Chem. Senses 35: 603–611, 2010
doi:10.1093/chemse/bjq058
Advance Access publication June 9, 2010

# Stingless Bees Use Terpenes as Olfactory Cues to Find Resin Sources

**S.D. Leonhardt[1], S. Zeilhofer[2], N. Blüthgen[1] and Thomas Schmitt[2]**

[1]Department of Animal Ecology and Tropical Biology, University of Würzburg, 97074 Würzburg, Germany and [2]Department of Evolutionary Biology and Animal Ecology, Faculty of Biology, University of Freiburg, 79104 Freiburg, Germany

*Correspondence to be sent to: Thomas Schmitt, Department of Evolutionary Biology and Animal Ecology, University of Freiburg, Hauptstrasse 1, 79104 Freiburg, Germany. e-mail: thomas.schmitt@biologie.uni-freiburg.de*

*Accepted May 3, 2010*

## Abstract

Insects largely rely on olfactory cues when seeking and judging information on nests, partners, or resources. Bees are known to use volatile compounds—besides visual cues—to find flowers suitable for pollen and nectar collection. Tropical stingless bees additionally collect large amounts of plant resins for nest construction, nest maintenance, nest defense, and to derive chemical constituents for their cuticular profiles. We here demonstrate that stingless bees of Borneo also use olfactory cues to find tree resins. They rely on volatile mono- and sesquiterpenes to locate or recognize known resin sources. Moreover, by modifying resin extracts, we found that stingless bees do not use the entire resin bouquet but relative proportions of several terpenes. In doing so, the bees are able to learn specific tree resin profiles and distinguish between tree species and partly even tree individuals.

**Key words:** olfactory cues, resin, stingless bees, terpenes

## Introduction

Insects use olfactory cues not only to recognize potential mates, relatives, nestmates, or enemies but also to find suitable nesting sites or resources for food and/or nest construction. Olfactory cues involved in finding and recovering resources for the supply of food or nesting substrate have been studied in ants (Roces 1994; Steck et al. 2009), wasps (Reid et al. 1995), and honeybees (Pham-Delègue et al. 1986, 1990, 1993; Thiery et al. 1990; Masson et al. 1993; Laloi et al. 2000; Wright, Lutmerding, et al. 2005; Wright, Thomson, and Smith 2005). In another group of highly social bees, the tropical stingless bees (Apidae: Meliponini), olfactory cues involved in resource location are largely unknown. Like honeybees, stingless bees collect pollen and nectar from flowers as food supply, but they also collect large amounts of plant resins to build, maintain, and defend their nests (Khoo and Yong 1987; Roubik 1989, 2006; Lehmberg et al. 2008; Duangphakdee et al. 2009; Leonhardt and Blüthgen 2009). Resin is collected from tree wounds, buds, fruits, or other plant parts (Armbruster 1984; Roubik 1989; Wallace and Trueman 1995) and mixed with wax to build the main nest material: cerumen (Wille 1983; Bankova et al. 2000; Patricio et al. 2002; Roubik 2006). Bees also use resin to coat the inner nest walls, which prevents the growth of bacteria and fungi (Wille 1983; Velikova et al. 2000). Alternatively, they directly apply it to the nest entrance tube to entangle intruders such as ants, termites, or foreign bees (Schwarz 1948; Wittmann 1985; Khoo and Yong 1987; Roubik 2006; Lehmberg et al. 2008; Leonhardt and Blüthgen 2009). When looking for resin, bees tend to collect from multiple resin wounds of different tree species (Leonhardt and Blüthgen 2009) and frequently engage in inter- and intraspecific fights over resin sources (Howard 1985; Leonhardt and Blüthgen 2009). Resin was therefore considered a limiting resource for stingless bees (Howard 1985).

The deterrent properties of resin are largely due to the presence of terpenes, mainly mono- and sesquiterpenes, which are produced by trees to protect themselves against herbivore— and/or microbial attack (Langenheim 2003; Gershenzon and Dudareva 2007). Some insects, such as the bark beetle *Dendroctonus ponderosae*, which exploit the protective resins for their own purpose, use terpenes to locate host trees (reviewed by Phillips and Croteau 1999). Terpenes are (among other compounds) also used by honeybees to recognize oilseed rape flowers (Blight et al. 1997) and snapdragon flowers (Wright, Lutmerding, et al. 2005). Moreover, the cuticular chemical profiles of stingless bees from Borneo comprise terpenes, which are derived from resins collected (Leonhardt et al. 2009). It is therefore highly likely that they also use terpenes to locate suitable resin sources, because terpenes represent the main constituents of resins from dipterocarp trees (Langenheim 2003), which dominate the rain forests of Borneo. Dipterocarp tree resin is therefore most frequently collected by Bornean stingless bees (Leonhardt and Blüthgen 2009).

In this study, we tested whether stingless bees use olfactory signals from resins to locate resin sources. Moreover, we investigated whether resin-derived volatile terpenes serve as olfactory cues. By modifying resin extracts, we further tested whether bees rely on/learn the whole resin bouquet or only particular compounds.

## Materials and methods

### Study sites and bees

Field experiments were conducted at the Rainforest Discovery Center (RDC) of Sandakan, in Sabah, Borneo (Malaysia), from September to November 2008. The RDC is a small education center located ~2 km West of the Kabili Sepilok Reserve (5°54′N, 118°04′E, 20–120 m above sea level), an area of 4294 ha with coastal dipterocarp and mangrove forest (Fox 1973), surrounded by oil palm plantations. The RDC itself comprises 148.6 ha of mainly secondary and planted vegetation including *Agathis borneensis* (Araucariaceae), a highland pine species normally absent from lowland rain forests. The climate is typically equatorial with a mean annual temperature of 26–30 °C and a yearly rainfall of 2600–3000 mm (Fox 1973).

Collections of bee specimens held by the Forestry Research Center in Sepilok as well as our own observations prelude between 15 and 20 stingless bee species in the RDC (species and genera names as in Moure 1961).

### Trees and resin secretion

We performed experiments with 3 tree species known from previous studies to easily secret resins that attract bees (see Leonhardt and Blüthgen 2009): *A. borneensis* (Araucariaceae: 3 individuals), *Shorea xanthophylla* (Dipterocarpaceae: 1 individual) and *Dryobalanops lanceolata* (Dipterocarpaceae: 2 individuals). We either created artificial resin wounds or maintained the resin flow of wounds already present using a nail and/or a knife to scratch the trees' bark. Resin was sampled from 3 to 10 different wounds per tree individual. Resin flow could be maintained for up to 5 days before running dry.

### Resin extracts

To test whether bees could be attracted by those components of tree resins that were solvable in hexane, hexane extracts of tree resins were prepared. For these extracts, we collected 1 ml resin from all 6 trees using a clean knife and transferred it into a 3 ml vial containing 2 ml pure hexane (Sigma-Aldrich). After 15 h, the hexane extract with the hitherto dissolved resin compounds was transferred into a new vial, whereas the nondissolved residue of the resin was discarded.

### Modification of resin extracts

To test whether stingless bees rely on mono- and/or sesquiterpenes to locate resin sources, we modified resin extracts by either adding purchased terpenes (previously identified in hexane extracts of tree resins and nest material from bees, Leonhardt SD and Schmitt T, unpublished data) or mixing extracts of 2 different tree individuals (1:1 mixtures).

Monoterpenes added comprised (1R)-(+)-α-pinene (≥97%), (–)-β-pinene (≥97%), myrcene (≥90%), γ-terpinene (≥95%), terpinolene (≥90%), (+)-camphene (95%), and *p*-cymene (≥97%) (all substances purchased from Sigma-Aldrich). Sesquiterpenes added comprised (–)-α-copaene (≥90%), β-caryophyllene (≥80%), and α-humulene (≥98%) (all substances purchased from Sigma-Aldrich) as well as mixture of 3 different farnesene isomers (7% *cis*-β-farnesene, 10% *trans*-β-farnesene, and 9% *trans*-*trans*-α-farnesene) and germacrene D (each ~40% v/v), which were both obtained from the department of Chemistry of the University of Würzburg. Both the farnesene mixture and germacrene D contained other nonpolar sesquiterpenes (in germacrene D: γ-muurolene and 4 unknown sesquiterpenes each accounting for more than 4%; in the farnesene mixture: 3 bisabolene isomers and 1 unknown sesquiterpene each accounting for more than 4%) and are hitherto only referred to as germacrene and farnesene. A mono- and a sesquiterpene mixture were produced by adding 0.3 ml of all mono- and all sesquiterpenes, respectively, in 3 ml hexane. We then added 0.3 ml of these mixtures to the 2 ml resin extracts. In doing so, normal concentrations of the mono- and sesquiterpenes in the resin extracts were increased between 4- (β-caryophyllene) and 41-fold (α-humulene) but never exceeded the concentration of terpenes naturally occurring in resin extracts. Because sesquiterpenes appeared to strongly affect the bees' choices, we additionally tested modified extracts with only one of the above-mentioned sesquiterpenes added or with mixtures of sesquiterpenes lacking germacrene plus farnesene or solely farnesene.

### Behavioral assays

We transferred 0.3 ml of pure or modified resin extracts on a clean filter paper (Melitta) of 3 cm in diameter. For control, the same amount of the solvent hexane or the pure resin extract, respectively, was put on another filter paper. Both test and control filter papers were then placed at a distance of 40–120 cm from the source tree. We installed them between 50 and 100 cm above the ground by putting them on the surrounding vegetation with a minimum distance of 60 cm between test and control filter papers. After 5 min, both filter papers were replaced by fresh ones to prevent the loss of highly volatile compounds from resin extracts. In general, filter papers were exchanged once during 1 observation. To prevent bees from learning the positions of the filter papers, we exchanged the positions of control and test filter papers after each observation or completely relocated them.

Each pairwise comparison of 2 extracts comprised 10–40 replicate observation periods (each 10 min) at 1–2 trees (Wilcoxon matched-pairs tests). During each 10-min period, we observed both filter papers and noted the number and duration of bee visits to anyone filter paper. We considered the approach of each bee individual as an independent "visit" when it hovered at a height of less than 2 cm above or landed on the filter paper. Thus, 1 bee individual may have been counted multiple times if it approached the filter paper more than once because discrimination between different bee individuals of the same species was impossible. Pure resin extracts were tested against hexane at 1 tree individual of each species (*A. borneensis*, *S. xanthophylla*, and *D. lanceolata*) (Table 1). Tests with extracts modified by adding terpenes

**Table 1** Results of preference tests with pure resin extracts versus hexane (control), modified resin extracts, pure resin extracts of a different tree, and resin extract mixtures

| Extract 1 | *N* (trees) | Tested against (extract 2) | *N* (observations) | Mean ± standard deviation | | *V* | *P* |
|---|---|---|---|---|---|---|---|
| | | | | Extract 1 | Extract 2 | | |
| Pure extracts against controls | | | | | | | |
| *Agathis borneensis* | 1 | Hexane | 11 | 5 ± 3.8 | 0 | 66 | **0.004** |
| *Shorea xanthophylla* | 1 | Hexane | 10 | 2.4 ± 1.9 | 0.1 ± 0.3 | 36 | **0.013** |
| *Dryobalanops lanceolata* | 1 | Hexane | 10 | 5.3 ± 6.7 | 0.1 ± 0.3 | 36 | **0.014** |
| Pure extracts against extracts modified by addition of terpenes | | | | | | | |
| *A. borneensis* | 2 | *A. borneensis* + all monoterpenes | 40 | 4.2 ± 2.9 | 3.2 ± 3.3 | 484 | **0.017** |
| *A. borneensis* | 2 | *A. borneensis* + all sesquiterpenes | 30 | 5.7 ± 4.5 | 2 ± 1.5 | 389 | **<0.001** |
| *A. borneensis* | 2 | *A. borneensis* + all sesquiterpenes but farnesene | 20 | 8.3 ± 5.1 | 3.2 ± 4.2 | 160 | **0.001** |
| *A. borneensis* | 2 | *A. borneensis* + all sesquiterpenes but germacrene and farnesene | 20 | 4.3 ± 4.4 | 4.7 ± 4.0 | 66 | 0.248 |
| *A. borneensis* | 1 | *A. borneensis* + *trans*-caryophyllene | 20 | 4.7 ± 3.9 | 3.9 ± 2.5 | 94 | 0.431 |
| *A. borneensis* | 1 | *A. borneensis* + α-humulene | 14 | 6.3 ± 8.6 | 4.8 ± 5.2 | 61 | 0.614 |
| *A. borneensis* | 1 | *A. borneensis* + α-copaene | 14 | 2.4 ± 2.9 | 3.1 ± 4.1 | 25 | 0.797 |
| *A. borneensis* | 1 | *A. borneensis* + germacrene | 10 | 2.7 ± 2.8 | 1.6 ± 2.4 | 36 | 0.12 |
| *A. borneensis* | 2 | *A. borneensis* + farnesene | 24 | 7.5 ± 5.6 | 2.2 ± 2.3 | 268 | **<0.001** |
| Pure extracts of different *A. borneensis* trees | | | | | | | |
| *A. borneensis* A | 1 | *A. borneensis* B | 10 | 5 ± 5.7 | 0.5 ± 0.5 | 21 | **0.036** |
| *A. borneensis* A | 1 | *A. borneensis* C | 10 | 6.6 ± 8.0 | 2.2 ± 2.4 | 25 | 0.076 |
| *A. borneensis* B | 1 | *A. borneensis* A | 10 | 5.1 ± 2.8 | 1.1 ± 1.0 | 55 | **0.006** |
| *A. borneensis* B | 1 | *A. borneensis* C | 10 | 4.5 ± 5.2 | 3.1 ± 2.1 | 32 | 0.682 |
| *A. borneensis* C | 1 | *A. borneensis* B | 10 | 11.8 ± 6.4 | 3.8 ± 3.2 | 53 | **0.011** |
| *A. borneensis* C | 1 | *A. borneensis* A | 10 | 5.8 ± 5.2 | 4.9 ± 40 | 23 | 0.575 |
| Pure extracts against mixtures | | | | | | | |
| *A. borneensis* A | 1 | *A. borneensis* A + *A. borneensis* B | 10 | 3.1 ± 2.3 | 4.5 ± 4.3 | 12 | 0.211 |
| *A. borneensis* A | 1 | *A. borneensis* A + *A. borneensis* C | 10 | 6.4 ± 7.2 | 2.3 ± 1.8 | 55 | **0.005** |
| *A. borneensis* B | 1 | *A. borneensis* A + *A. borneensis* B | 10 | 5.3 ± 4.8 | 1.1 ± 1.1 | 35 | **0.025** |
| *A. borneensis* B | 1 | *A. borneensis* B + *A. borneensis* C | 10 | 11.2 ± 6.1 | 10.7 ± 8.6 | 33 | 0.609 |
| *A. borneensis* C | 1 | *A. borneensis* A + *A. borneensis* C | 10 | 5.1 ± 4.0 | 4.2 ± 3.8 | 39 | 0.251 |
| *A. borneensis* C | 1 | *A. borneensis* B + *A. borneensis* C | 10 | 5.2 ± 6.1 | 9.8 ± 8.2 | 7 | 0.139 |

The mean numbers of bees visiting each extract/control are given. Bold *P* values mark significant preferences for 1 of the 2 extracts tested.

as well as with extract mixtures were conducted for *A. borneensis* resin only, because *A. borneensis* was the only tree species with more than 2 individuals present at RDC (Table 1). Pure extracts were tested against modified extracts at 2 *A. borneensis* individuals (Table 1). Preference tests between pure extracts and extract mixtures (of different *A. borneensis* individuals) were performed at all 3 *A. borneensis* trees (Table 1).

### Chemical analyses of resin extracts

Besides the pure and modified or mixed extracts of the 3 tree species used for observations, 1–2 ml fresh resin was obtained from wounds (1 wound per tree) of 23 further tree individuals (14 tree species), 17 of which (10 species) had been visited by bees for resin collection in 2007. To control for the success of extract preparation, modification, and mixing, all extracts were analyzed using a Hewlett Packard HP 6890 Series Gas Chromatograph (GC) System coupled to a Hewlett Packard HP 5973 Mass Selective Detector (Agilent Technologies). The GC was equipped with a DB-1 fused silica capillary column (30 m × 0.25 mm inner diameter; degrees of freedom = 0.25 μm; J&W). Temperature was programed from 60 to 300°C with 5°C/min heating rate and held for 10 min at 300°C. Helium was used as carrier gas with a constant flow of 1 ml/min. Injection was carried out at 250°C in the splitless mode for 1 min. The electron impact mass spectra were recorded at 70 eV and 230°C source temperature. We used the Windows version of the ChemStation software package (Agilent Technologies) for data acquisition.

For comparison, compounds found in resin extracts were characterized by their mass spectra and retention times. Peaks with identical mass spectra and retention times were regarded as the same substance. We used 3 commercially available mass spectra libraries (Wiley 275, NIST 98, and Adams EO library 2205) and—where available—standards (purchased from Sigma-Aldrich) to identify substances of *A. borneensis* resin with regard to their mass spectra and retention indices. Because only mono- and sesquiterpenes were expected to be volatile enough to serve as olfactory cues to bees, we confined our analyses, identifications, and comparisons to compounds with retention times below 30 min.

### Statistical analyses

To see whether differences in the attractiveness (visited vs. nonvisited) between trees correlated with differences in their chemical composition, the volatile compounds of resin extracts from the 23 tree resins were compared by an "Adonis" test (R command for multivariate analysis of variance based on dissimilarities). The test was based on the Bray–Curtis distance matrix of the proportions of each compound that accounted for more than 0.5% of the total peak area in all samples. Proportions of compounds were calculated by dividing the peak area of each compound by the total area of all peaks included in the analysis. Overall, 264 compounds were used for the analysis. To test whether trees could be differentiated by sesquiterpenes, a separate Adonis test was performed.

To test for inter- and intraspecific variation in the chemical composition of volatile compounds from different *A. borneensis* individuals, 2–3 resin samples from different wounds of each of the 3 individuals used for the experiments were analyzed and compared by an Adonis test. We further compared Bray–Curtis distances of volatile compounds across extracts of the individual *A. borneensis* trees and their mixtures.

All statistical analyses were performed in R (R Development Core Team 2009).

## Results

### Attractiveness of pure resin extracts and resin extracts modified by addition of terpenes

Bees visited filter papers with pure resin extracts of all 3 tree species significantly more often than control filter papers with hexane only (Table 1), indicating that volatiles extracted from resin attract stingless bee resin foragers.

When *A. borneensis* resin extracts were modified by adding all mono- or sesquiterpenes, bees visited filter papers with the known, pure resin extracts significantly more often than modified ones (Table 1). However, when *A. borneensis* resin extract was modified by only one of the following sesquiterpenes: (–)-α-copaene, β-caryophyllene, α-humulene, and germacrene, no preference was found (Table 1), suggesting that no single terpene influenced the bees' choices. When only farnesene was added to *A. borneensis* resin extract, bees did prefer pure over modified resin extract (Table 1). Bees also preferred pure *A. borneensis* resin extract over modified extract containing all sesquiterpenes except farnesene (Table 1). However, they showed no such preference when the modified extract contained all sesquiterpenes except germacrene and farnesene (Table 1).

### Attractiveness of pure resin extracts of different *A. borneensis* trees

When bees, collecting resin at 1 of the 3 *A. borneensis* individuals, were presented with pure resin extract from their collecting tree and resin extract from 1 of the other 2 tree individuals, they either preferred resin extract from their collecting tree (in 3 out of 6 trials) or showed no preference between the 2 extracts (Table 1).

Resin extracts of *A. borneensis* tree individuals B and C were more similar in their chemical composition to each other than to tree A (Bray–Curtis distances), especially when only monoterpenes were included in the analysis (Figure 1).

### Attractiveness of pure versus mixtures of *A. borneensis* resin extracts

When bees were presented with pure resin extract from their collecting tree and mixtures of this tree and another tree, they

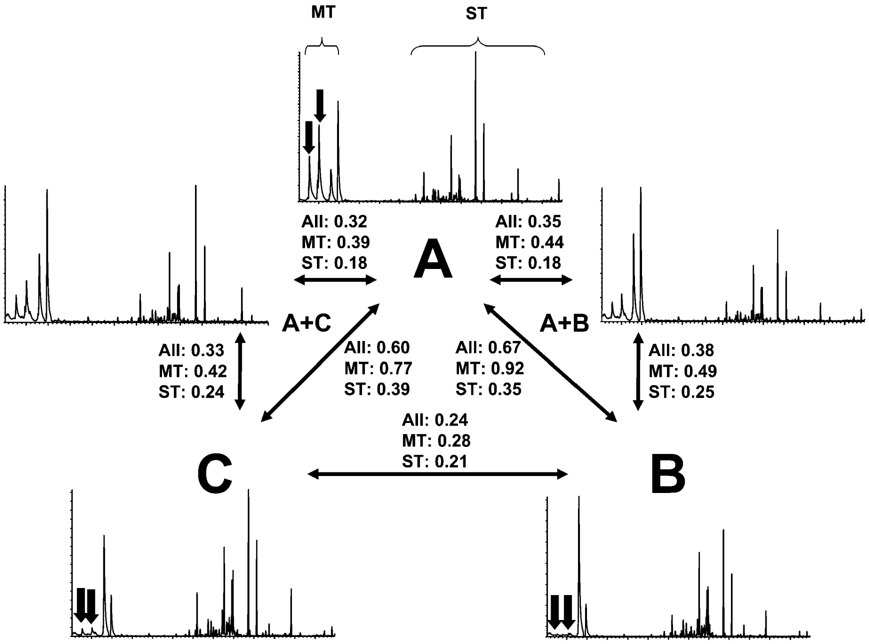

**Figure 1** Chromatograms of 3 pure resin extracts (A, B, and C) and 2 extract mixtures (AB and AC) from 3 *Agathis borneensis* trees (comprising only volatile compounds). Bray–Curtis distances between extracts and extract mixtures are given for all volatile compounds (All), only monoterpenes (MT), and only sesquiterpenes (ST). Arrows indicate highly volatile monoterpenes that quantitatively differ between pure resin extracts.

showed a clear preference for the resin extract from their collecting tree in only 2 out of 6 trials, whereas no preferences were found in the remaining trials (Table 1).

As expected, the mixtures of resin extracts AB and AC were intermediate in their chemical similarity between extracts of the original tree resins A, B, and C regarding all compounds as well as only mono- and only sesquiterpenes (Figure 1). Hexane extract of *A. borneensis* A resin contained considerably higher proportions of the most volatile monoterpenes ($\alpha$-pinene, sabinene, $\beta$-phellandrene, and $\gamma$-terpinene) than extracts of *A. borneensis* B and C (Table 2, Figure 1), whereas extract mixtures AB and AC were intermediate in the levels of these monoterpenes (Figure 1).

### Chemical analyses of resin extracts from *A. borneensis*

The chemical composition of *A. borneensis* resin differed both within and between individuals (Table 2). Interindividual variation was, however, more pronounced than intraindividual variation (Table 2) and was sufficient to distinguish between the 3 *A. borneensis* trees (Adonis: $R^2 = 0.78$, $P = 0.035$, Table 2). Notably, interindividual differences were even more pronounced when the analysis was confined to monoterpenes (Adonis: $R^2 = 0.84$, $P = 0.004$) or sesquiterpenes (Adonis: $R^2 = 0.78$, $P = 0.003$).

### Chemical analyses of resin extracts from different tree species

The 17 trees visited by bees and the 6 trees not visited by bees were poorly separated by their chemical compositions (Adonis—all compounds: $R^2 = 0.08$, $P = 0.041$, Figure 2a; only sesquiterpenes: $R^2 = 0.08$, $P = 0.031$, Figure 2b), indicating that the whole resin bouquet is a weak indicator of the attractiveness of tree species to bees. The resin extracts from the 23 trees strongly varied in their chemical compounds (Figure 2). Different tree individuals of the same species (e.g., *S. faguetiana*) were more similar to trees of other tree species (e.g., *S. parvifolia*) than to each other (Figure 2).

## Discussion

Stingless bees in Borneo use olfactory cues to find and recognize tree resins, which they exploit for their chemical and physical properties. We extracted resin-derived volatiles by the solvent hexane and attracted bees to filter papers with these extracts. When resin extracts were modified by adding terpenes or mixing them, the bees often did not show a clear preference for anyone extract, although they tended to preferentially visit the familiar unmodified/pure extract of their collecting tree. This preference was particularly pronounced when extracts were modified by adding a whole mixture of mono- or sesquiterpenes, whereas the addition of single terpenes did not influence the bees' behavioral choices. Bees further discriminated between pure resin extracts and extracts enriched by germacrene and/or farnesene, which were not available in pure forms but additionally contained other sesquiterpenes. Moreover, strong qualitative differences between 2 monoterpenes in resin extracts of the 3 *A. borneensis* individuals (Figure 2) were not sufficient to

**Table 2** Percentages [±standard deviation] of tentatively identified substances found in hexane extracts of resins from 3 *Agathis borneensis* individuals listed according to molecular weight (MW), retention indices (RI), and retention times (RT)

| No. | MW | Class | Compound | RI | RT | *A. borneensis* A (%) | *A. borneensis* B (%) | *A. borneensis* C (%) |
|---|---|---|---|---|---|---|---|---|
| 1 | 136 | MT | Tricyclene | 921 | 5.03 | 0.83 ± 0.26 | 0.07 ± 0.03 | 0.12 ± 0 |
| 2 | 136 | MT | α-Pinene[a] | 932 | 5.16 | 10.08 ± 4.17 | 0.49 ± 0.19 | 1.37 ± 0.22 |
| 3 | 136 | MT | Sabinene | 970 | 6.03 | 9.48 ± 6.29 | 0 ± 0 | 1.05 ± 0.41 |
| 4 | 136 | MT | β-Pinene[a] | 974 | 6.18 | 0 ± 0 | 0.45 ± 0.07 | 0.52 ± 0.05 |
| 5 | 136 | MT | *para*-Cymene[a] | 1020 | 7.08 | 1.62 ± 0.79 | 0.26 ± 0.1 | 0.38 ± 0.1 |
| 6 | 136 | MT | β-Phellandrene | 1025 | 7.21 | 7.13 ± 5.67 | 36.44 ± 2.2 | 26.15 ± 3.75 |
| 7 | 136 | MT | γ-Terpinene[a] | 1054 | 7.91 | 33.81 ± 18.45 | 4.23 ± 2.55 | 6.65 ± 1.91 |
| 8 | 136 | MT | Terpinolene[a] | 1086 | 8.61 | 0.19 ± 0.02 | 0.15 ± 0.02 | 0.11 ± 0 |
| 9 | 132 | MT | — | 1089 | 8.72 | 0.24 ± 0.12 | 0.06 ± 0 | 0.05 ± 0.01 |
| 10 | 204 | ST | δ-Elemene | 1335 | 15.21 | 0.08 ± 0.03 | 0.05 ± 0.01 | 0.12 ± 0.06 |
| 11 | 196 | MT | Terpinyl acetate | 1346 | 15.55 | 0.52 ± 0.28 | 0.14 ± 0.03 | 0.07 ± 0.01 |
| 12 | 204 | ST | α-Cubebene | 1345 | 15.59 | 0.18 ± 0.01 | 0.5 ± 0.04 | 0.53 ± 0.03 |
| 13 | 204 | ST | α-Ylangene | 1373 | 16.2 | 0.31 ± 0.13 | 0.4 ± 0.03 | 0.52 ± 0.04 |
| 14 | 204 | ST | α-Copaene[a] | 1374 | 16.37 | 1.93 ± 0.73 | 1.92 ± 0.25 | 3.07 ± 0.38 |
| 15 | 204 | ST | β-Cubebene | 1387 | 16.67 | 0.44 ± 0.11 | 0.78 ± 0.04 | 0.88 ± 0.03 |
| 16 | 204 | ST | — | — | 16.82 | 0.1 ± 0.04 | 0.13 ± 0.01 | 0.14 ± 0.01 |
| 17 | 204 | ST | Sibirene | 1400 | 17.16 | 0.06 ± 0.01 | 0.29 ± 0.01 | 0.21 ± 0.04 |
| 18 | 204 | ST | Sesquithujene | 1405 | 17.29 | 0.65 ± 0.17 | 0.58 ± 0.1 | 0.3 ± 0.16 |
| 19 | 204 | ST | — | — | 17.46 | 0.7 ± 0.21 | 1.43 ± 0.16 | 1.36 ± 0.07 |
| 20 | 204 | ST | β-Caryophyllene[a] | 1417 | 17.54 | 0.32 ± 0.1 | 0.29 ± 0.16 | 0.28 ± 0.07 |
| 21 | 204 | ST | — | — | 17.64 | 0.04 ± 0.02 | 0.1 ± 0.01 | 0.09 ± 0.01 |
| 22 | 204 | ST | — | — | 17.76 | 0.62 ± 0.19 | 1.01 ± 0.22 | 0.89 ± 0.37 |
| 23 | 204 | ST | — | — | 17.79 | 0.25 ± 0.06 | 0.6 ± 0.21 | 0.52 ± 0.33 |
| 24 | 204 | ST | β-Copaene | 1430 | 17.84 | 0.14 ± 0.06 | 0.14 ± 0.01 | 0.14 ± 0.03 |
| 25 | 204 | ST | — | — | 17.79 | 0.14 ± 0.11 | 0.27 ± 0.04 | 0.62 ± 0.03 |
| 26 | 204 | ST | — | — | 18.13 | 0.28 ± 0.09 | 0.48 ± 0.04 | 0.49 ± 0 |
| 27 | 204 | ST | *trans*-β-Farnesene[a] | 1454 | 18.24 | 0.31 ± 0.1 | 0.87 ± 0.12 | 0.6 ± 0.2 |
| 28 | 204 | ST | — | — | 18.29 | 0.26 ± 0.09 | 0.45 ± 0.04 | 0.46 ± 0 |
| 29 | 204 | ST | α-Humulene[a] | 1452 | 18.4 | 0.11 ± 0.03 | 0.11 ± 0.01 | 0.15 ± 0.03 |
| 30 | 204 | ST | *cis*-Cadina-1(6),4-diene | 1461 | 18.56 | 0.54 ± 0.18 | 0.8 ± 0.1 | 0.89 ± 0.07 |
| 31 | 204 | ST | — | — | 18.67 | 0.06 ± 0.03 | 0.1 ± 0.01 | 0.1 ± 0 |
| 32 | 204 | ST | Dauca-5,8-diene | 1471 | 18.79 | 0.14 ± 0.07 | 0.21 ± 0.01 | 0.25 ± 0 |
| 33 | 204 | ST | — | — | 18.86 | 0.76 ± 0.37 | 1.68 ± 0.19 | 2.25 ± 0.17 |
| 34 | 204 | ST | γ-Muurolene | 1478 | 19.03 | 3.81 ± 1.34 | 7.24 ± 0.81 | 7.01 ± 0.24 |
| 35 | 204 | ST | Germacrene D | 1484 | 19.29 | 0.59 ± 0.25 | 1.39 ± 0.12 | 1.49 ± 0.02 |
| 36 | 204 | ST | — | — | 19.42 | 0.54 ± 0.12 | 0.89 ± 0.11 | 1.24 ± 0.14 |
| 37 | 204 | ST | γ-Amorphene | 1495 | 19.48 | 0.35 ± 0.11 | 0.49 ± 0.05 | 0.53 ± 0.06 |
| 38 | 204 | ST | — | — | 19.55 | 0.62 ± 0.32 | 0.84 ± 0.08 | 1.24 ± 0.09 |

**Table 2** Continued

| No. | MW | Class | Compound | RI | RT | *A. borneensis* A (%) | *A. borneensis* B (%) | *A. borneensis* C (%) |
|---|---|---|---|---|---|---|---|---|
| 39 | 204 | ST | — | — | 19.72 | 0.06 ± 0.05 | 1.52 ± 0.49 | 1.18 ± 0.17 |
| 40 | 204 | ST | — | — | 19.8 | 1.83 ± 0.85 | 3.84 ± 0.45 | 4.08 ± 0.04 |
| 41 | 204 | ST | δ-Amorphene | 1511 | 19.9 | 1.47 ± 0.38 | 4.43 ± 0.55 | 4.48 ± 0.25 |
| 42 | 220 | ST | — | — | 19.95 | 0.46 ± 0.1 | 2.81 ± 2.24 | 1.03 ± 0.02 |
| 43 | 222 | ST | — | — | 20.07 | 0.09 ± 0.15 | 1 ± 0.23 | 0.38 ± 0.09 |
| 44 | 204 | ST | *trans*-Cadina-1,4-diene | 1533 | 20.26 | 0.13 ± 0.04 | 0.3 ± 0.03 | 0.29 ± 0 |
| 45 | 220 | ST | — | — | 20.34 | 0.3 ± 0.27 | 0.36 ± 0.02 | 0.49 ± 0.03 |
| 46 | 222 | ST | — | — | 21.42 | 8.84 ± 3.66 | 9.05 ± 0.37 | 12.29 ± 0.11 |
| 47 | 222 | ST | — | — | 21.57 | 0.32 ± 0.18 | 0.34 ± 0.05 | 0.43 ± 0.01 |
| 48 | 222 | ST | — | — | 22.24 | 4.56 ± 1.88 | 4.81 ± 0.13 | 6.61 ± 0.41 |
| 49 | 220 | ST | — | — | 22.69 | 0.05 ± 0.06 | 0.08 ± 0.07 | 0.07 ± 0.03 |
| 50 | 222 | ST | epi-α-Cadinol | 1638 | 22.81 | 0.01 ± 0.01 | 0.11 ± 0.07 | 0.1 ± 0.03 |
| 51 | 222 | ST | epi-α-Murrolol | 1640 | 22.85 | 0.07 ± 0.04 | 0.19 ± 0.07 | 0.24 ± 0.03 |
| 52 | 222 | ST | α-Muurolol | 1644 | 22.91 | 0.09 ± 0.08 | 0.2 ± 0.1 | 0.15 ± 0.01 |
| 53 | 222 | ST | α-Cadinol | 1652 | 23.12 | 0.27 ± 0.28 | 0.48 ± 0.26 | 0.45 ± 0 |
| 54 | 222 | ST | — | — | 23.44 | 0.12 ± 0.04 | 1.18 ± 0.22 | 0.97 ± 0.15 |
| 55 | 222 | ST | — | — | 23.76 | 0.09 ± 0.06 | 0.22 ± 0.1 | 0.15 ± 0.01 |
| 56 | 220 | ST | — | — | 24.37 | 0.43 ± 0.65 | 0 ± 0 | 0.4 ± 0.11 |
| 57 | 220 | ST | — | — | 25.09 | 0.23 ± 0.16 | 0.2 ± 0.03 | 0.32 ± 0.01 |
| 58 | 220 | ST | — | — | 25.45 | 0.18 ± 0.12 | 0.15 ± 0.05 | 0.2 ± 0 |
| 59 | 222 | ST | — | — | 25.51 | 0.02 ± 0.03 | 0.21 ± 0.05 | 0.08 ± 0.12 |
| 60 | 222 | ST | — | — | 25.61 | 2.14 ± 0.95 | 2.22 ± 0.22 | 2.81 ± 0.55 |

[a] Substances that were confirmed by synthetic standards.

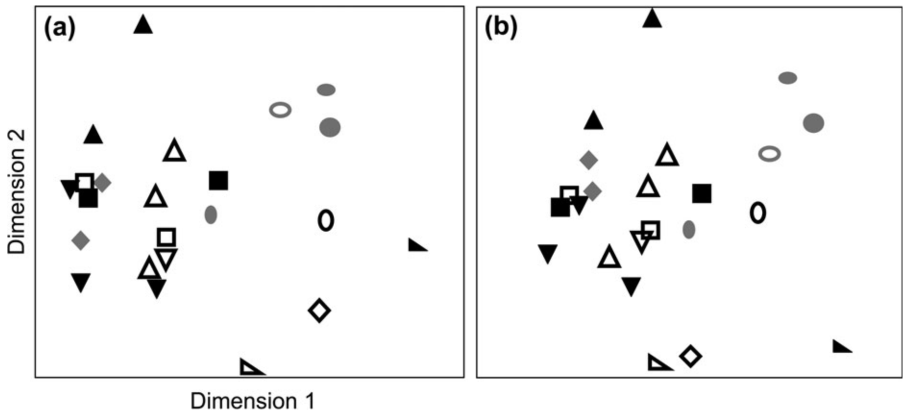

**Figure 2** Ordination figures (based on Bray–Curtis distance matrices) of **(a)** all compounds and **(b)** only sesquiterpenes from resin extracts from 23 trees (14 species). Different symbols indicate different tree species—dipterocarp trees: closed triangle, *Shorea pilosa*; open triangle, *Parashorea melanonan*; closed triangle upside down, *Hopea nervosa*; open triangle upside down, *P. tomentella*; closed square, *S. smithiana*, open square, *S. parvifolia*, closed diamond, *S. faguetiana*; closed circle, *Dryobalanops aromatica*; open ellipse standing, *Dryobalanops lanceolata*; closed ellipse standing, *S. ferruginea*; open ellipse lying, *Dipterocarpus geniculatus*; non-dipterocarp trees: closed ellipse lying, *Mangifera rufocostata* (Anacardiaceae); open diamond, *Canarium denticulatum* (Burseraceae); closed triangle lying, *Dacryodes* spec. (Burseraceae); open triangle lying, *Agathis borneensis* (Araucariaceae). Black symbols indicate trees visited by bees and gray symbols indicate trees not visited by bees.

explain the differences in the bees' behavioral choices between these extracts, indicating that other compounds (sesquiterpenes) must (also) have played a role.

All these findings suggest that stingless bees do not rely on/learn the entire resin bouquet because they showed neither a response to slight modifications of the bouquet (e.g., by adding only 1 terpene) nor a consistent preference for their known collecting tree as would be expected if they used/learned the entire bouquet. Instead, they responded to relatively strong modifications of the resin bouquet (e.g., by adding terpene mixtures), suggesting that they use not only one but several specific mono- and sesquiterpenes to locate known and/or preferred resin sources. They likely learn the proportions of these compounds within the resin bouquet of the visited tree individual/species and use them to recognize partly even individual trees. Given the vast number and diversity of as well as the often strong intra/interindividual variation among volatile compounds in resin bouquets or floral scents, relying on several specific compounds—at the expense of recognition acuity—appears to be a useful strategy for bees searching for resources. Such a reliance on the proportion of several resin terpenoids has also been shown for the moth *Dioryctria sylvestrella* that preferred trees with resin containing low concentrations of β-pinene and high concentrations of β-caryophyllene (Kleinhentz et al. 1999). Honeybees also use several specific compounds to recognize flowers (Pham-Delègue et al. 1990; Masson et al. 1993; Blight et al. 1997; Laloi et al. 2000; Wright, Lutmerding, et al. 2005). Blight et al. (1997) found that a mixture of terpenes (α-pinene, *p*-cymene, α-terpinene, linalool, (*E*,*E*)-α-farnesene, and 3-carene), alcohols, and aldehydes elicited the highest conditioned proboscis extension responses. A nearly equally strong response could be provoked by a mixture of the 3 most active compounds (linalool, 2-phenylethanol, and (*E*,*E*)-α-farnesene), which likely play a key role in honeybee recognition of oilseed rape flowers (*Brassica napus*) (Blight et al. 1997). To recognize snapdragon flowers (*Antirrhinum majus*), honeybees seem to use 3 monoterpenes (myrcene, *E*-β-ocimene, and linalool) and 5 phenylpropanoids (methylbenzoate, acetophenone, dimethoxytoluene, *cis*-methylcinnamate, and *trans*-methylcinnamate) but were only able to discriminate between different snapdragon cultivars when their floral scents showed relatively strong quantitative differences (Wright, Lutmerding, et al. 2005). Interestingly, some of the terpenes used in these studies (α-pinene, (+)-3-carene, *p*-cymene, myrcene, and farnesene) were also used in our study, and farnesene even affected the behavioral choices of resin foragers, indicating that the same terpenes might be utilized by flower- and resin-seeking bees. However, our study does not allow for a precise identification of terpenes used by bees foraging on resin sources. Depending on the context, olfactory receptors of insects are often highly sensitive to specific compounds and are even able to distinguish between different enantiomers of a given substance (e.g., Ulland et al. 2006). Given the importance of resin, it is possible that stingless bees show a similar acuity for resin volatiles, but whether they even rely on specific enantiomers needs further investigation.

Summarizing our results, stingless bees appear to use the same mechanisms and compounds to locate and recognize resin sources as honeybees (and therefore most likely also stingless bees) do to locate and recognize flowers: they rely on proportions of several specific mono- and sesquiterpenes instead of the whole odor bouquet. Moreover, stingless bees tend to prefer known over modified extracts, suggesting some kind of "resin constancy." Although we cannot rule out that visual cues are also involved in the location and/or recognition of resin sources—as they are in the location of floral resources (Villa and Weiss 1990)—we could reliably demonstrate that stingless bees use volatile terpenes.

## Funding

German Excellence Initiative to the Graduate School of Life Science, University of Würzburg to S.D.L., German Academic Exchange Program (Deutscher Akademischer Austauschdienst) to S.Z.; Sonderforschungsbereich SFB 554 (Mechanisms and Evolution of Arthropod Behavior) of the Deutsche Forschungsgemeinschaft to T.S.

## Acknowledgements

We thank the Economic Planning Unit for giving us permission to perform research in Malaysia and the Royal Society for their help with planning and conducting field work in Borneo. Dr Chey Vun Khen, Dr Robert Ong, and Dr Arthur Chung (Forestry Research Center, Sandakan) kindly supported this project. The comments of 2 anonymous reviewers greatly improved the manuscript.

## References

Armbruster WS. 1984. The role of resin in angiosperm pollination—ecological and chemical considerations. Am J Bot. 71:1149–1160.

Bankova VS, de Castro SL, Marcucci MC. 2000. Propolis: recent advances in chemistry and plant origin. Apidologie. 31:3–15.

Blight MM, LeMetayer M, Delegue MHP, Pickett JA, MarionPoll F, Wadhams LJ. 1997. Identification of floral volatiles involved in recognition of oilseed rape flowers, *Brassica napus*, by honeybees, *Apis mellifera*. J Chem Ecol. 23:1715–1727.

Duangphakdee O, Koeniger N, Deowanish S, Hepburn HR, Wongsiri S. 2009. Ant repellent resins of honeybees and stingless bees. Insectes Soc. 56:333–339.

Fox JD, editor. 1973. A handbook to Kabili-Sepilok forest reserve. Sandakan (Malaysia): Sabah Forestry Department.

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

Howard JJ. 1985. Observations on resin collecting by six interacting species of stingless bees (Apidae, Meliponinae). J Kans Entomol Soc. 58: 337–345.

Khoo SG, Yong HS. 1987. Nest structure and colony defence in the stingless bee *Trigona terminata* Smith. Nat Malays. 12:4–15.

Kleinhentz M, Jactel H, Menassieu P. 1999. Terpene attractant candidates of *Dioryctria sylvestrella* in maritime pine (*Pinus pinaster*) oleoresin, needles, liber and headspace samples. J Chem Ecol. 25:2741–2756.

Laloi D, Bailez O, Roger B, Pham-Delegue MH, Wadhams LJ. 2000. Recognition of complex odors by restrained and free-flying honeybees, Apis mellifera. J Chem Ecol. 26:2307–2319.

Langenheim JH, editor. 2003. Plant resins: chemistry, evolution, ecology and ethnobotany. Portland, (OR): Timber Press.

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

Leonhardt SD, Blüthgen N. 2009. A sticky affair: resin collection by Bornean stingless bees. Biotropica. 41:730–736.

Leonhardt SD, Blüthgen N, Schmitt T. 2009. Smelling like resin: terpenoids account for species-specific cuticular profiles in Southeast-Asian stingless bees. Insect Soc. 56:157–170.

Masson C, Pham-Delègue MH, Fonta C, Gascuel J, Arnold G, Nicolas G, Kerszberg M. 1993. Recent advances in the concept of adaptation to natural odor signals in the honeybee, *Apis mellifera* L. Apidologie. 24: 169–194.

Moure JS. 1961. A preliminary supra-specific classification of the old world meliponine bees (Hymenoptera, Apoidea). Studia Entomol. 4:181–242.

Patricio E, Cruz-Lopez L, Maile R, Tentschert J, Jones GR, Morgan ED. 2002. The propolis of stingless bees: terpenes from the tibia of three *Frieseomelitta* species. J. Insect Physiol. 48:249–254.

Pham-Delègue MH, Bailez O, Blight MM, Masson C, Picardnizou AL, Wadhams LJ. 1993. Behavioral discrimination of oilseed rape volatiles by the honeybee *Apis mellifera* L. Chem Senses. 18:483–494.

Pham-Delègue MH, Etievant P, Guichard E, Marilleau R, Douault P, Chauffaille J, Masson C. 1990. Chemicals involved in honeybee-sunflower relationship. J Chem Ecol. 16:3053–3065.

Pham-Delègue MH, Masson C, Etievant P, Azar M. 1986. Selective olfactory choices of the honeybee among sunflower aromas—a study by combined olfactory conditioning and chemical analysis. J Chem Ecol. 12: 781–793.

Phillips MA, Croteau RB. 1999. Resin-based defenses in conifers. Trends Plant Sci. 4:184–190.

R Development Core Team. 2009. R: a language and environment for statistical computing. ISBN 3-900051-07-0 [Internet]. Vienna (Austria): R Foundation for Statistical Computing. Available from: URL http://www.R-project.org.

Reid BL, Macdonald JF, Ross DR. 1995. Foraging and spatial dispersion in protein scavenging workers of *Vespula germanica* and *V. maculifrons* (Hymenoptera, Vespidae). J Insect Behav. 8:315–330.

Roces F. 1994. Odor learning and decision making during food collection in the leaf cutting ant *Acromyrmex lundi*. Insect Soc. 41:235–239.

In: Roubik DW, editor. Ecology and natural history of tropical bees. New York: Cambridge University Press.

Roubik DW. 2006. Stingless bee nesting biology. Apidologie. 37:124–143.

Schwarz HF. 1948. Stingless bees of the western hemisphere. Bull Am Mus Nat Hist. 90:1–546.

Steck K, Hansson BS, Knaden M. 2009. Smells like home: desert ants, *Cataglyphis fortis*, use olfactory landmarks to pinpoint the nest. Front Zool. 6:1–8.

Thiery D, Bluet JM, Pham-Delègue MH, Etievant P, Masson C. 1990. Sunflower aroma detection by the honeybee—study by coupling gas-chromatography and electroantennography. J Chem Ecol. 16:701–711.

Ulland S, Ian E, Borg-Karlson AK, Mustaparta H. 2006. Discrimination between enantiomers of linalool by olfactory receptor neurons in the cabbage moth *Mamestra brassicae* (L.). Chem Senses. 31:325–334.

Velikova M, Bankova V, Tsvetkova I, Kujumgiev A, Marcucci MC. 2000. Antibacterial ent-kaurene from Brazilian propolis of native stingless bees. Fitoterapia. 71:693–696.

Villa JD, Weiss MR. 1990. Observations on the use of visual and olfactory cues by *Trigona* spp foragers. Apidologie. 21:541–545.

Wallace HM, Trueman SJ. 1995. Dispersal of *Eucalyptus torelliana* seeds by the resin-collecting stingless bee, *Trigona carbonaria*. Oecologia. 104:12–16.

Wille A. 1983. Biology of the stingless bee. Annu Rev Entomol. 28:41–46.

Wittmann D. 1985. Aerial defense of the nest by workers of the stingless bee *Trigona (Tetragonisca) angustula* (Latreille) (Hymenoptera, Apidae). Behav Ecol Sociobiol. 16:111–114.

Wright GA, Lutmerding A, Dudareva N, Smith BH. 2005. Intensity and the ratios of compounds in the scent of snapdragon flowers affect scent discrimination by honeybees (*Apis mellifera*). J Comp Physiol A. 191: 105–114.

Wright GA, Thomson MGA, Smith BH. 2005. Odour concentration affects odour identity in honeybees. Proc R Soc Lond B Biol Sci. 272:2417–2422.