx.

Wollenweber, E., Wehde, R., Dörr, M., Lang, G. & Stevens, J. F. 2000: C-Methyl-flavonoids from the leaf waxes of some Myrtaceae. *Phytochemistry* **55**, 965–970.

Zhang, S., Koh, T. H., Seah, W. K., Lai, Y. H., Elgar, M. A. & Li, D. 2012: A novel property of spider silk: chemical defence against ants. *Proc. R. Soc. B* **279**, 1824–1830.

Table 1. Mixed-effects model investigating the sources of variation in the number of ants in the barrier, and central section of the filter paper. The reduced models, derived from sequentially removing non-significant ($P > 0.1$) interaction terms, for (a) the number of ants in the barrier ($r^2 = 0.64$, AIC = 840.9, $n = 120$; full model: $r^2 = 0.66$, AIC = 860.8). and (b) number of ants in the central section ($r^2 = 0.54$, AIC = 951.1; $n = 120$; full model: $r^2 = 0.57$, AIC = 962.8).

	Estimate	SE	df	Statistics	Probability
a) Ants in the barrier section					
Parameter Estimates				t Ratio	> t
<i>Term</i>					
Intercept	-1.684	2.677	9.6	-0.63	0.54
Solvent [Ethanol]	-0.799	0.682	108.3	-1.17	0.24
Source [Control]	0.925	0.958	108.1	0.97	0.34
Source [Propolis]	0.797	0.990	108.8	0.80	0.42
Solvent [Ethanol] x Source [Control]	-2.732	0.976	108.5	-2.80	0.006
Solvent [Ethanol] x Source [Propolis]	0.382	0.959	108.1	0.40	0.69
Time	0.649	0.125	108.8	5.20	<.0001
Number outside	0.846	0.130	110.0	6.49	<.0001
Fixed Effects Tests					
<i>Main effects</i>				F ratio	> F
Solvent [Hexane, Ethanol]			108.3	1.373	0.24
Source [Control, Propolis, Resin]			108.6	1.522	0.22
Time			108.8	27.074	<.0001
Number outside			110.0	42.092	<.0001

Interactions

Solvent x Source	108.3	4.586	0.012
------------------	-------	-------	-------

b) Ants in the central section

Parameter Estimates

Term

Intercept	16.538	4.666	8.4	3.54	0.007
Solvent [Hexane]	0.205	1.112	108.2	0.18	0.85
Source [Control]	3.192	1.561	108.1	2.04	0.043
Source [Propolis]	2.726	1.614	108.7	1.69	0.094
Solvent [Hexane] x Source [Control]	5.149	1.591	108.5	3.23	<0.002
Solvent [Hexane] x Source [Propolis]	-4.685	1.564	108.1	-3.00	<0.004
Time	0.280	0.203	108.6	1.38	0.17
Number outside	0.995	0.213	111.3	4.68	<.0001

Fixed Effects Tests

Main effects

Solvent [Hexane, Ethanol]	108.2	0.0341	0.85
Source [Control, Propolis, Resin]	108.5	6.767	0.002
Time	108.6	1.903	0.17
Number outside	111.3	21.891	<.0001

Interactions

Solvent x Source	108.3	6.495	0.002
------------------	-------	-------	-------

Table 2. Mixed-effects model investigating the variation in the number of ants in the central section of the filter paper with extracts using (a) Hexane ($r^2 = 0.49$, AIC = 489.5; $n = 60$) and (b) Ethanol ($r^2 = 0.65$, AIC = 462.7; $n = 60$) solvents.

Estimate	SE	df	Statistics	Probability
----------	----	----	------------	-------------

a) Hexane

Parameter Estimates

t Ratio > |t/

Term

Intercept	14.538	5.406	17.7	2.68	0.016
Source [Control]	7.946	2.515	52.2	3.16	0.003
Source [Propolis]	-2.367	2.519	52.2	-0.94	0.35
Time	0.318	0.328	53.0	0.97	0.34
Number outside	1.267	0.397	44.3	3.19	0.003

Fixed Effects Tests

Main effects			F ratio	> F
Source [Control, Propolis, Resin]		52.7	5.081	0.010
Time		52.96	0.944	0.34
Number outside		44.3	10.186	0.003

b) Ethanol

Parameter Estimates				<i>t Ratio</i>	<i>> t </i>
<i>Term</i>					
Intercept	18.472	5.647	8.2	3.27	0.011
Source [Control]	-2.155	1.862	51.2	-1.16	0.25
Source [Propolis]	7.732	1.907	51.3	4.05	0.0002
Time	0.194	0.236	51.2	0.82	0.41
Number outside	0.859	0.223	54.1	3.86	0.0003

Fixed Effects Tests

Main effects			F ratio	> F
Source [Control, Propolis, Resin]		51.2	8.845	0.0005
Time		51.17	0.677	0.41
Number outside		54.1	14.869	0.0003

Table 3. Mixed-effects models investigating the variation in the number of ants in the barrier section of the filter paper with extracts using (a) Hexane ($r^2 = 0.78$, AIC = 411.5; n = 60) and (b) Ethanol ($r^2 =$

0.60, AIC = 422.6; n = 60) solvents.

	Estimate	SE	df	Statistics	Probability
a) Hexane					
Parameter Estimates				<i>t Ratio</i>	<i>> t/</i>
<i>Term</i>					
Intercept	-1.965	2.765	14.1	-0.71	0.49
Source [Control]	3.022	1.230	51.8	2.46	0.017
Source [Propolis]	-0.242	1.232	51.8	-0.20	0.84
Time	0.506	0.160	52.6	3.15	0.003
Number outside	1.283	0.200	47.6	6.52	<.0001
Fixed Effects Tests					
<i>Main effects</i>				<i>F ratio</i>	<i>> F</i>
Source [Control, Propolis, Resin]			52.3	3.431	0.040
Time			52.56	9.940	0.003
Number outside			44.3	42.460	<.0001
b) Ethanol					
Parameter Estimates				<i>t Ratio</i>	<i>> t/</i>
<i>Term</i>					
Intercept	-1.334	3.318	12.3	-0.40	0.695
Source [Control]	-2.193	1.322	51.3	-1.66	0.10
Source [Propolis]	1.805	1.354	51.6	1.33	0.19
Time	0.722	0.167	51.3	4.32	<.0001
Number outside	0.579	0.158	55.0	3.67	0.0005
Fixed Effects Tests					
<i>Main effects</i>				<i>F ratio</i>	<i>> F</i>
Source [Control, Propolis, Resin]			51.4	1.527	0.23
Time			51.31	18.656	<.0001
Number outside			55.0	13.482	0.0005

Figures

Fig. 1 The mean ($\pm$ SE) number of ants observed in either the central (a, b) or barrier (c, d) sections of the filter paper, which had chemicals extracted with either hexane (a, c) or ethanol (b, d) solvents. Different lowercase letters above each column indicates significant differences (Tukey HSD post-hoc test, $P < 0.05$) between treatments (the variation in (d) was not significantly different). (For statistical tests, see Tables 2 and 3).

a) Centre | Hexane

b) Centre | Ethanol

c) Barrier | Hexane

d) Barrier | Ethanol

Minerva Access is the Institutional Repository of The University of Melbourne

Author/s:

Wang, S;Wittwer, B;Heard, TA;Goodger, JQD;Elgar, MA

Title:

Nonvolatile chemicals provide a nest defence mechanism for stingless bees *Tetragonula carbonaria* (Apidae, Meliponini)

Date:

2018-09-01

Citation:

Wang, S., Wittwer, B., Heard, T. A., Goodger, J. Q. D. & Elgar, M. A. (2018). Nonvolatile chemicals provide a nest defence mechanism for stingless bees *Tetragonula carbonaria* (Apidae, Meliponini). *ETHOLOGY*, 124 (9), pp.633-640. <https://doi.org/10.1111/eth.12768>.

Persistent Link:

<http://hdl.handle.net/11343/284253>