Resin collection and use in stingless bees

Wie stachellose Bienen Pflanzenharze sammeln und nutzen

Doctoral Thesis for a doctoral degree at the Graduate School of Life Sciences, Julius-Maximilians-Universität Würzburg
Section: Integrative Biology

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The Bee

His labor is a chant,
His idleness a tune;
Oh, for the bee's experience
Of clovers and of noon!

Emily Dickinson – Poems XV

Submitted on/ eingereicht am:

……...

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Würzburg ……...

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So long, and thanks for all the bees…

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Thesis is based on the following manuscripts:

(1) Leonhardt SD, Zeilhofer S, Blüthgen N, Schmitt T (under review, **Chemical Senses**) Stingless bees use terpenes as olfactory cues to find resin sources.

(2) Leonhardt SD, Blüthgen N (2009) A sticky affair: resin collection by Bornean stingless bees. **Biotropica** 41 (6): 730-736.

(3) Leonhardt SD, Blüthgen N, Schmitt T (2009) Smelling like resin: terpenoids account for species-specific cuticular profiles in Southeast-Asian stingless bees. **Insectes Sociaux** 56 (2): 157-170.

(4) Leonhardt SD, Blüthgen N, Schmitt T (submitted to **Journal of Chemical Ecology**) Tree resin's trace: chemical profiles of body surfaces and nests from six Bornean stingless bee species.

(5) Leonhardt SD, Schmitt T, Blüthgen N (submitted to **New Phytologist**) Chemodiversity: tree resin composition, collection behaviour and selective filters shape chemical profiles of tropical bees.

(6) Leonhardt SD, Jung LM, Schmitt T, Blüthgen N (in press) Terpenoids tame aggressors: role of chemicals in stingless bee communal nesting. **Behavioral Ecology and Sociobiology.**

(7) Leonhardt SD, Schmitt T (submitted to **Austral Ecology**) The cuticular profiles of Australian stingless bees mirror the unusual resin of their resin source (*Corymbia torelliana*).

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I. Summary

Resin, a sticky sap emitting terpenoids and other volatiles, is produced by various plant species to seal wounds and protect themselves against herbivores and microbes. Among several other insects, bees have evolved the surprising ability to handle the repellent plant sap and use it to construct and defend their nests. Whereas the collection of pollen and nectar has been intensively studied in bees, resin collection has received only little attention. The aim of this dissertation was to better understand how the physiological and chemical properties of resin and resin-derived compounds (terpenes) affect the ecology of stingless bees. I therefore asked why, where and how stingless bees of Borneo (seven study-species), Australia (eight) and Costa Rica (27) collect and process plant resins, addressing the importance of a largely neglected resource not only for building and defensive properties, but also for the bees' chemical diversity.

Stingless bees are highly opportunistic resin foragers with all species collecting resin from a similar set of tree species. They locate and/or recognize resin sources on the basis of several volatile mono- and sesquiterpenes. I found that different bee species and even colonies significantly varied in the amount of resin collected. Predator attack (e.g., by ants) had the strongest affect on resin intake, whereas manual nest destruction only slightly increased the number of resin foragers. Resin is used to build, maintain and defend nests, but also as source for chemical compounds (terpenes) which stingless bees include in their surface profiles (chemical profiles). They directly transfer resin-derived compounds to their body surfaces (cuticular terpenes), but only include a subset (8 %) of the large number (>> 1000) of terpenes found in tree resins. This phenomenon can only be explained by a hitherto unknown ability to filter environmentally derived compounds which results in species-specific terpene profiles and thus in an increased chemical heterogeneity among species. Moreover, due to the addition of resin-derived substances the diversity of compounds on the bees' body surfaces by far exceeds the chemical diversity of profiles in other hymenopterans.

Because stingless bees filter but do not modify resin-derived compounds, species from Borneo, Australia and Costa Rica all resemble the characteristic resin of typical trees in their regions of origin. This chemical similarity reveals a strong correlation between the diversity of tree resins and the diversity of cuticular terpenes among stingless bees in a given habitat. Because different tree species are found in different tropical regions, the chemical composition of tree resins varies between tropical regions as does the composition of cuticular terpenes in bee species from these

regions. Cuticular terpenes are however most common among stingless from Borneo, with 100 % of species studied having resin-derived terpenes in their chemical profiles. They are least common in Costa Rica, with only 40 % of species having terpenes. Likewise, resin collection was found to be highest in *Tetragonilla collina* colonies of Borneo where occasionally up to 90 % of foragers collected resin. By contrast, resin collection was only performed by 10 % of foragers of a given colony in Australia and by a maximum of 40 % in Costa Rica. The dominance of resin and resin-derived compounds in the chemical ecology of bees from Borneo may mirror the dominance of a particular Southeast Asian tree family: the highly resinous dipterocarps. Such a correlation between the chemistry of bees and the chemistry of tree resins therefore underlines the close relationship between stingless bees and the trees of their habitat.

Cuticular terpenes are assumed to protect bees against predators and/or microbes. Sesquiterpenes, a specific group of terpenes, most vary between species and impair inter-specific aggression by reducing aggressive behavior in species without sesquiterpenes, thereby providing a novel mechanism to achieve interspecific tolerance among insects. Reduced interspecific aggression may also be an important factor enabling the non-aggressive aggregation of nests from stingless bee colonies of up to four different species, because such aggregations frequently comprise both species with and species without sesquiterpenes.

Given its various functions, resin represents a highly important resource for stingless bees which directly affects their chemical ecology, defensive properties and inter-specific communication. It remains to be investigated how the bees influence the resin-derived terpene profiles on their body surface and in their nests, particularly how they manage to exclude entire groups of terpenes. Whether bees actually need a high diversity of different resin sources and therefore tree species to maintain the homeostasis of their colonies or whether they would do equally well with a limited amount of resin sources available, should also be addressed in future studies. Answers to this question will directly impair bee and forest management in (sub)tropical regions.

II. Zusammenfassung

Harz ist ein klebriges Pflanzenprodukt mit einem oft intensiven aromatischen Geruch. Es wird von Bäumen produziert, um Wunden zu verschließen und schädliche Besucher abzuwehren. Einige Insektenarten haben jedoch die erstaunliche Fähigkeit entwickelt, mit der klebrigen Substanz umzugehen und sie sich gar zu Nutzen zu machen. So verwenden Bienen Harz beispielsweise zum Nestbau und zur Verteidigung ihrer Kolonien. Während allgemein bekannt ist, dass Bienen Pollen und Nektar sammeln, wird der Tatsache, dass sie auch Harz sammlen, allerdings sehr viel weniger Beachtung geschenkt. Ziel meiner Dissertation war es daher, herauszufinden, warum, wie und wo stachellose Bienen in Borneo (sieben untersuchte Bienenarten), Australien (acht Arten) und Costa Rica (27 Arten) Pflanzenharze sammeln und verwerten. Diese Arbeit behandelt somit die enge Beziehung zwischen einer eusozialen Insektengattung und einem chemisch und physiologisch hoch komplexen Pflanzenprodukt, das Bienen nicht nur als Nestmaterial und zur Verteidigung dient, sondern auch eine wesentliche Bedeutung für deren chemische Diversität hat.

Stachellose Bienen verhalten sich hochgradig opportunistisch, wenn sie Harz sammeln, d.h. verschiedene Bienenarten sammeln Harz von denselben Baumarten, wobei sie nahezu jede verfügbare Harzquelle nutzen. Dabei finden und erkennen sie Harzquellen anhand einiger charakteristischer Mono- und Sesquiterpene, nutzen jedoch nicht das gesamte Harz-Bouquet. Die Menge an eingetragenem Harz unterscheidet sich zwischen verschiedenen Bienenarten und -kolonien und varriert mit verschiedenen Umweltbedingungen. Insbesondere eine Bedrohung durch Fressfeinde (z. B. Ameisen) führt zu einer massiven Steigerung des Harzeintrages; eine manuelle Zerstörung des Nesteinganges hat dagegen relativ wenig Einfluss. Das eingetragene Harz wird zum Nestbau und zur Verteidigung gegen Fressfeinde und Mikroben genutzt. Darüber hinaus dient es als Quelle für Terpene, die von den Bienen in ihre chemischen Oberflächenprofile eingebaut werden (kutikuläre Terpene). Dabei übertragen sie nur einen Bruchteil (8 %) der gewaltigen Menge (>> 1000) an Terpenen, die man im Harz von Bäumen findet, auf ihre Oberfläche. Die übertragenen Terpene bleiben in ihrer Struktur unverändert, allerdings unterscheiden sich die Bienenarten in der Zusammensetzung der Terpenprofile auf ihrer Oberfläche, obwohl alle untersuchten Arten Harz von denselben Bäumen sammeln. Die unterschiedlichen Terpenprofile sowie die Tatsache, dass nur wenige Terpene aus dem Harz aufgenommen werden, deuten auf einen artspezifischen und bisher unbekannten Filterungsmechanismus bei stachellosen Bienen hin. Auch übersteigt durch die Aufnahme von Terpenen die chemische Diversität der Oberflächenprofile von stachellosen Bienen die zahlreicher anderer Hymenopteren.

Da Bienen die Terpene aus dem Harz nur „filtern“, sie dabei aber nicht verändern, sind sämtliche Bienenarten aus Borneo, Australien und Costa den charakteristischen Harzprofilen von Bäumen aus ihren Ursprungsgebieten chemisch sehr ähnlich. Da in jeder tropischen Region andere Baumarten vorkommen, varriert die chemische Zusammensetzung der vorkommenden Harze und damit der kutikulären Terpene von dort vorkommenden Bienen. Die meisten Bienenarten mit kutikulären Terpenen findet man in Borneo, wo nahezu 100 % der untersuchten Arten aus Baumharzen gewonnene Terpene in ihre chemischen Profilen einbauen. Im Gegensatz dazu sind es in Costa Rica nur 40 % der untersuchten Arten. Auch sammeln in Borneo gelegentlich 9 von 10 Arbeiterinnen einer *Tetragonilla collina* Kolonie Harz, wohingegen in Australien maximal 10 % und in Costa Rica maximal 40 % der Arbeiterinnen einer Kolonie Harz sammeln. Das Vorherrschen von Harz und aus Harz gewonnenen Terpenen in der chemischen Ökologie von Bienen auf Borneo spiegelt das Vorherrschen einer bestimmten südostasiatischen Baumfamilie wieder: der Dipterocarpaceen, deren Holz ungewöhnlich harzig ist. Ein solch enger Zusammenhang zwischen der Chemie von Bienen und der von Baumharzen verdeutlicht die enge Beziehung zwischen stachellosen Bienen und den Bäumen in ihrem Habitat.

Die kutikulären Terpene schützen ihre Träger vor Angreifern (z.B. Ameisen) und Mikrobenbefall. Dabei variiert eine bestimmte Gruppe – Sesquiterpene – am meisten zwischen den Arten. Diese Terpengruppe manipuliert die natürlichweise auftretende zwischen-artliche Aggression, indem sie letztere bei jenen Arten verringert, die selbst keine Sesquiterpene in ihrem Profil haben. Aggressionsminderung durch chemische Komponenten, welche aus der Umwelt aufgenommen werden, stellt somit einen bisher unbekannten Mechanismus dar, um Toleranz zwischen sonst aggressiven Arten zu erreichen. Eine derarte Herabsetzung von aggressiven Verhalten bei stachellosen Bienen kann darüber hinaus ein entscheidender Faktor für das Entstehen sogenannter Nestaggregationen sein. Dabei nisten Kolonien von Bienenarten mit und Bienenarten ohne Sesquiterpene in ihrem chemischen Profil in unmittelbarer Nachbarschaft, ohne gegeneinander aggressiv zu sein.

Im Hinblick auf die zahlreichen Funktionen, die Harze und/oder aus dem Harz gewonnene Substanzen für stachellose Bienen haben, stellt Harz zweifelsohne eine bedeutende Ressource in der Welt der Bienen dar – eine Ressource, die einen direkten Einfluss auf deren chemische Ökologie, Verteidigungsmechanismen und zwischen-artliche Kommunikation ausübt. Wie genau die Bienen ihre artspezifischen Terpenprofile erzeugen, insbesondere, wie es ihnen gelingt, dabei ganze Terpengruppen auszuschließen, muss in zukünftigen Studien genauer untersucht werden. Auch stellt sich die Frage, wie wichtig eine hohe Diversität an

Harzquellen und damit Baumarten für die Bienen ist! Es ist durchaus möglich, dass neben einer Vielfalt an Blütenpflanzenarten auch der „Harzreichtum“ für das Wohlergehen der Bienen eine entscheidende Rolle spielt.

III. Synopsis

Given the enormous diversity of plants and insects worldwide, it may not be surprising that many scientific studies focus on plant-insect interactions. These studies frequently address mutualistic interactions, such as between plants and their pollinators, or antagonistic interactions, such as between plants and their herbivores. Probably most interactions between plants and insects are however neither mutualistic nor antagonistic and yet affect one or the other in often striking ways. My dissertation addresses such an interaction between resources (resin from trees) and consumers (social bees) without a negative effect on the resource. I thereby reveal a surprising connection between the chemical ecology and diversity of bees and the chemical ecology and diversity of trees.

Many bees visit plants not only for pollen and nectar gathering, but also to collect resin, a sticky sap that is secreted by open wounds, young leaves, buds or other plant parts. Resin collection is particularly pronounced in tropical stingless bees that use the sticky material to construct, maintain and defend their nests. To better understand, how resin and resin-derived compounds (terpenes) affect the ecology and behavior of stingless bees, I studied the chemical and behavioral patterns of foraging and defense as well as intra- and interspecific interactions of stingless bees from Borneo and Costa Rica. I further analyzed the chemical profiles of body surfaces and nests from seven stingless bee species of Borneo, eight species of Australia and 27 species of Costa Rica.

Dealing with a toxic plant product

Resin is an often aromatic, highly sticky plant product that can be fatal for incautious insects. Both its stickiness and its chemical composition, particularly the presence of mono- and sesquiterpenes, render it an efficient defense against invertebrates and microbes (Gershenzon and Dudareva 2007). Stingless bees have evolved the striking ability to handle resin and use it not only to build their nests and defend their colonies against predators, like termites or ants (chapter VI), but also as source for environmentally derived compounds (terpenes) which they include in their own chemical surface profiles (chapter VII – IX). Up to 60 % (118 compounds) of the surface compounds from stingless bees in Borneo and up to 50 % (81 compounds) from bees in Australia could be directly allocated to compounds from tree resins. Because terpenes are acquired in addition to genetically derived chemical compounds already present on the bees' body surfaces, they strongly increase the chemical diversity of their profiles.

The bees' ability to deal with the sticky and partly toxic plant sap likely rendered them able to prosper in humid and warm environments, such as the wet tropics. Here, the employment of resin and resin-derived terpenes may strongly improve the effectiveness of defenses against microbial pathogens and infections of their brood and food storage which is crucial for their survival (Michener 1974; Roubik 1983; 1989). Stingless bees further use volatile mono- and sesquiterpenes to locate new or recognize known resin sources (chapter V), whereas many other insects are repelled by terpenoid compounds, e.g. in floral bouquets (Junker et al. 2007; Junker and Blüthgen 2008). Bees are also known to rely on terpenoids, among other volatiles, when seeking flowers for pollen and nectar collection (see chapter V). Due to their obligate dependency on floral resources, Junker and Blüthgen (2010) suggested that obligate flower visitors, like bees, evolved a general tolerance against these deterrent, repellent or even toxic floral substances. This adaptation might have been a prerequisite for evolving an even broader tolerance including the deleterious effect of resinous compounds. Such a broad tolerance might also explain why stingless bees are highly opportunistic foragers and visit a broad range of plants for both resin collection (H_2' = 0.20, chapter IX) and pollen/nectar collection (H_2' = 0.11, Dworschak and Blüthgen 2010).

The origin, diversity and functions of cuticular terpenes

Stingless bees in Southeast Asia are not unique in having resin-derived terpenes in their chemical profiles, although previous studies on chemical profiles of particularly neotropical stingless bee species, that did not reveal any terpenes, suggested so (see chapter VII). Cuticular terpenes were however also found in seven stingless bee species from Australia (chapter XI) and eleven species from Costa Rica (chapter XII). Like in Borneo, the cuticular terpenes of Costa Rican and Australian bees showed a highly species-specific distribution, suggesting that stingless bee species from all over the world are able to specifically filter resin-derived compounds. In contrast to Borneo, fewer species of Australia and particularly Costa Rica had resin-derived compounds on their body surfaces (chapter XII), indicating that the ability to acquire resin-derived compounds is particularly pronounced in the Indo-Malayan stingless bee clade (Fig. 1). According to Rasmussen and Cameron (2007; 2010), the two major sister clades of Southeast Asian and neotropical stingless bees likely split up approximately 50-60 Mya ago. Indo-Malayan stingless bees may have been faced with an extremely high diversity and abundance of plant resins that likely exceeded those of other tropical regions, because they were living in forests dominated by a highly resinous tree family: the dipterocarps. The hypothesis that resin availability is highest in Southeast Asia is supported by the comparatively low resin intake in colonies from both Australia (max. 10 %

of foragers; Wallace and Lee 2010) and Costa Rica (max. 40 % of foragers; chapter XII) compared to colonies from Borneo (up to 90 %; chapter VI). However, only a direct assessment of the density of resin producing trees visited by stingless bees across the three tropical regions may reveal, whether resin abundance is actually higher in Southeast Asia and thus supports a broader distribution of cuticular terpenes among Southeast Asian stingless bee species.

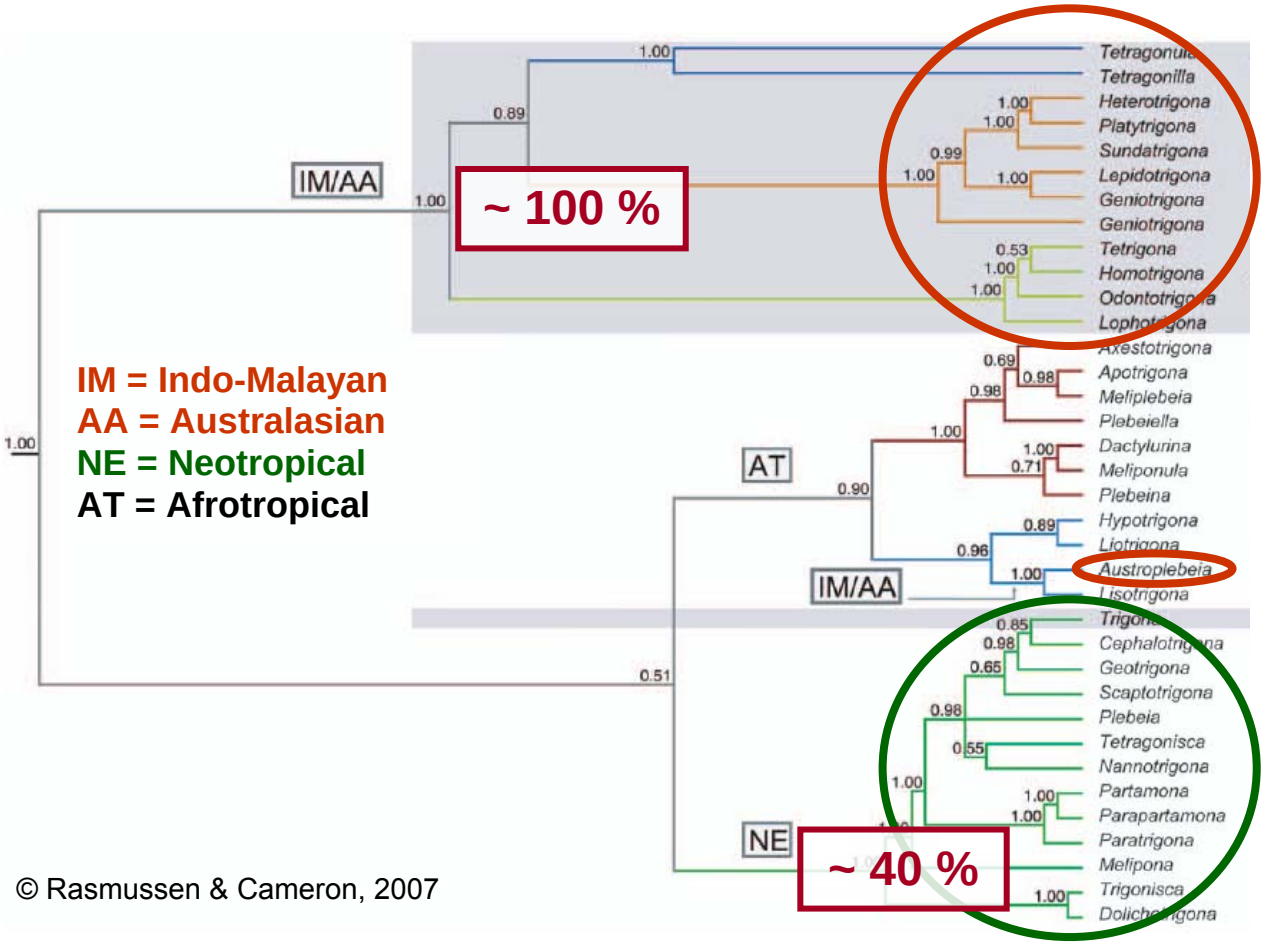

Figure 1. The two major sister clades of stingless bees comprising Old World species (Indomalayan and Australasian) and New World species (Neotropical and Afrotropical). Note that the genus Austroplebeia falls within the New World clade. Percentages of species with terpenes in their chemical surface profiles are given for the two clades. The graph was obtained from Rasmussen & Cameron, Systematic Entomology, 32, 2007.

Stingless bees from all three regions addressed in this dissertation chemically resemble the characteristic resin which is typical for their regions of origin: While compounds from *Corymbia torelliana* seed resin are most prominent among Australian stingless bees (chapter XI), bees from Borneo mirror the characteristic chemical profile of dipterocarp resins (comprising mono-, sesqui- and triterpenes, chapter VII) and surface profiles of neotropical bees contain the same diversity of terpene groups that is found in tree resins of Costa Rican forests (chapter XII). The congruence of bee profiles and tree resin chemistry with regard to

terpene groups underlines the observation that stingless bee species specifically filter resin-derived compounds without subsequently modifying them. It further suggests a strong correlation between the diversity of tree resins in a given habitat and the diversity of cuticular terpenes among stingless bees living in this habitat.

The selective transfer of resin-derived terpenoids to the bees' body surfaces does not only increase the chemical diversity of their surface profiles (chapter IX), but also protects them against predators (Lehmberg et al. 2008) and most likely against microbial attack. If protection was however the only reason for the acquisition of terpenes, the bees would probably do equally well without species-specific terpene profiles (chapter VII). The species-specific distribution of terpenes on the body surfaces of bees all over the world (chapter XI & XII) suggests that acquired terpenes play a role in the inter- and intraspecific communication system of stingless bees. This hypothesis is further supported by the appeasing effect of sesquiterpenes (chapter X). Sesquiterpenes vary most among species and are present in some, but absent in other species (chapter X). Due to their appeasing effect on species without this particular group of terpenes, they may mediate interspecific tolerance. Such chemically mediated tolerance may play a role in the formation of nest aggregations where colonies of up to four different species can be found in close proximity, because these aggregations often comprise both species with and species without sesquiterpenes (chapter X).

Given its importance for the bees' nesting ecology (Roubik 2006), defensive properties, interspecific interactions (chapter X) and chemical diversity (chapter IX), resin can truly be considered a limiting resource for stingless bees (Howard 1985). Its high significance and various purposes may explain why occasionally up to 90 % of foragers from a colony collect resin (chapter VI) and why several plant species offer resin as reward for their bee pollinators (Armbruster 1984) or seed dispersers (Nunez et al. 2008; Wallace et al. 2008). Resin availability may further impair the distribution of stingless bees. Its absence may explain why stingless bees are entirely lacking in oil palm plantations, although these plantations provide a variety of floral resources (personal observation).

IV. General Introduction

Various insects use plants or plant parts as food source, shelter or as "stock" for materials that can be employed for nest construction (e.g., leaves by leaf-cutting bees: Hasenkamp 1974) and/or defense (e.g.; resin by a bee assassin bug: Choe and Krust 2007). Several insect species even sequester toxic plant compounds which render them unpalatable to predators (Eisner et al. 1974; Duffey 1980; Fordyce et al. 2005; Fordyce and Nice 2008). In turn, many plants species depend on insects as pollinators, protectors (e.g., myrmecophilous plants) or seed dispersers, underlining the often close relationship between plants and insects.

Because plants are rarely visited by only one individual insect, but by multiple individuals of the same or different species, insect-insect encounters frequently occur at plant resources, resulting in either mutual tolerance or aggressive competition. Olfactory cues often represent the major inter-mediators in these encounters (Blum and Brand 1972) as well as between plants and insects in general (Wright and Schiestl 2009; Schiestl et al. 2010). Chemical cues therefore play a highly important role in plant-insect interactions.

Studies of plant-insect interactions frequently address plants and their parasites (e.g., herbivores) or flowers and their visitors. The collection and use of floral resources (e.g., pollen) by pollinators has been thoroughly investigated, whereas resin, another important plant resource particularly collected by bees, has been largely neglected. This dissertation focuses on a hitherto unknown close interaction between resin-collecting bees and resin-providing trees which are often heavily competed for by different bee species. While all bee species appear to use the same chemical cues for resin collection and thus largely visit the same trees, different species greatly vary in their "chemical outfit", thus adding a new level of (chemical) complexity to this particular interaction network of bees, trees and tree resin-derived chemical compounds.

General and nesting ecology of stingless bees

Stingless bees (Meliponini: Apidae) comprise a large monophyletic group of at least 600 species (61 described genera) found in tropical regions worldwide, with their highest abundance and diversity in the Neotropics (South and Central America), but further distributions in tropical Africa, Southeast (SE) Asia and Australia (Michener 1979; Rasmussen and Cameron 2010). They are eusocial and live in large colonies with one physogastric queen and between a few dozen and up to several thousand workers (Roubik 1989; Michener 2000). New colonies are founded by a young queen that leaves the parental nest and builds a new nest in its close proximity, exhibiting a species-specific time of

dependence on the mother colony during which workers and resources are frequently exchanged (Inoue et al. 1984). Nests are built in the soil, holes of other animals or within crevices in tree trunks, rocks or human-made buildings (reviewed by Souza et al. 2006) and comprise a large diversity of species-specific structures and shapes (Wille 1983) (Fig. 1). The inner brood cells are surrounded by storage pots with honey, pollen and nectar (Fig. 1) and the whole nest is surrounded by a thick layer of batumen (involucrum) (Roubik 2006). Many species build a more or less long entrance tube (Fig. 1b) that channels the traffic in and out of the nest and allows for an easier and more effective defense against predators, such as ants, true bugs, spiders, termites, wasps or other bee species (Roubik 1998, 2006).

Figure 1. Nesting characteristics of stingless bees: (a) brood cells and pollen storage pots of a hived *Tetragonula carbonaria* colony (Elonora, Australia), (b) guards at the nest entrance tube of a *Nannotrigona perilampoides* colony (Santa Cruz, Costa Rica, © Dylan Burge), (c) *T. carbonaria* workers consuming honey from a honey storage pot (Elonora, Australia) and (d) *Scaptotrigona pectoralis* queen on sealed brood cells (Santa Cruz, Costa Rica, © Dylan Burge).

Stingless bees are considered crucial pollinators in tropical forests (Roubik 1989; Momose et al. 1998; Corlett 2004) and visit flowers of more than 100 plant species in a given habitat (Wilms et al. 1996). Habitat destruction and conversion by humans can alter the composition

of stingless bee communities which might affect the reproductive success of plants and thus forest composition (Samejima et al. 2004; Winfree et al. 2009), further stressing their importance as generalist pollinators.

Resource allocation in stingless bees

The main resources collected by stingless bees are pollen and nectar, but they also collect a variety of other resources (Fig. 2), such as sap, oils, honeydew, water, urine, carrion, soil, rotten wood, bark, mud, feces, spores, blood, paint, salts, gums and plant resins (Fig. 2b,c) for nutrition or nest construction (Roubik 1989). Pollen is the main protein source for bee larvae and adults. It is added to brood cells, but also exchanged between adults in a liquid suspension via trophallaxis (Sommeijer et al. 1985). Nectar represents the main energy source for adult bees, but is also used by foragers to attach pollen to their hindlegs (Roubik 1989; Leonhardt et al. 2007). Stingless bees temporarily specialize on either pollen, nectar or resin collection (Sommeijer et al. 1983; Biesmeijer and Toth 1998; Leonhardt et al. 2007) and often tend to return to a previously occupied lucrative site ("central-place" foraging) (Roubik 1989). Depending on their body size, stingless bees can cover flight ranges of up to 3 km (Roubik 1989).

Different bee species employ different foraging strategies with some species showing high recruitment rates and/or aggression to monopolize lucrative food sources, while others are more effective in finding new and also scattered resources, but show rather low aggressive and/or recruitment behavior (Hubbell and Johnson 1978; Johnson 1983; Nagamitsu and Inoue 1997; Biesmeijer and Slaa 2004). At resources, individuals of the same or different colonies/species can often be observed simultaneously (reviewed by Biesmeijer and Slaa 2004), and inter- and intraspecific aggression occasionally occurs, especially at high quality resources where two bees may fight until one or both opponents die (Johnson and Hubbell 1974; Howard 1985; Nagamitsu and Inoue 1997; Leonhardt and Blüthgen 2009).

Figure 2. Resource allocation in stingless bees: (a) *Heterotrigona erythrogaster* forager killed at a resin wound by a true assassin bug (Reduviidae) (Sepilok, Borneo), (b) returning *Tetragonilla collina* resin forager with a resin load attached to its corbicula (Sepilok, Borneo), (c) *Tetrigona binghami* forager collecting resin from *Hopea nervosa* (Danum Valley, Borneo) and (d) *T. binghami* foragers collecting pollen from inflorescences of a perennial herb (Sepilok, Borneo).

The chemical basis of nestmate recognition, communication and defense in bees

Bees, like most other insects, possess a large repertoire of chemical compounds stored in specialized glands or secreted on their body surface (reviewed by e.g.`; Blum and Brand 1972; Hefetz 1987; Howard 1993; Ayasse et al. 2001). These compounds serve for mate recognition and attraction (sex pheromones, reviewed by Ayasse et al. 2001), to render brood cells waterproof (Hefetz 1987), as defense against intruders (Hefetz et al. 1979), as alarm pheromones and for colony defense (Johnson et al. 1985; Pankiw 2004), as well as for other types of communication (Breed 1983; 1998; Pankiw 2004; Barth et al. 2008). Most chemical compounds are produced by the bees themselves in specialized glands (genetically determined compounds, e.g.; Breed et al. 1988a), but some compounds, especially on the bees' body surfaces (cuticular compounds), are (additionally) acquired from the environment

(environmentally derived compounds, e.g.; Dressler 1982; Downs et al. 2000; Leonhardt et al. 2009).

Cuticular compounds

Cuticular compounds are produced in dorsal epithelial gland cells and are generally thought to protect insects from desiccation, cuticle abrasion and infection (Lockey 1988; St. Leger 1995). In several insect taxa, they became further involved in inter- and intraspecific communication (Wilson 1971; Fletcher and Michener 1987; Howard 1993). The main substance classes of cuticular compounds are hydrocarbons, such as non-polar long-chain linear n-alkanes, alkenes, and mono-, di- and trimethyl-branched alkanes, as well as polar compounds like carboxylic acids, esters and long-chain alcohols and aldehydes (Buckner 1993; Howard 1993). Non-polar n-alkanes, alkenes and methyl-branched alkanes are predominantly found in ants (Hölldobler 1995; Martin and Drijfhout 2009), termites (Howard et al. 1982; Kaib et al. 2004), social wasps (Espelie et al. 1994) and bumblebees (Ayasse et al. 1995), whereas, besides non-polar compounds, compounds with functional groups (alcohols, aldehydes, esters, carboxylic acids, lactones) are frequently present in the cuticular profiles of bees (Ayasse et al. 1999; Paulmier et al. 1999; Fröhlich et al. 2000b; Abdalla et al. 2003; Jungnickel et al. 2004; Kerr et al. 2004; Mant et al. 2005; Nunes et al. 2008). The cuticular chemistry of stingless bees has hitherto received only little attention. Those few studies that investigated cuticular profiles of exclusively neotropical stingless bee species revealed mainly, partly even exclusively, non-polar aliphatic hydrocarbons (n-alkanes, alkenes and branched alkanes) and, to a lesser extent, compounds with functional groups (esters, carboxylic acids, aldehydes) (Abdalla et al. 2003; Jungnickel et al. 2004; Kerr et al. 2004; Nunes et al. 2008; 2009a; 2009b). The chemical composition of these compounds differed between species and/or colonies of the same species (Abdalla et al. 2003; Jungnickel et al. 2004; Kerr et al. 2004; Nunes et al. 2008) – a prerequisite for the ability to distinguish between nest members and foreign individuals (nestmate recognition). A sophisticated nestmate recognition system in stingless bees based on colony-specific chemical signals is further indicated by behavioural studies in both neotropical and paleotropical bees (Inoue and Roubik 1990; Breed and Page 1991; Suka and Inoue 1993; Bowden et al. 1994; Suka et al. 1994; Nagamitsu and Inoue 1997; Inoue et al. 1999; Kirchner and Friebe 1999; Dworschak and Blüthgen 2010). In honeybees, nestmate recognition cues were suggested to comprise saturated and unsaturated fatty acids (Breed 1998; Breed et al. 2004a; 2004b). Fatty acids also seem to function as recognition cues in the stingless bee *Trigona fulviventris*, but are complemented by alkanes and floral oils (Buchwald and Breed 2005).

Glandular compounds

Bees have exocrine glands (modifications of epidermal cells in the integument) over their entire body (Da Cruz-Landim et al. 2005). These glands produce a high diversity of chemical compounds used for intraspecific communication, nest construction or defense (Table 1). The amount and composition of these compounds varies not only between different species, but also within the same species (Table 1; reviewed by Da Cruz-Landim et al. 2005).

Wax, a mixture of multiple compounds (Table 1; Hepburn 1986; Fröhlich et al. 2000a), for comb production is, for instance, produced in wax glands on the inner sites of the bees' sternites. In *Apis mellifera*, wax is softened and cleaned by labial gland secretions (Simpson 1960), whereas these secretions serve for scent trail marking in several stingless bee species (Jarau 2009). Labial and mandibular glands in heads of Brazilian stingless bees contain a huge variety of different compounds (Table 1; Francke et al. 2000). Mandibular glands also have various functions across bee species and individuals (reviewed by Da Cruz-Landim et al. 2005): In nursing honeybees, they produce part of the food for larvae, whereas they secrete the well-known "queen pheromone" in honeybee queens, and defensive/repellent secretions (e.g.; citral in *Lestrimelitta limao*) or trail pheromones in some stingless bees (Table 1). In stingless bees, attractive food sources are further marked with secretions from tendon glands that open at the legs' tips (Table 1; Hrncir et al. 2004; Jarau 2009). Similar compounds were also found in tarsal glands of bumblebees (e.g., Schmitt et al. 1991).

A hydrophobic lining used for brood protection or nest marking is secreted by the Dufour gland (reviewed by Hefetz 1987; and Da Cruz-Landim et al. 2005) which is found in solitary bees and honeybees, but is frequently missing in stingless bees (Table 1; Da Cruz-Landim et al. 2005; Abdalla 2006). The composition of Dufour gland secretions is highly species-specific (Hefetz 1987). A variety of terpenoid compounds that attract workers is further produced by the Nassanoff gland of honeybees (Butler and Calam 1969) which is also absent in stingless bees (Table 1). Many additional glands have been described in bees, but their functions and compositions are less well known.

Due to this huge variety of glands, bees were frequently considered "small chemical factories" that produce a tremendous number of different substances serving various important ecological functions.

Table 1. Glandular compounds of stingless bees (Meliponini) and *Apis mellifera*.

Gland	Product		Function		References
	Apis mellifera	Meliponini	*Apis mellifera*	Meliponini	
Wax gland	wax (aliphatic hydrocarbons, esters, alcohols, acids)		comb cell production		Fröhlich et al. 2000a, Hepburn 1986
Labial gland	unknown	esters, alcohols, carboxylic acids, terpenoids, aldehydes, ketones, aromatic lactones	wax softening	scent trail marking	Simpson 1960, Jarau 2009, Francke et al. 2000
Mandibular gland	alcohols, carboxylic acids, aromatic compounds	esters, alcohols, carboxylic acids, terpenoids, aldehydes, ketones, aromatic lactones	production of larvae food in workers and queen pheromone in queens	production of defensive/repellent secretions, scent trail marking	Jarau 2009, Francke et al. 2000, Da Cruz-Landim et al. 2005
Tendon gland	unknown	aliphatic hydrocarbons, esters, acids, aldehydes	unknown	food source marking	Hrncir et al. 2004, Jarau 2009
Dufour gland	aliphatic hydrocarbons, lactones, esters, aldehydes, alcohols, triglycerids, terpenoids	(frequently) absent	brood protection	(frequently) absent	Hefetz 1987, Da Cruz-Landim et al. 2005, Abdalla 2006
Nassanoff gland	terpenoid compounds	absent	worker attraction	absent	Butler & Calam 1969

Resin and terpenes

Resin – a sticky and often aromatic plant sap – is produced by a large number of tree families (Langenheim 2003). It is secreted in response to an injury of plant parts, but can also occur spontaneously (Langenheim et al. 1978). Resin primarily seals wounds, thereby preventing infections (Langenheim 2003), but also repels herbivorous insects (e.g.; lepidopteran larvae by *Hymenaea* resin: Langenheim and Stubblebine 1983), ants (pine resins: Codella and Raffa 1995), termites (guayule pine resin: Bultman et al. 1998), bacteria (*Clusia* resin: Lokvam and Braddock 1999) and fungi (dipterocarp resin: Messer 1985; guayule pine resin: Bultman et al. 1991). The wound-sealing and repellent functions are due to a synergism of different compound classes, frequently mono-, sesqui-, di- and triterpenes (also known as terpenoids or isoprenoids; Gershenzon and Dudareva 2007; Langenheim 2003). In conifer resin, the lower molecular weight monoterpenes are believed to act as solvents enabling the rapid transport of the higher molecular weight diterpene acids (Gershenzon and Dudareva 2007). The latter act as toxins and feeding deterrents to herbivores, but further polymerize on exposure to oxygen, thereby sealing the wound, whereas monoterpenes repel herbivores and inhibit fungal growth (Gershenzon and Dudareva 2007).

Although resin typically contains toxic and deterrent compounds, some animals are able to utilize resin for their own benefits. The sawfly larva (*Neodiprion sertifer*) sequesters terpenes obtained from resin of its host plant *Pinus sylvestris* to deter predators (Eisner et al. 1974). *Vollenhovia* ants build their whole nests out of resin (Brühl 2003), and *Formica paralugubris* ants carry solidified conifer resin pieces into their nests to protect themselves against pathogens (Christe et al. 2003; Chapuisat et al. 2007), a prophylaxis that further decreases their immune activity (Castella et al. 2008a; 2008b). Similarly, in honeybees, resin collection significantly decreases the expression of two immune-related genes as well as the bacterial loads within their nests, thus directly enhancing their social immune system (Simone et al. 2009).

An alternative to obtaining resin or resin-derived terpenes from the environment is the *de novo* production of these compounds (Eisner 1970; Pasteels et al. 1983). Self-produced volatile irritants, like monoterpenes, and resinous secretions serve as defense particularly in slow-moving arthropods that are highly exposed to predation, e.g.; several termite species (Eisner et al. 1976; Prestwich 1979), small ants (Maschwitz 1975), carrion beetles (Eisner et al. 1986), onychophorans and glomerid millipedes (Eisner 1970). These secretions are predominantly aimed against large predating ants (Prestwich 1979; Pasteels et al. 1983). Besides their repellent or deterrent function, insects further secrete self-produced terpenes to

attract mates, communicate within and between species or mark resources (see "the chemical basis of nestmate recognition, communication and defense in bees").
Overall, the diversity and functions of terpenes in nature is striking and represents a good example of the synergistic benefits of 'chemodiversity' (Gershenzon and Dudareva 2007) from which plants and animals do benefit alike.

Thesis outline

Between 2007 and 2010, I studied the behavioral, chemical and ecological role of resin collection in tropical stingless bees on three different continents (Fig. 3), focusing on the following aspects:

1. *Olfactory cues used by stingless bee foragers to find resin sources:*

To investigate whether stingless bees rely on terpenes to locate resin sources in the field, foraging bees were offered pure resin-extracts as well as resin extracts modified by single or multiple mono- or sesquiterpenes.

2. *General use of plant resins by stingless bees:*

To find out which factors impair resin intake in stingless bees, I observed the proportion of resin foragers at nest entrances from several species and colonies and noted whether different events (such as the manual destruction of the bees' entrance tube or an ant attack) influenced the colonies' resin intake.

3. *Cuticular profiles and nest profiles of stingless bees from Borneo:*

The chemical surface profiles of seven stingless bee species were characterized by GC-MS analysis, revealing the presence of cuticular terpenes which have as yet not been found on the body surface of any other social insect. In addition to the bees' surfaces, I further analyzed the chemical profiles of the bees' nest material to compare bees and nests and relate the findings to equivalent analyses in other bees.

4. *The origin of cuticular terpenes and chemical diversity in stingless bees:*

The addition of resin-derived terpenes increases the chemical diversity of surface profiles from stingless bees which by far exceeds the chemical diversity of cuticular profiles in ants and bumblebees. To find out whether different stingless bee species collected resin from different tree species (specialized) or from the same tree species (generalized), I observed bees at trees (sources of chemical compounds) and nest entrances. Two-dimensional network analyses were then used to compare chemical (tree resin compounds – tree species & cuticular compounds – bee species) and foraging networks (bee species – tree species/resins).

If bees merely transferred resin-derived terpenes to their surfaces without filtering or modifying them, their species-specificity of resin collection would directly predict the specificity of their chemical profiles. However, if bees filtered or modified resin-derived terpenes before sequestering them on their surface, their chemical profiles would be independent of their resin foraging behavior.

5. *Use of cuticular terpenoids:*

To investigate the role of the cuticular terpenoids, I performed recognition assays by modifying the surface profile of the stingless bee *Tetragonula melanocephala*. Because sesquiterpenes were found to be most variable between different bee species, I used this class of terpenes for the modification experiments, showing that the addition of sesquiterpenes significantly reduced aggression in *T. melanocephala*. To test for a general correlation between sesquiterpenes and reduced interspecific aggression which may facilitate the frequent formation of nest aggregations of several bee species in Borneo, additional behavioral aggression assays were performed including bees from the same and different nest aggregations.

6. *Resin collection and cuticular terpenes in Australian and neotropical stingless bees:*

Stingless bees are found all over the world's tropical and subtropical regions. To compare the collection and use of resin and resin-derived compounds between Borneo and other parts of the world, I additionally observed resin collection in stingless bees from Australia and Costa Rica and analyzed the chemical profiles of their body surfaces and nests.

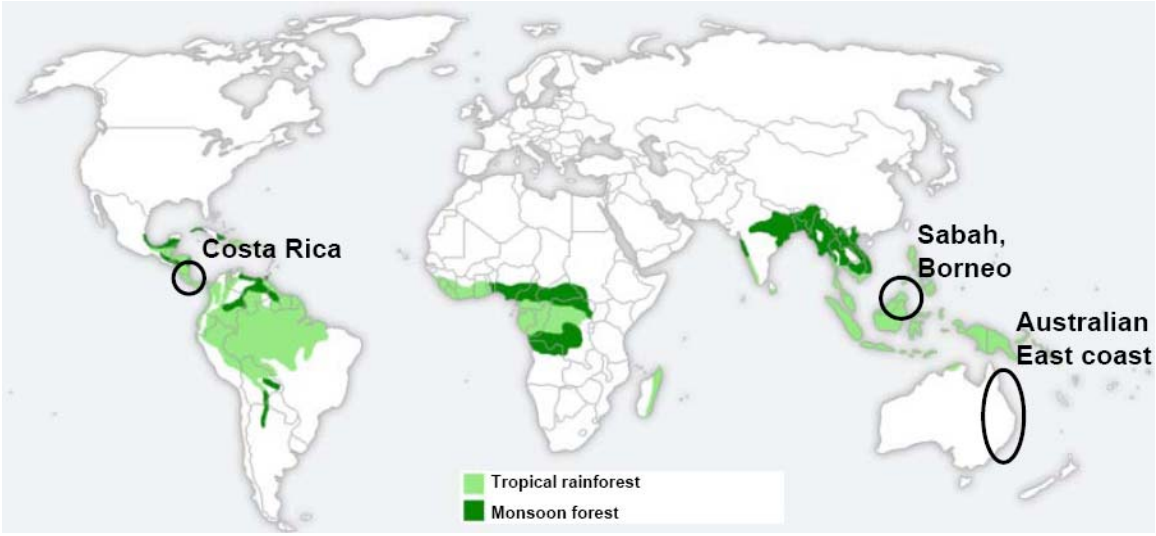

Figure 3. Sites of data collection: Borneo (Malaysia), Australia and Central America.

V. How stingless bees find resin sources

This chapter has been submitted as:
Leonhardt SD, Zeilhofer S, Blüthgen N & Schmitt T – Stingless bees use terpenes as olfactory cues to find resin sources.

V. 1 Summary

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.

V. 2 Introduction

Insects use olfactory cues 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; Thiery et al. 1990; Masson et al. 1993; Pham-Delègue et al. 1993; Laloi et al. 2000; Wright et al. 2005a; 2005b). 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; Souza et al. 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; Souza et al. 2006). Bees also use resin to coat the inner nest walls which prevents the growth of bacteria and fungi (Wille 1983;

Velikova et al. 2000b). 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; Souza et al. 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 intra-specific 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 et al. 2005a). Moreover, the cuticular chemical profiles of stingless bees comprise terpenes which are derived from resins collected (Leonhardt et al. 2009). It is therefore highly likely that stingless bees also use terpenes to locate suitable resin sources, especially since mono-, sesqui- and triterpenes represent the main constituents of resins from dipterocarp trees (Langenheim 2003) which dominate the rain forests of Borneo.

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.

V. 3 Methods

Study sites and bees

Field experiments were conducted at the Rainforest discovery centre (RDC) of Sandakan, in Sabah, Borneo (Malaysia), from September to November 2008. The RDC is a small education centre located ~ 2 km West of the Kabili Sepilok Reserve (KSR: 5°54’ N, 118°04’ E, 20-120 m asl), 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 Centre 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 three tree species known from previous studies to easily secret resins that attract bees (see Leonhardt and Blüthgen 2009): *Agathis borneensis* (Araucariaceae: three individuals), *Shorea xanthophylla* (Dipterocarpaceae: one individual) and *Dryobalanops lanceolata* (Dipterocarpaceae: two 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 3-10 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 six trees using a clean knife and transferred it into a 3 ml vial containing 2 ml pure hexane (Sigma-Aldrich, Munich, Germany). After 15 h, the hexane extract with the hitherto dissolved resin compounds was transferred into a new vial, whereas the non-dissolved 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, unpublished data) or mixing extracts of two 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, Munich, Germany). Sesquiterpenes added comprised (-)-α-copaene (≥ 90 %), β-caryophyllene (≥ 80 %) and α-humulene (≥ 98 %) (all substances purchased from Sigma-Aldrich, Munich, Germany) as well as mixture of three different farnesene isomers (7 % cis-β-farnesene, 10% trans-β-farnesene, 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 non-polar sesquiterpenes (in germacrene: γ -Muurolene and four unknown sesquiterpenes each accounting for more than 4 % D; in the farnesene mixture:

three Bisabolene isomers and one unknown sesquiterpene each accounting for more than 4 %) are hitherto only referred to as germacrene and farnesene. We produced a mono- and a sesquiterpene mixture by adding 0.3 ml of all mono- and all sesquiterpenes, respectively, in 3 ml hexane. Three drops of these mixtures were then added 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, Germany) 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 located 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 paper. 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 one 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 pair-wise comparison of two 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 any one 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, one 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 one tree individual of each species (*A. borneensis*, *S. xantophylla*, and *D. lanceolata*) (Table 1). Tests with extracts modified by adding terpenes as well as with extract mixtures were conducted for *A. borneensis* resin only, because *A. borneensis* was the only tree species with more than two individuals present at RDC (Table 1). Pure extracts were tested against modified extracts at two *A. borneensis* individuals (Table 1). Preference tests between pure extracts and extract

mixtures (of different *A. borneensis* individuals) were performed at all three *A. borneensis* trees (Table 1).

Chemical analyses of resin extracts

Besides the pure and modified or mixed extracts of the three tree species used for observations, 1-2 ml fresh resin was obtained from wounds (one wound per tree) of 23 further tree individuals (14 tree species), 17 of which (ten 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 GC System coupled to a Hewlett Packard HP 5973 Mass Selective Detector (Agilent Technologies, Böblingen, Germany). The GC was equipped with a DB-1 fused silica capillary column (30m x 0.25 mm ID; df = 0.25 μm; J & W, Folsom, CA, USA). Temperature was programmed from 60°C 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 (EI-MS) were recorded at 70 eV and 230°C source temperature. We used the Windows version of the ChemStation software package (Agilent Technologies, Böblingen, Germany) 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 three commercially available mass spectra libraries (Wiley 275, NIST 98 and Adams EO library 2205) and – where available – standards (purchased from Sigma-Aldrich, Munich, Germany) 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.

Table 1. Results of preference tests with pure resin extracts vs. hexane (control), modified resin extracts, pure resin extracts of a different tree, and resin extract mixtures. The mean numbers of bees visiting each extract/control are given. Bold *p*-values mark significant preferences for one the two extracts tested.

				Mean ± SD			
Extract 1	**N (trees)**	**Tested against (extract 2)**	**N (observations)**	**Extract 1**	**Extract 2**	***V***	***p***
Pure extracts against controls							
A. borneensis	1	hexane	11	5 ± 3.8	0	66	**0.004**
S. xantophylla	1	hexane	10	2.4 ± 1.9	0.1 ± 0.3	36	**0.013**
D. 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 & 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	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

Statistical analyses

To see whether differences in the attractiveness (visited vs. non-visited) between trees correlated with differences in their chemical composition, we performed a two-dimensional NMDS (non-metric dimensional scaling) analysis followed by an "Adonis" test (R command for multivariate analysis of variance based on dissimilarities) with the volatile compounds of resin extracts from the 23 tree resins. The NMDS was based on Bray-Curtis distance of the proportions of all volatile compounds that accounted for more than 0.5 % of the total peak area in all samples (start configuration: PCoA, 1000 iterations). 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 two-dimensional NMDS and "Adonis" analysis were performed. A further NMDS was performed to compare extracts of individual *A. borneensis* trees and their mixtures. 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 three individuals used for the experiments were analyzed and compared by an "Adonis" test.

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

V. 4 Results

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

Bees visited filter papers with pure resin extracts of all three 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 (Fig. 1).

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).

Figure 1. *Tetrigona apicalis* visiting filter paper with *Agathis borneensis* resin extract.

Attractiveness of pure resin extracts of different A. borneensis trees

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

Attractiveness of pure vs. 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 showed a clear preference for the resin extract from their collecting tree in only two out of six 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 (Fig. 2). Hexane extract of *A. borneensis* A resin contained considerably higher proportions of the most volatile monoterpenes (α-pinene, sabinene, β-phellandrene and γ-terpinene) than extracts of *A. borneensis* B and C (Table 2, Fig. 2), whereas extract mixtures AB and AC were intermediate in the levels of these monoterpenes (Fig. 2).

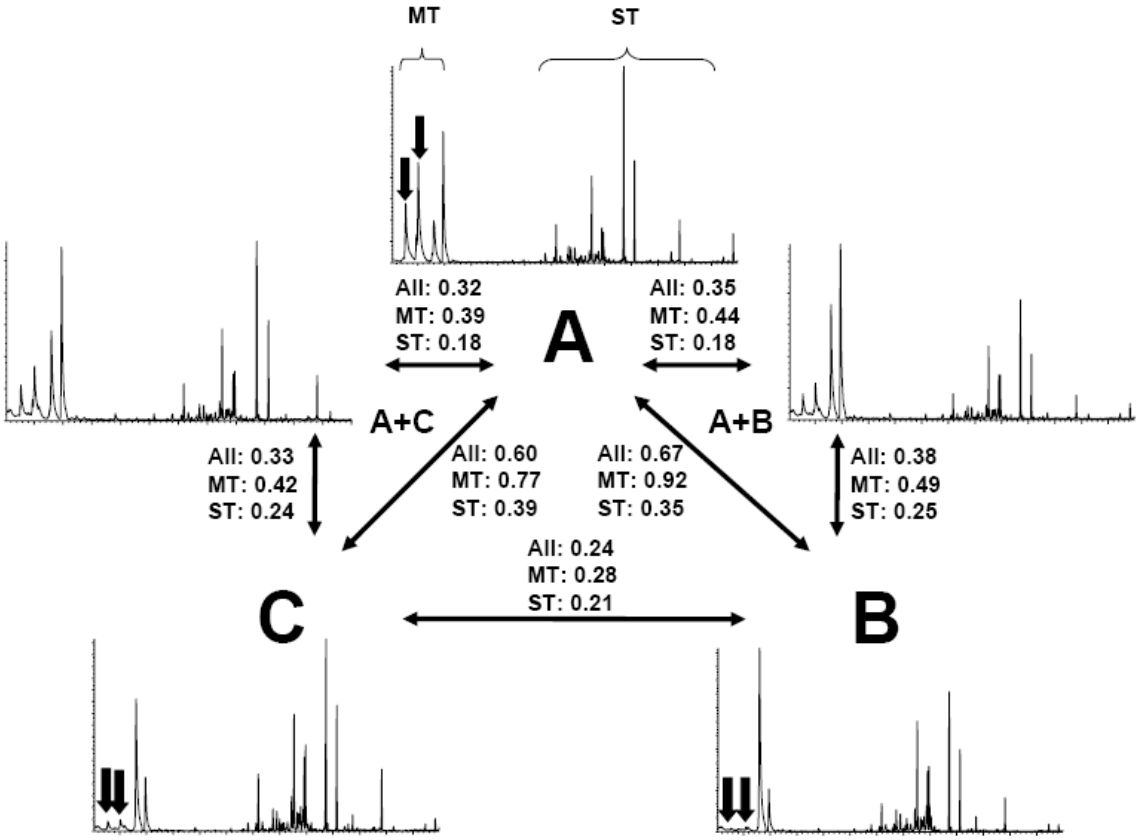

Figure 2. Chromatograms of three pure resin extracts (A, B and C) and two extract mixtures (AB and AC) from three *A. borneensis* trees (comprising only volatile compounds). Bray-Curtis distances between extracts and extract mixtures are given for all volatile compounds (All), only monoterpenes (MT)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

Chemical analyses of resin extracts from A. borneensis

The chemical composition of *A. borneensis* resin differed both within and between individuals (Table 2). Inter-individual variation was however more pronounced than intra-individual variation (Table 2) and was sufficient to distinguish between the three *A. borneensis* trees (Adonis: $R^2 = 0.78$, $p = 0.035$, Table 2). Notably, inter-individual 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 six trees not visited by bees were poorly separated by their chemical compositions (Adonis: all compounds: $R^2 = 0.08$, $p = 0.041$, Fig. 3a; only sesquiterpenes: $R^2 = 0.08$, $p = 0.031$, Fig. 3b), 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 (Fig. 3). Different tree individuals of the

same species (e.g. *Shorea faguetiana*) were more similar to trees of other tree species (e.g. *Shorea parvifolia*) than towards each other (Fig. 3).

Table 2. Percentages [± SD] of tentatively identified substances found in hexane extracts of resins from three *A. borneensis* individuals listed according to molecular weight (MW), retention indices (RI) and retention times (RT). An asterisk indicates substances that were confirmed by synthetic standards.

Nr.	MW	Class	Compound	KI	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*	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*	974	6.18	0 ± 0 %	0.45 ± 0.07 %	0.52 ± 0.05 %
5	136	MT	para-Cymene*	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*	1054	7.91	33.81 ± 18.45 %	4.23 ± 2.55 %	6.65 ± 1.91 %
8	136	MT	Terpinolene*	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*	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*	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*	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*	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 %
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 %

Table 2. continued.

Nr.	MW	Class	Compound	KI	RT	*A. borneensis* A	*A. borneensis* B	*A. borneensis* C
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 %

V. 5 Discussion

Stingless bees in Borneo use olfactory cues to find and recognize tree resins which they exploit for their chemical and physiological 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 any one extract, although they tended to preferentially visit the familiar un-modified/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 two monoterpenes in resin extracts of the three *A. borneensis* individuals (Fig. 2) were not sufficient to explain the differences in the bees' behavioral choices between these extracts, indicating that other compounds (sesquiterpenes) must (also) have played a role.

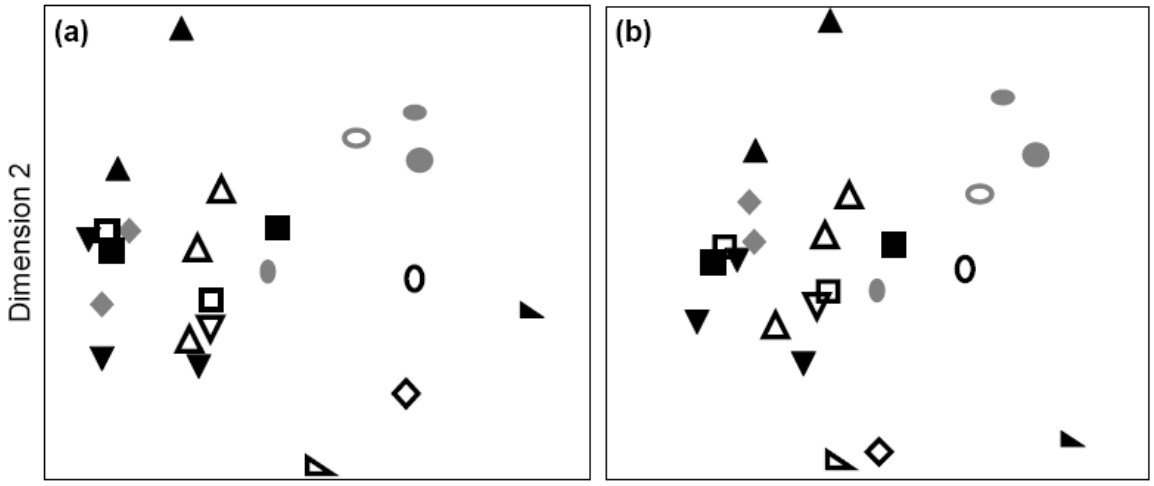

Figure 3. NMDS analyses of (a) all compounds and (b) only sesquiterpenes from resin extracts from 23 trees (14 species). Different symbols indicate different tree species: dipterocarp trees: closed triangle = *Shorea pilosa*, open triangle = *Parashorea melanonan*, closed triangle upside down = *Hopea nervosa*, open triangle upside down = *Parashorea tomentella*, closed square = *Shorea smithiana*, open square = *Shorea parvifolia*, closed diamond = *Shorea faguetiana*, closed circle = *Dryobalanops aromatica*, open ellipse standing = *Dryobalanops lanceolata*, closed ellipse standing = *Shorea 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 grey symbols

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 one 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/inter-individual 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 et al. 2005a). 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 (CPE) responses. A nearly equally strong response could be provoked by a mixture of the three 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 three monoterpenes (myrcene, E-*β*-ocimene, and linalool) and five 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 et al. 2005a). 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.

VI. What for? Use of plant resins by stingless bees

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V. 1 Summary

Plant resins are used by stingless bees for nest construction and maintenance. To reveal factors that influence the bees' decision about where and when to collect resin, resin collection was studied in ten stingless bee species (Apidae: Meliponini) collecting resin at natural and artificially induced wounds of nine tree species in Borneo. Artificially induced wounds were found by bees within 1–2 days. The number of foragers at artificial wounds increased during the subsequent 5 days until resin secretion stopped or the resin hardened. At natural resin wounds, species identity and number of foragers remained constant during the observation period. Bees collected resin from some trees and ignored others. *Agathis borneensis* (Araucariaceae) was the most attractive resin source. The bees' visitation rate did not correlate significantly with resin wound size. Inter- and intraspecific aggression occurred at ten resin wounds. In *Tetragonilla collina* and *Tetragonula melanocephala*, we additionally recorded resin intake at colony entrances. The proportion of workers retuning with resin varied considerably between colonies. We observed attacks by ants at three of our eight focal colonies which resulted in a significant increase in resin intake while the nest was under attack and until 1–2 days after the attack had stopped. The increase in resin collection triggered by ant attacks was even stronger than the increase following a manual destruction of the nest entrance tube.

V. 2 Introduction

Resin, a sticky plant sap, is produced by various tree families and is secreted in response to an injury or infection of plant parts. However, resin secretion can also occur spontaneously as has been shown for the tropical legume *Hymenaea* (Caesalpinioideae) (Langenheim *et al.* 1978). Resin serves as a repellent against herbivorous insects, such as lepidopteran larvae (*Hymenaea* resin: Langenheim and Stubblebine 1983), as well as against ants (pine resins: Codella and Raffa 1995), termites (guayule pine resin: Bultman et al. 1998), bacteria (*Clusia* resin: Lokvam and Braddock 1999) and fungi (dipterocarp resin: Messer 1985; guayule pine resin: Bultman et al. 1991). This repellent or defensive function is most likely due to the

presence of terpenes, especially mono- and sesquiterpenes (Gershenzon and Dudareva 2007). However, some animals manage to utilize resin. A known example is the sawfly larva (*Neodiprion sertifer*) that sequesters terpenes obtained from the resin of its host plant *Pinus sylvestris* to deter predators (Eisner et al. 1974). *Formica paralugubris* ants carry solidified conifer resin pieces into their nests to protect themselves against pathogens (Christe et al. 2003; Chapuisat et al. 2007), and *Vollenhovia* ants even build their whole nests out of resin (Brühl 2003).

Stingless bees (Apidae, Meliponini) also use resin as nest building material. They collect resin from wounded trees or other plant parts such as buds, fruits (Souza et al. 2006) or even inflorescences (Armbruster 1984). Several larger stingless bee species actively bite into the resin wounds and are able to stimulate and maintain resin secretion for days or even weeks (e.g.; Schwarz 1948). However, most species, especially the smaller ones, depend on either other animals, or injury through breakage, or spontaneous resin secretion for resin collection (Howard 1985). Bees collect resin with their mandibles and transfer it to their corbiculae - a unique transportation structure on the hindlegs (Roubik 1989) - (Schwarz 1948; Bassindale 1955; Michener 1974), thus applying the same strategy as used for the transport of pollen.

At resin wounds several species are often found collecting resin at the same time. Howard (1985) observed that six Costa Rican stingless bee species showed a 10–20 fold increased density and an increased frequency of aggressive behavior while they were collecting tree resin compared to other foraging situations (*e.g.*, pollen or nectar collection), suggesting that resin is a limiting resource in the colony size and growth of stingless bees. Moreover, Roubik (1989) emphasized its potential significance for the evolution of eusociality in tropical bees.

Resin appears to serve several functions in stingless bees:

(1) Unlike honeybees which build their nests primarily or even solely out of wax (Michener 1974; Ghisalberti 1979), most stingless bees incorporate various plant materials for nest constructions as plant gums, resin, pollen, seeds, or even mud and feces which are likely to sustain nest stability (Michener 1974; Roubik 1989; van Veen and Arce 1999; Patricio et al. 2002; Eltz et al. 2003). Cerumen is resin mixed with wax (Wille 1983; Bankova et al. 2000; Patricio et al. 2002) and is used to build protective and supporting nest structures as well as honey pots (Wille and Michener 1973). The inner nest walls are further coated with a resin based lining (Wille 1983).

(2) Besides its usage for nest construction, bees most likely benefit from the repellent properties of resin. Resin, deposited in the vicinity of the colony's nest entrance tube, entangled termites as well as ants and thus successfully prevented nest invasions (Schwarz

1948; Wittmann 1985; Khoo and Yong 1987; Souza et al. 2006; Lehmberg et al. 2008). Moreover, resin barriers enable some stingless bee species to construct and maintain their own nests within ant nests without being attacked (Sakagami *et al.* 1989). Besides applying resin at the entrance, stingless bees also place the sticky substance on the body of non-nestmate intruders (*Melipona panamica*: Inoue et al. 1999; *Scaptotrigona bipunctata*: Jungnickel et al. 2004) or predators (Roubik 2006). In addition to the repellence of invertebrates, resin may also serve as a germicide, preventing growth of microbes and fungi (honeybees: Ghisalberti 1979; stingless bees: Velikova et al. 2000a; 2000b).

Despite these potentially important functions of resin, only Howard (1985) has so far described behavioral strategies involved in resin collection by neotropical stingless bees. In the present study, we therefore examined resin collection in ten Bornean stingless bee species. We investigated their preferences for different tree taxa as well as recruitment speed, visitation rates and aggressive interactions at resin wounds. We further studied the factors which influence resin intake at eight nests of the two species *Tetragonilla collina* Moure and *Tetragonula melanocephala* Moure.

V. 3 Methods

Study sites and bees

Observations were conducted at three different field sites in Borneo (Malaysia) from September to December 2007: Danum Valley Conservation Area (DVC), Kabili Sepilok Reserve (KSR) and Rainforest discovery centre (RDC), and Lambir Hills National Park (LHN). All field sites have a typical equatorial rainforest climate with a mean annual temperature of 26–30°C and a yearly rainfall of 2600–3000 mm (Fox 1973; Sakai et al. 1997). DVC (Sabah: 4°55’ N, 117°40’ E; 100 m asl) comprises 43,800 ha and represents one of the major remaining patches of Sabah’s primary lowland dipterocarp rainforest (Marsh and Greer 1992). KSR (Sabah: 5°54’ N, 118°04’ E; 20–120 m asl) covers an area of 4294 ha of coastal dipterocarp forest with more than one-third of it consisting of mangrove forest (Fox 1973), whereas the Rainforest discovery centre (RDC) is a small (148.6 ha) human made education centre about 2 km west of the Kabili Sepilok Reserve. At the RDC, various rainforest trees had been planted or maintained around a system of gravel paths, including *Agathis borneensis* (Araucariaceae), a highland pine species normally absent from lowland rainforests. LHN (Sarawak: 4°20’ N, 113°50’ E; 150 m asl) comprises 6952 ha of intact mixed-dipterocarp forest. At LHN, a well-maintained canopy observation system that consists of two towers and

nine aerial walkways (Inoue et al. 1995) allowed for observation of bees at resin wounds up to a tree height of 40 m.

Fifteen stingless bee species had previously been recorded in DVC (Eltz 2004; Dworschak and Blüthgen 2010). Inoue and colleagues (1994) had described 27 stingless bee species in LHN. To our knowledge, there is no reference on the number of bee species in KSR, but collections of specimens kept by the Forestry Research Centre in Sepilok prelude between 15 and 20 species.

Resin foragers of ten stingless bee species (species and genus names as in Moure 1961) were observed. In three cases, bees could not be identified to the species level. These ten species represent about 40 % of the local stingless bee fauna and are commonly found in disturbed and undisturbed forests of Sabah and Sarawak (Eltz et al. 2003). For observations at nest entrances, the two species *Tetragonilla collina* and *T. melanocephala* were used as focal species.

Resin wounds

Across the three sites (DVC, KSR and LHN), we found 18 trees with naturally occurring wounds secreting resin by searching for such trees following the park's path system. Artificial wounds were inflicted to further 59 trees as well as to two trees which additionally also had naturally occurring resin wounds by hammering nails (5 mm diam.) into the tree trunk. Nails were inserted between 1–2 m height and were removed immediately after insertion to allow resin to flow out of the wound. Between two and four holes per tree were punched to increase the probability of hitting a resin vein. The 61 trees punched belonged to nine different tree families (Dipterocarpaceae, Ebenaceae, Fabaceae, Fagaceae, Burseraceae, Anacardiaceae, Euphorbiaceae, Meliaceae, Proteaceae) with most of them (46 trees) representing dipterocarps. Of these 61 trees, 19 trees actually secreted resin (two of which additionally had natural resin wounds). Thus, bee visitation was monitored for a total of 35 trees (15 species of six families, 22 dipterocarps) with either artificially induced or naturally occurring resin wounds (Table 1), comprising both small trees of about 5 m height and large trees of up to 30 m height.

Bee visitation rates at resin wounds

All wounds were observed for at least three days. Trees without any resin flow until the third day were discarded. Trees with naturally occurring or artificially induced resin flow were observed for 2–5 d after discovery or wound infliction, respectively, and again for another 1-3 d after a period of 1–2 months if the tree was still secreting resin. It was tested whether the visitation rate corresponded to the quantity of resin. Visitation rate was defined as the

number of bees present at the wound plus those arriving during the observation period (3–15 min) divided by the time of observation. The wound area (corresponding to the quantity of resin secretion) was visually assessed and scored as 1–4, with 1 = invisible resin flow, 2 = area of < 2 cm^2 covered with resin, 3 = 2–5 cm^2, and 4 = > 5 cm^2.

Aggression at resin wounds

We observed whether interspecific and/or intraspecific aggression occurred at resin wounds. The following behaviors were considered as aggressive: opening mandibles, running towards another bee with open mandibles, and biting. Among all observation periods in which two species occurred at the same wound, we calculated the proportion of periods with aggressive encounters to determine whether different bee species differed in the amount of aggressive behavior expressed towards others and whether aggression correlated with bee size. Bee size was measured as bee head width (defined as the narrowest distance between the eyes) as given by Dworschak and Blüthgen (2010) for the species observed. This measure is considered to be the most constant measure for bee size.

Observations at bee nests

Observations at nest entrances were conducted for eight colonies: four colonies of *T. melanocephala* in Sepilok ($N = 1$), Danum Valley (2) and Lambir Hills (1); four colonies of *T. collina* in Sepilok (3) and Danum Valley (1). During each survey, 10–22 individual foragers returning to their colony were caught using a butterfly net. Each worker was assigned as either resin forager, pollen forager, nectar forager or forager without load. However, the latter often had thin linings of a propolis-like substance on their hind tibia (Leonhardt et al. 2007). Resin foragers could clearly be distinguished from the rest as they carried large amount of sticky resin on their corbiculae. Over 3 months, a total of 181 surveys were performed for all eight colonies. Each colony was observed one to three times during 0600–1800 h to determine whether the number of resin foragers was dependent on the time of day. To test whether artificially induced nest damage influenced resin collection, each colony was observed before and after the nest entrance tube had been completely removed. Before damage, no bees were observed being engaged in entrance tube elongation. During the study period, three of our eight focal colonies were attacked by ants. All colonies had already been surveyed for 4–13 d before the ant attack, and continued to be observed afterwards. To test whether an ant attack influenced resin collecting behavior of a given colony, the frequency of resin foragers during the attack was compared with the pooled surveys before and after the attack.

Data analysis

To evaluate the attractiveness of *Agathis borneensis* trees, dipterocarps and other tree families as resin sources, the bee visitation rates were compared between the three groups of trees using Kruskal-Wallis ANOVA. *Agathis borneensis* was treated as a separate group because this species attracted large numbers of resin foragers, although, as a highland species, it does not normally occur in SE Asian lowland rainforests. To evaluate the potential affect of resin wound size on the bee visitation rate we tested for correlation between these two variables using the Spearman Rank correlation test.

To reveal which factors affect the proportion of resin foragers among bees returning to the nest, general linear mixed models (GLMMs) with binomial error distribution and a logic link function were fitted for each species separately. The explanatory variables were 'time of day' (morning, midday and late afternoon), 'location' (Danum, Sepilok and Lambir), 'ant attack' (during ant attack and during periods without ant attack), and 'nest damage' (before nest damage and after nest damage until the bees had finished repairing their entrance tube). 'Colony' was included as random factor in all models to avoid pseudoreplication. We followed a forward stepwise procedure to fit a minimal adequate model. Likelihood ratio tests (LRT) were used to find those variables which reduced Akaike's information criterion (AIC) when included in the model. To correct for multiple comparisons (four explanatory variables), False Discovery Rate (FDR) was applied within each GLMM. All statistical analyses were performed in R Version 2.4 (R-Development-Core-Team 2009).

V. 4 Results

Foragers of the following ten bee species were observed at resin wounds: *Lophotrigona canifrons*, *T. collina*, *Tetrigona binghami*, *Heterotrigona erythrogaster*, *Homotrigona fimbriata, Odontotrigona haematoptera*, *Tetragonula laeviceps*, *T. melanocephala*, *Tetragonula melina* and *Geniotrigona thoracica*. These species collected resin from 21 trees (nine species) with either naturally occurring (14) or artificially induced (seven) resin wounds (Table 1). Six tree species secreting resin remained unvisited (Table 1).

Table 1. List of tree species with natural or artificial (marked with an asterisk) resin wounds (N_T indicates the number of trees observed for each tree species, in brackets trees that were actually visited by bees, SS indicates the bee species observed at each tree species with Gt = G. thoracica, He = H. erythrogaster, Hf = H. fimbriata, Oh = O. haematoptera, Tb = T. binghami, Lc = L. canifrons, Tc = T. collina, Tl = T. laeviceps/geissleri group, Tmo = T. melanocephala, Tmi = T. melina, Tsp = unidentified bee species, (*) marks trees with both artificially induced and naturally occurring wounds, two trees that could not be identified are not listed here).

Location	Tree species	Family	N_T	SS
Danum	*Dialium indum******	Fabaceae	**2(0)**	**-**
	Dryobalanops lanceolata(*****)	Dipterocarpaceae	**2(2)**	**Tl**
	Hopea nervosa(*****)	Dipterocarpaceae	**2(2)**	**Tb,Tmo**
	*Mangifera rufo costata******	Anacardiaceae	**1(0)**	**-**
	Parashorea malanonan	Dipterocarpaceae	**2(1)**	**Tsp**
	*Shorea faguetiana******	Dipterocarpaceae	**2(0)**	**-**
	*Shorea parvifolia******	Dipterocarpaceae	**2(1)**	**Tb,Tsp**
Lambir	*Dryobalanops aromatica******	Dipterocarpaceae	**1(0)**	**-**
	Horsfieldia palidicaula	Myristicaceae	**1(1)**	**Tl,Tmo,Tmi**
	*Shorea parvifolia**	Dipterocarpaceae	**1(1)**	**Hf**
	*Shorea pilosa******	Dipterocarpaceae	**1(0)**	**-**
	*Shorea smithiana******	Dipterocarpaceae	**1(0)**	**-**
Sepilok	*Agathis borneensis*	Araucariaceae	**6(6)**	**Gt,Lc,He,Tc,Oh**
	Dacryodes spec.	Burseraceae	**1(1)**	**Lc,Tl**
	*Dryobalanops lanceolata******	Dipterocarpaceae	**1(1)**	**Tl**
	*Hopea nervosa******	Dipterocarpaceae	**1(1)**	**He,Oh,Tsp**
	Parashorea tomentella	Dipterocarpaceae	**3(2)**	**Lc,Oh,Tc,Tb**
	*Shorea almon******	Dipterocarpaceae	**1(0)**	**-**
	Shorea smithiana	Dipterocarpaceae	**2(1)**	**Tc**

Bee visitation rates at resin wounds

The seven artificially induced resin wounds were discovered by bees within 1–2 d. Four of the seven artificial wounds secreted resin over more than three days (*Hopea nervosa*, *Dryobalanops lanceolata*, *Shorea parvifolia* and *S. smithiana*). At these four wounds, the number of bees collecting resin increased until the 3rd–7th day after wound insertion and then declined (Fig. 1), due to either resin depletion or resin hardening. At natural resin wounds, species identity and number of foragers remained relatively constant during the observation period. Across different plant species, there was no significant correlation between visitation rate of bees at wounds and wound size (Spearman $r_S = 0.08$, $p = 0.62$).

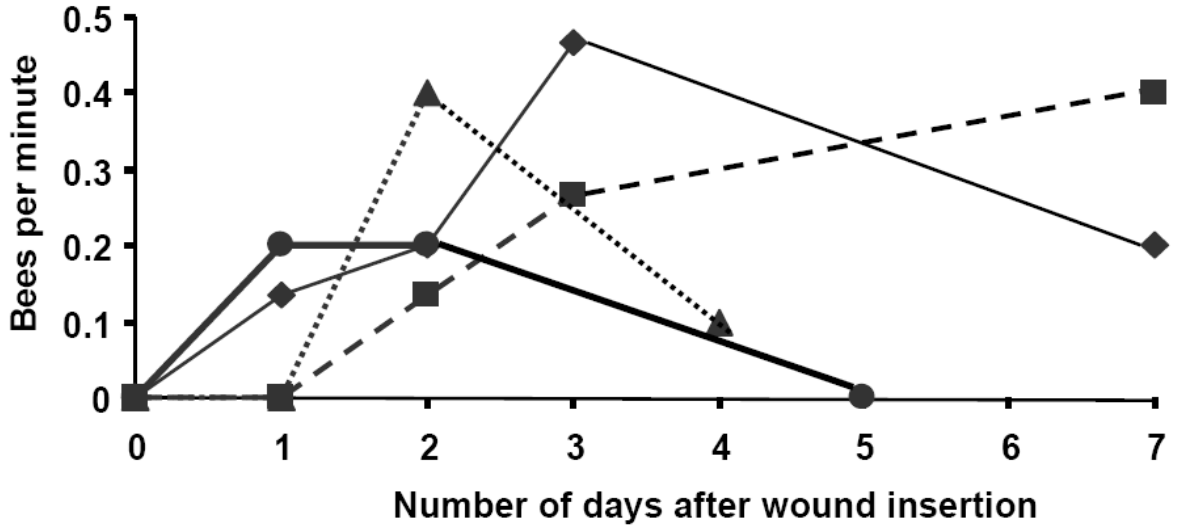

Figure 1. Change of visitation rate of four bee species at four resin wounds observed from the 1st to the 7th day after wound insertion (diamonds = *T. binghami* at *Hopea nervosa*; squares = *T. laeviceps* at *Dryobanalops lanceolata*; triangles = *H. fimbriata* at *Shorea parvifolia*; circles = *T. collina* at *Shorea smithiana*).

Attractiveness of different trees

Attractiveness differed significantly between *A. borneensis* (wound size range: 2–4), dipterocarps (wound size range: 1–4) and other tree families (wound size range: 2–4) (χ^2 = 11.0, $p < 0.001$; Fig. 2). *Agathis borneensis* was visited by the highest number of bee species (five), with up to four different species collecting resin from the same wound at the same time.

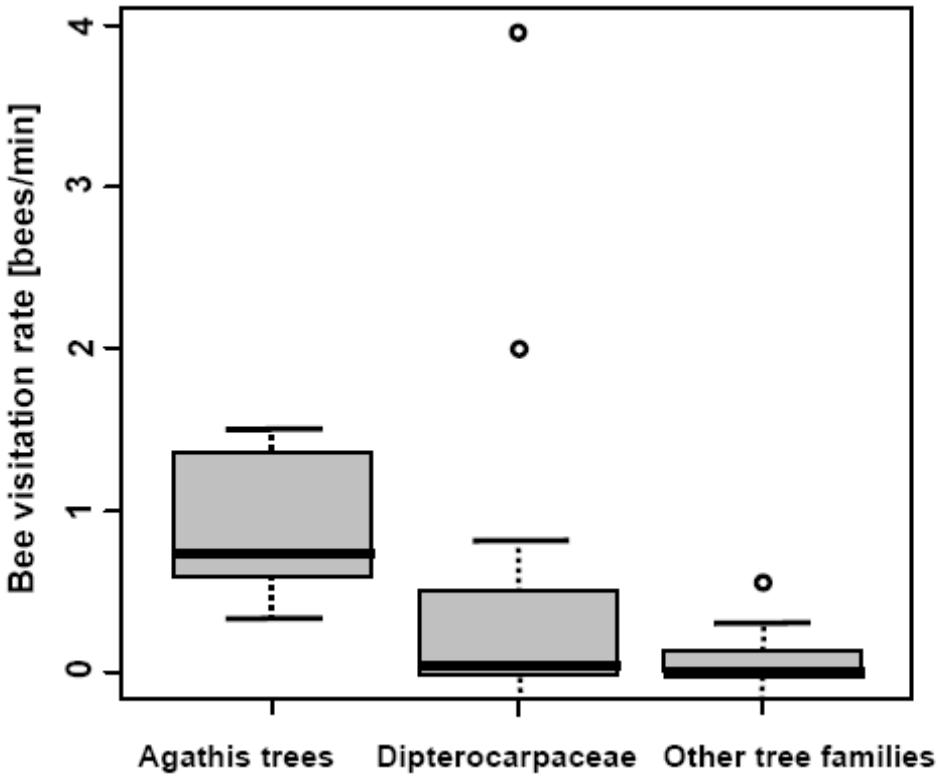

Figure 2. Bee visitation of resin wounds at three groups of trees.

Aggression at resin wounds

Inter- and intraspecific aggression between individuals was observed at ten resin wounds (five tree species). Nine of these ten wounds were small resin wounds with an area of < 2 cm^2 covered by resin. All bee species observed at resin wounds (except *T. collina* and *H. fimbriata*) showed inter- (5) and/or intraspecific (5) aggression (Fig. 3). Larger species showed a higher proportion of aggressive encounters and bees of an intermediate size tended to be the least aggressive (Fig. 3). In 61 % of the aggressive interactions, aggressors were larger than receivers.

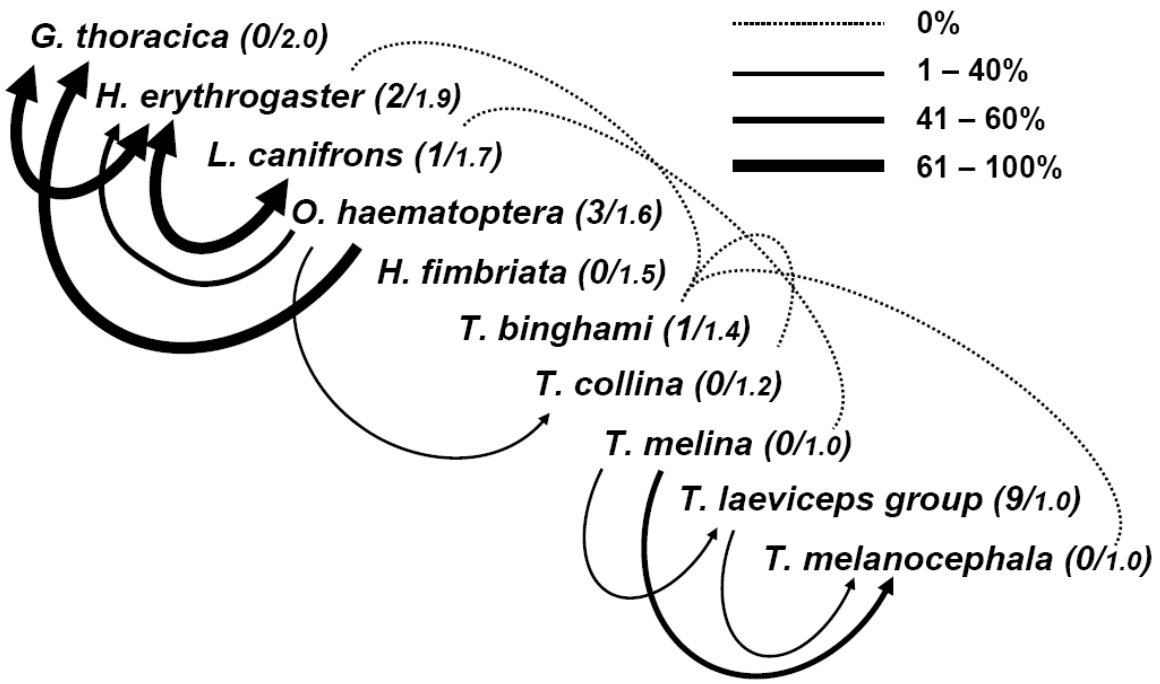

Figure 3. Inter- and intraspecific aggression observed at resin wounds. Arrow strength indicates proportion of observation periods with aggressive encounters between two species among all observation periods where both species co-occurred. Arrow heads indicate the direction of attacks. Numbers in parentheses indicate total number of observation periods with intraspecific aggression (first number) and bee head width (mm; second number). Bees are ordered by their size (head width decreasing from top to bottom).

Observation of resin foragers at undisturbed nests

Colonies of *T. collina* and *T. melanocephala* were found at all three field sites except for *T. collina* which was not recorded in LHN. Foragers of both species were also seen collecting resin at resin wounds.

Bees transporting resin back to their colony were recorded in all four *T. collina* and all four *T. melanocephala* colonies, but the relative proportion of resin foragers strongly differed between colonies (Table 2; Fig. 4a). In *T. collina*, diurnal variation was significant with most bees collecting resin in the late afternoon (Table 2).

Table 2. General linear mixed models (GLMMs) to examine effects on the proportion of resin foragers. Each stingless bee species (T. collina and T. melanocephala) was analyzed separately. LRT = likelihood ratio test, bold P-values indicate significant effects after correction for multiple comparisons (False Discovery Rate) within each model. Three of the eight colonies studied were attacked by ants during the study period.

	T. collina			*T. melanocephala*		
Parameter	df	*LRT*	*p*	df	*LRT*	*p*
Location	2	< 0.1	0.99	2	7	0.03
Time of day	2	8.7	0.01	2	1.7	0.42
Ant attack	1	15.7	< 0.0001	1	4.1	0.04
Nest damage	1	5.3	0.02	1	1.9	0.17

Observations at nests attacked by ants or artificially damaged

During the study, ant attacks occurred at three of our eight focal colonies and lasted 1–3 days (both neighboring *T. collina* colonies in Sepilok; one *T. melanocephala* colony in Danum Valley). All three attacks were launched by *Tapinoma melanocephalum* ants (Dolichoderinae). Despite their small size (*ca* 2 mm) these ants successfully extracted brood of the bees' nests. When bees were attacked by ants, the number of returning foragers loaded with resin significantly increased from a median of 19 to 42 % in *T. collina* colonies (Table 2; Fig. 4b) within a few hours. Ant attacks also led to an increased resin intake in the *T. melanocephala* colony attacked, but this result was not significant after correction for multiple comparisons. In case of an ant attack, up to eight workers were observed simultaneously elongating the entrance tube using fresh resin brought by resin foragers. Ants frequently got entangled in resin droplets placed by the bees at the entrance tube.

Destructive removal of the nest entrance tube in *T. collina* was also followed by a significant, albeit minor (compared to ant attacks), increase in the proportion of resin foragers (Table 2; Fig. 4c).

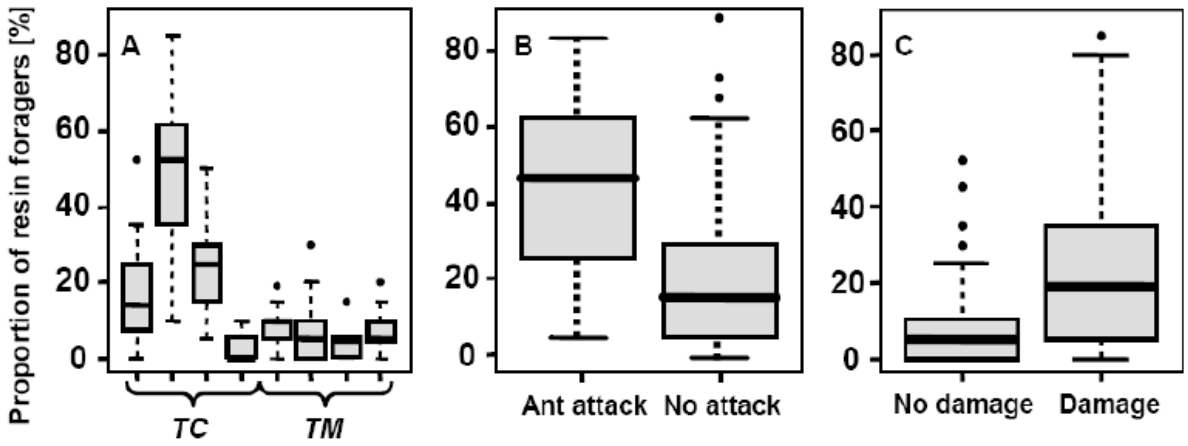

Figure 4. Proportion of resin foragers in *T. collina* (*TC*) and *T. melanocephala* (*TM*) (A) at eight different colonies, (B) in response to ant attacks (only showing the three affected colonies) and (C) before and after nest damage (all eight colonies).

V. 5 Discussion

Plant resins are highly important to stingless bees. They use resin for nest construction, as defense against intruding parasites and predators, and to inhibit the growth of bacteria and fungi within the nest (reviewed by Roubik 2006).

Bee visitation at resin wounds

We found foragers of ten stingless bee species collecting resin at natural and artificial resin wounds. Resin foragers were very efficient in finding even small freshly induced resin wounds within 1–2 days and in recruiting other bees as shown by the rapid increase of resin foragers at artificially induced wounds (Fig. 1). At natural wounds, the number of bees remained relatively constant. At one *Hopea nervosa* tree and one unidentified tree, both with naturally occurring resin wounds, we observed beetle larvae inside the resin that were likely responsible for the resin secretion due to their burrowing activity. In doing so, these beetles could provide a continuous resin source to resin seeking bees which may last for weeks or even months.

Attractiveness of different trees

If all trees were pooled resin wound size did not correlate with bee visitation rate, indicating that other attributes of resin than quantity may be important for the bees' foraging decisions. Moreover, resin from seven tree species did not attract any bees during our study (Table 1), although these species produced copious amounts of resin and were often located only few meters away from trees where bees actually collected resin, thus clearly within the bees' flight range. Most of the trees attended for resin collection by the bees belonged to the dipterocarp family (Table 1) which represents by far the most dominant tree family in SE Asian forests

(Soepadmo et al. 2004). Dipterocarps are highly resinous (Turner 2001) and their resin is known to inhibit the growth of pollen associated fungi (Messer 1985). They may therefore represent the most commonly utilized resin sources of SE Asian stingless bees, whereas trees of the legume family (particularly the Caesalpinioideae and the Papilionoideae) may serve as common source of resin in the Neotropics (Langenheim 1969; Roubik 1989). However, the most striking example of a preference for a specific resin source was *Agathis borneensis*, a highland pine species that had been planted at the Sepilok RDC and attracted five bee species (Table 1; Fig. 2). At *A. borneensis*, large numbers of foragers were frequently observed fighting over small resin wounds, indicating that resin of this particular species was highly preferred by several stingless bee species. This preference for highland pine trees has also been described by Roubik (1989) and may be due to differences in the composition of terpenes.

Inter- and Intraspecific aggression at resin wounds

Especially at small wounds, eight of the ten bee species observed showed aggression towards individuals of the same or another species in up to 100 % of their encounters (Fig. 3), suggesting that these wounds represent limited resources to the bees which are worth defending. Likewise do floral resources of high quality (*e.g.*, higher amounts or concentrations of nectar) provoke higher levels of aggression in stingless bees (Johnson and Hubbell 1974). On flowers, dominant species often exclude less aggressive ones (Johnson and Hubbell 1974; Hubbell and Johnson 1978; Nagamitsu and Inoue 1997). Among different bee species foraging at flowers and artificial feeders in Sarawak (Nagamitsu and Inoue 1997), a similar dominance hierarchy was found as in our study at resin sources, *e.g.*, *T. canifrons* and *T. melina* severely defended their resources whereas *T. melanocephala* did not show any aggression towards other bee species. Despite the high level of aggression at resin wounds, dominant bee species were often unable to effectively monopolize wounds and, thus, shared some wounds with up to four additional species, *e.g.*, at *A. borneensis*.

Our findings are in accordance with Howard's observations in six stingless bee species from Costa Rica (1985). Howard observed hundreds of foragers of stingless bees collecting as well as fighting over resin from naturally occurring resin wounds of a single *Castilla elastica* tree (Moracea). By contrast, in our study resin wounds of 35 trees (18 with naturally occurring wounds) greatly varied in the number of bees attracted, and only ten wounds were actually defended by bees. These differences in the number of resin wounds found may indicate that resin represents a more available resource in Borneo compared to Costa Rica, which may at

least partly be due to the dominance of the highly resinous dipterocarps. This hypothesis needs, however, further investigation in both the Paleotropics and the Neotropics.

Resin intake at nests

The proportion of resin foragers from undisturbed colonies was similar to the level recorded for the same colonies in 2005 and 2004 (see Leonhardt *et al.* 2007) with about 10–30 % of the foragers returning with resin, suggesting a relatively constant resin intake in established colonies. The highest intensity of resin collecting (90 % of returning foragers) was observed at two neighboring nests of *T. collina* located in a wall recently built at the Sepilok RDC, suggesting that these two colonies may still have been in the nest building process.

Freshly collected resin appears to play a pronounced role in nest defense against intruders such as ants. Whereas the removal of the entrance tube did trigger only a slight increase in resin intake, especially the two *T. collina* colonies attacked by ants immediately doubled the proportion of resin foragers. Resin was used by bees to elongate the entrance tube and to build barriers of resin droplets placed at the nest entrance which effectively entangled ants. Further attacks on two non-focal colonies (*T. collina* and *T. terminata*) were observed in KSR. These attacks were launched by the invasive ant *Anoplolepis gracilipes* (Formicinae). *Anoplolepis gracilipes* invaded the *T. collina* colony, but was effectively prevented from entering the *T. terminata* colony due to resin droplets on the nest entrance tube. Following ant attacks, nest tube elongation was relatively fast whereas repairing activity after nest damage was comparatively slow and sometimes even completely absent during the time of observation. It is likely that the freshly collected resin may serve best for nest defense because fresh resin still contains monoterpenes (Gershenzon and Dudareva 2007) which have been shown to effectively repel ants and other invertebrates (Eisner et al. 1986; Junker and Blüthgen 2008). Moreover, resinous compounds may play a role in the chemical defense of the bees' bodies as well, since bees whose terpene-rich cuticle had been washed with solvents were increasingly attacked by ants (Lehmberg et al. 2008). An increased proportion of resin foragers may, however, be associated with costs of reduced food intake, *i.e.*, fewer bees collecting pollen or nectar. If and how this change in resource intake affects the colonies' well-being needs further investigation. Moreover, future studies on the bees' preferences as well as the chemical composition and properties of different plant resins would be helpful to better understand the use of resin in SE Asian stingless bees.

VII. Resin-derived terpenes make up for bee species-specific terpene profiles

As demonstrated in the previous chapter, plant resin is a highly important resource for stingless bees. They use resin not only for nest construction and maintenance, but also to defend their nests against intruders, predators and microbes. However, stingless bees make use of resinous compounds in a hitherto unknown manner: They transfer terpenes, derived from resin, to their body surface and include them in their chemical profiles.

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Leonhardt SD, Blüthgen N & Schmitt T (2009) Smelling like resin: terpenoids account for species-specific cuticular profiles in Southeast-Asian stingless bees. ***Insectes Sociaux*** *56 (2): 157-170.*

VI. 1 Summary

Insects may be unique in having a cuticle with a species-specific chemical profile. In social insects, colony survival depends not only on species-specific but also on colony-specific cuticular compounds with hydrocarbons playing an important role in the communication systems of ants, termites, wasps and bees. We investigated inter- and intraspecific differences in the composition of compounds found on the body surface of seven paleotropical stingless bee species (Apidae: Meliponini) at two different sites in Borneo (Sabah, Malaysia). Besides hydrocarbons, the body surface of all seven stingless bee species comprised terpenoid compounds, a substance class that has not been reported for chemical profiles of any social insect so far. Moreover, the chemical profile of some species differed fundamentally in the composition of terpenoids with one group (e.g. sesquiterpenes) being present in one species, but missing in another. Chemical profiles of different colonies from the same species showed the same hydrocarbon- and terpenoid compounds over different regions, as tested for *Tetragonilla collina* and *Tetragonula melanocephala*. However, chemical profiles differed quantitatively between the different colonies especially in *T. melanocephala*. It is likely that the terpenoids are derived from plant resins because stingless bees are known to collect and use large amounts of resins for nest construction and defence, suggesting an environmental origin of the terpenoids in the chemical profile of paleotropical stingless bees.

VI. 2 Introduction

All insects express and depend on a lipid based waxy layer on the cuticle fulfilling various important ecological functions. Cuticular lipids are thought to preserve insects from desiccation, cuticle abrasion and infection, thus directly ensuring their survival (Lockey 1988; St. Leger 1995). In several insect taxa, cuticular lipids have become further involved in the communication system by enabling them to reliably differentiate between friend and foe or find a mate based on differences in the chemical composition of cuticular profiles (Wilson 1971; Fletcher and Michener 1987; Howard 1993). The main substance classes of cuticular lipids are hydrocarbons such as non-polar long-chain linear n-alkanes, alkenes, and mono-, di- and trimethyl-branched alkanes, as well as polar compounds like carboxylic acids, esters and long-chain alcohols and aldehydes (Buckner 1993; Howard 1993). The non-polar n-alkanes, alkenes and methyl-branched alkanes appear to play a dominant role in ants (Hölldobler 1995), termites (Howard et al. 1982; Kaib et al. 2004), social wasps (Espelie et al. 1994) and bumblebees (Ayasse et al. 1995). However, in the cuticular profiles of bees, compounds with functional groups (alcohols, aldehydes, esters, carboxylic acids) are frequently found besides non-polar compounds (Ayasse et al. 1999; Paulmier et al. 1999; Fröhlich et al. 2000b; Abdalla et al. 2003; Jungnickel et al. 2004; Kerr et al. 2004; Mant et al. 2005; Nunes et al. 2008). In honeybees, cuticular hydrocarbons appear to be genetically determined surface and nest comb wax compounds as well as, although in negligible amounts, compounds acquired from the environment (e.g., from floral resources) (Francis; Francis et al. 1989; Page et al. 1991; Breed et al. 1992; Breed and Stiller 1992; Breed et al. 1998; Fröhlich et al. 2001). These factors seem to act together in forming the bees' colony profile (Breed et al. 1998), although floral compounds appear to be of minor importance in the recognition system of honeybees (Downs et al. 2000).

Whereas several studies exist on cuticular hydrocarbons and their effect on the recognition system in honeybees, the cuticular profiles of another group of highly eusocial bees, the tropical stingless bees (Hymenoptera: Apidae, Meliponini), have received little attention. Stingless bees are the largest group of eusocial bees, comprising more than 400 species (Michener 2000). They are very common throughout the tropics worldwide, but the majority of Trigonini and all Meliponini are found in the Neotropics (Sakagami and Camargo 1964; Roubik 1989). Not surprisingly, the few studies that investigated cuticular profiles of stingless bees were performed in neotropical species (Abdalla et al. 2003; Jungnickel et al. 2004; Kerr et al. 2004; Nunes et al. 2008). Similarly to honeybees, these studies revealed mainly, partly even exclusively, non-polar aliphatic hydrocarbons (n-alkanes, alkenes and branched

alkanes), but in addition, although to a lesser extent, compounds with functional groups (esters, carboxylic acids, aldehydes) as constituents of cuticular lipids, and further demonstrated clear differences between species and/or different colonies from the same species (Abdalla et al. 2003; Jungnickel et al. 2004; Kerr et al. 2004; Nunes et al. 2008). Several behavioural studies in both neotropical and paleotropical stingless bees also showed a clear indication of nestmate recognition, suggesting colony-specific chemical signals (Inoue and Roubik 1990; Breed and Page 1991; Suka and Inoue 1993; Bowden et al. 1994; Suka et al. 1994; Nagamitsu and Inoue 1997; Inoue et al. 1999; Kirchner and Friebe 1999; Dworschak and Blüthgen 2010), but no study has analyzed the composition of chemical profiles from paleotropical stingless bee species so far.

We investigated the chemical profiles of seven Southeast-Asian stingless bee species which comprises both cuticular hydrocarbons as well as substances acquired from the nest environment. We focused on qualitative and quantitative differences between species and colonies. In particular, we examined the distribution of terpenoids that appear to be common in the chemical profile of Southeast-Asian meliponines, but are not found in neotropical stingless bee species (Abdalla et al. 2003; Jungnickel et al. 2004; Kerr et al. 2004; Nunes et al. 2008), let alone any other social insect. These terpenoids are likely to be derived from plant resins which are known to contain terpenes and are frequently collected and used for nest construction and defence by Bornean stingless bees (Leonhardt et al. 2007; Leonhardt and Blüthgen 2009).

VI. 3 Methods

Study sites and bee sampling

Bee samples were collected at two different field sites in Borneo (Malaysia) from 08.03. until 28.03.2006 at the Danum Valley Conservation Area (DVC) and the Kabili Sepilok Reserve (KSR). DVC (Sabah, 4°55'N 117°40'E, 100 m asl) comprises one of the major remaining patches of Sabah's primary lowland rainforest (43 800 ha) (Marsh and Greer 1992). KSR (Sabah, 5°54'N, 118°04'E, 20-120 m asl) covers an area of 4294 ha of coastal dipterocarp forest with more than one-third of it consisting of mangrove forest (Fox 1973). Both field sites have typical equatorial rainforest climate with a mean annual temperature of 26 - 30°C and a yearly rainfall of 2600 - 3000 mm (Fox 1973).

In order to investigate whether different stingless bee species differed in their chemical profiles, we sampled 32 colonies comprising seven different species. Eighteen colonies were located in DVC and 14 in KSR. For 21 colonies, 3-13 individuals were pooled in one sample

per colony. To examine whether different colonies of the same species could be distinguished based on differences in their chemical profiles, we analyzed samples of individual workers from six *Tetragonilla collina* colonies (two in DVC, four in KSR) and five *Tetragonula melanocephala* colonies (four in DVC, one in KSR). Bees were caught with plastic bags placed over the colonies' nest entrance tube to catch only departing workers.

Extraction and fractionation

Bags with bees were put into a freezer to kill the bees before they were transferred into 2 ml sample vials containing pure hexane for surface extraction. All specimens were extracted for ten minutes. To ensure that compounds identified in hexane extracts of bees were not due to contamination with resin, pollen or other substances recently collected by the bees and still attached to the bees' legs or other body parts, we compared whole body extracts to extracts from the bees' wings. This comparison was done for *T. collina* and *T. melanocephala*. We pooled wings of six individuals for each extract.

To test whether terpenoids were non-polar or had functional groups we fractionated pooled extracts using 6ml SiOH polypropylene columns (CHROMABOND®, 500mg, Macherey-Nagel, Düren, Germany). Columns were conditioned with pentane before adding about 40µl of surface extract. Non-polar and polar fractions of extracts were eluted with three column equivalents of pentane (non-polar) and two column equivalents of dichloromethane (polar), respectively.

Chemical analysis of extracts

Compounds found in the chemical profiles were characterized by their mass spectra and their retention times. Peaks with identical mass spectra and retention times were regarded as the same substance. We used three commercially available mass spectra libraries (Wiley 275, NIST 98 and Adams EO library 2205) to determine substance classes with regards to their mass spectra and retention times. Alkanes were additionally confirmed by synthetic standards (Sigma-Aldrick, Munich, Germany). Aldehydes, alcohols and esters as well as mono- and sesquiterpenes have been tentatively identified by comparison of the obtained mass spectra with the mass spectra from the libraries with regard to their diagnostic ions. Comparisons with synthetic standards (Sigma-Aldrich, Munich, Germany) were performed if standards were available. Potential triterpenes were tentatively determined by their molecular mass, typical diagnostic ions and the range of retention times where they normally elute. Data of triterpenes were further compared with those of dipterocarp resins that typically comprise sesqui- and triterpenes (Langenheim 2003).

For characterization we used a Hewlett Packard HP 6890 Series GC System coupled to a Hewlett Packard HP 5973 Mass Selective Detector (Agilent Technologies, Böblingen, Germany). The GC was equipped with a J & W, DB-1 fused silica capillary column (30m x 0.25 mm ID; df = 0.25 µm; J & W, Folsom, CA, USA). Temperature was programmed from 60°C 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 (EI-MS) were recorded at 70 eV and 230°C. We used the Windows version of the ChemStation software package (Agilent Technologies, Böblingen, Germany) for data acquisition.

Statistical analysis

Prior to analysis, trace compounds for which mass spectra could not be interpreted as well as compounds which accounted for less than 0.5 % of the total peak area in all samples were removed from the dataset (if a compound accounted for more than 0.5 % in one samples, this compound was included in the analysis although it may have accounted for less than 0.5 % in other samples). The analysis is based on a total of 146 compounds for all species and 79 compounds for *T. collina* as well as 55 compounds for *T. melanocephala*, respectively. These compounds were quantified as proportions by dividing the peak area of each compound by the total area of all peaks included in the analysis.

Three analyses were performed to examine the chemical variation (a) among the seven species, (b) among the six colonies of *T. collina* and (c) among the five colonies of *T. melanocephala*. For each level, a separate two-dimensional NMDS (non-metric dimensional scaling) analysis was performed based on Bray-Curtis distance of the proportions of each compound (start configuration: PCoA, 1000 iterations). We then analyzed the variation between bee species using a discriminant analysis with the two NMDS axes together as explanatory variables, in order to determine whether the axes can explain variation between species or colonies (only those bee species with more than one colony sampled were included). We report Wilks' λ values and the percentage of correctly assigned samples (classification matrix). For the analysis at the species level (a), we used the pooled bee extracts, whereas extracts of individual bees were used to discriminate between colonies of the same species (b and c). Two samples of *T. melanocephala* were excluded from analyses, as they contained a large number of compounds that were not found in any of the other 36 samples from this species, indicating contamination. All statistical analyses were performed using R (R-Development-Core-Team 2009).

To test whether chemical profiles could also be discriminated based on non-terpenoid compounds alone and whether terpenoid compounds were sufficient to discriminate between the chemical profiles of the seven bee species we performed two further NMDS analyses excluding terpenoid compounds or non-terpenoid compounds, respectively.

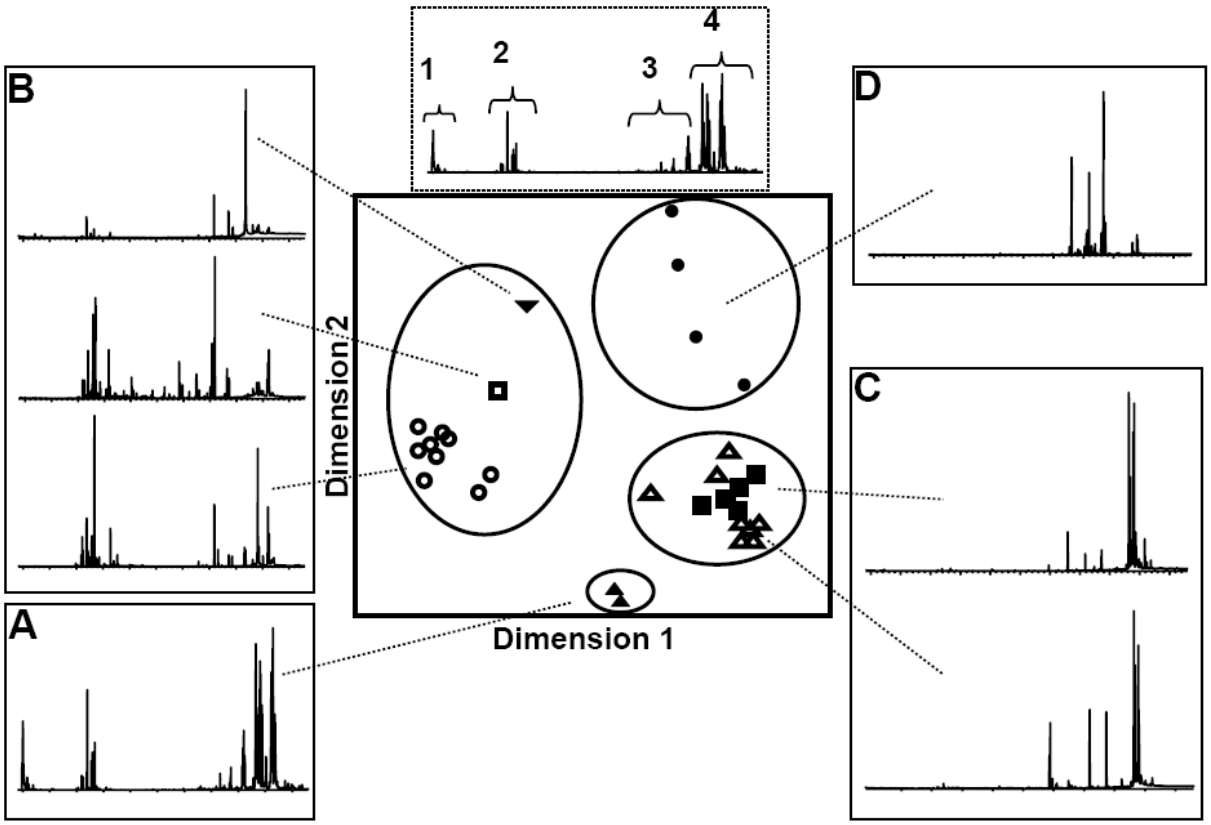


Figure 1. NMDS analysis and chromatograms of the cuticular compounds from hexane extracts of seven bee species. Different symbols indicate different species (each symbol represents one colony): open circles = *T. collina*, squares = *T. melanocephala*, open triangles = *T. fuscobalteata*, triangles = *T. geissleri*, circles = *L. terminata*, open square = *T. melina*, triangle upside down = *P. pendleburyi*; upper chromatogram represents *T. geissleri* and indicates areas where different compound groups elute: 1 = mainly monoterpenes, 2 = mainly sesquiterpenes, 3 = mainly alkanes, alkenes, methylbranched alkanes and esters, 4 = mainly triterpenes, alkanes and esters. Boxes to the right and left of the NMDS graph show chromatograms representative of the seven species: A = species with mono-, sesqui- and triterpenes, B = species with sesqui- and triterpenes, C = species with triterpenes, D = species with no terpenes or small amounts of triterpenes.

VI. 4 Results

Interspecific differences

Compounds found in the chemical profile of the seven stingless bee species comprised n-alkanes, alkenes, alkadienes, methylbranched alkanes, esters, carboxylic acids, aldehydes and alcohols (Table 1). Besides these compounds which are commonly found in the chemical profile of eusocial bees, we tentatively identified considerable amounts of terpenoids

including monoterpenes, sesquiterpenes and triterpenes (Table 1, Fig. 1). Monoterpenes and the most prominent sesquiterpenes were mainly found in the non-polar fraction, indicating sesquiterpene hydrocarbons. By contrast, triterpenes were exclusively found in the polar fraction, indicating compounds with functional groups. The NMDS analysis showed a clear discrimination of chemical profiles among species (Table 2) which can be partly explained by qualitative differences between the three terpenoid groups. Most notably, monoterpenes were mainly found in *Tetragonula geissleri* (Table 1, Fig. 1). The chemical profiles of *T. collina* and *Tetragonula melina* contained considerable amounts of sesquiterpenes which only occurred in small amounts in the chemical profiles of *Pariotrigona pendleburyi, T. melanocephala*, *Tetragonula fuscobalteata* and *Lepidotrigona terminata* (Table 1, Fig. 1). The chemical profiles of all species comprised variable amounts of triterpenes.

When the NMDS analysis was confined to terpenoids, species-specific profiles still differed significantly (Table 2, Fig. 2a). Discrimination between the different species was even more pronounced (100 % of samples correctly assigned) if only non-terpenoids were used in the NMDS (Table 2, Fig. 2b).

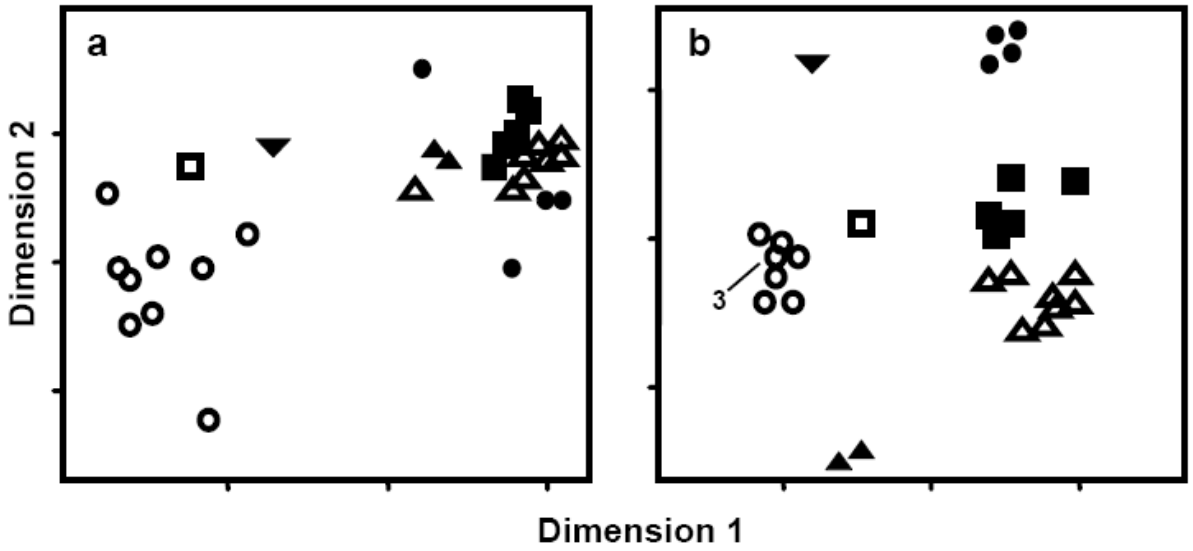


Figure 2. NMDS analyses of (a) only terpenoid and (b) only non-terpenoid cuticular compounds from hexane extracts of seven bee species. Different symbols indicate different species. Each symbol represents one colony; symbol codes as in Fig. 1. The number (3) indicates where three symbols were entirely overlapping.

In *T. collina*, 96 % of the compounds present in extracts from body rinses (94 % of the terpenoids) could also be found in extracts from wings (Fig. 3a). Similarly, the number of compounds (terpenoids) found in both whole bee and wing extracts comprised 65 % (69 %) in *T. melanocephala* (Fig. 3b).

Table 1. Percentages [±SD] of tentatively identified substances found in the chemical profiles of seven Southeast-Asian meliponine species listed according to retention times (RT) and molecular weight (MW); MT = monoterpene, ST = sesquiterpene, TT = triterpenes, UT = unknown terpenoid, E = esters, A = alkanes, MA = methylalkanes, AD = aldehydes, AO = alcohols, AE = alkenes, ADE = alkadienes, AC = acids, U = unknown compounds.

MW	Class	Substance	RT	*T.collina*	*T.fuscobalteata*	*T.geissleri*	*T.melanocephala*	*P.pendleburyi*	*L.terminata*	*T.melina*
136	MT	-	4.9	< 0.01	-	1 ± 0.57	-	-	-	-
136	MT	-	5.0	0.03 ± 0.02	-	4.24 ± 2.76	0.06 ± 0.12	0.06	0.03 ± 0.02	-
136	MT	-	5.7	-	-	0.48 ± 0.24	-	-	-	-
136	MT	beta-Pinene	5.8	< 0.01	-	0.79 ± 0.14	-	-	-	-
204	ST	-	15.9	0.6 ± 0.24	0.1 ± 0.2	0.32 ± 0.12	0.09 ± 0.15	0.18	0.09	1.21
204	ST	-	16.1	0.78 ± 1.01	-	0.01 ± 0	0.02 ± 0.04	0.10	0.02 ± 0.04	0.14
204	ST	-	16.2	1.48 ± 0.72	0.17 ± 0.41	0.33 ± 0.23	0.12 ± 0.12	0.34	0.11	1.28
204	ST	-	16.9	4.12 ± 1.35	0.58 ± 1.25	2.85 ± 0.35	0.3 ± 0.2	3.94	1.17 ± 1.75	3.29
204	ST	beta-Copaene	17.2	1.03 ± 0.44	0.09 ± 0.26	0.08 ± 0.03	0.04 ± 0.06	0.13	0.03 ± 0.06	0.53
204	ST	-	17.5	0.82 ± 0.3	0.04 ± 0.11	0.03 ± 0.01	0.01 ± 0.02	-	-	0.25
204	ST	alpha-Humulene	17.7	1.43 ± 0.57	0.16 ± 0.36	0.75 ± 0.12	0.09 ± 0.05	0.88	0.30	1.29
204	ST	-	17.9	1.74 ± 1.16	0.05 ± 0.12	0.58 ± 0.61	0.11 ± 0.04	0.27	0.1 ± 0.13	5.06
204	ST	Germacrene-D	18.3	10.09 ± 6.69	1.49 ± 4.07	1 ± 0.39	0.51 ± 0.61	1.57	0.34 ± 0.69	6.32
204	ST	Muurola-4(14),5-diene	18.6	1.93 ± 5.15	0.01 ± 0.04	0.02 ± 0.01	-	-	-	0.12
204	ST	-	18.7	0.99 ± 0.48	0.15 ± 0.42	0.12 ± 0.06	0.05 ± 0.07	0.21	0.04 ± 0.09	0.78
204	ST	-	18.8	0.12 ± 0.26	-	-	-	-	-	-
214	E	methyl Dodecanoate	19.2	-	-	-	-	-	-	1.04
204	ST	delta-Cadinene	19.3	0.62 ± 0.15	0.12 ± 0.27	0.04 ± 0	0.1 ± 0.08	0.34	0.08	0.70
220	ST	-	20.2	0.31 ± 0.16	-	-	-	-	-	-
220	ST	-	20.4	0.32 ± 0.17	0.03 ± 0.07	-	0.03 ± 0.05	-	-	0.80
220	ST	-	20.5	0.22 ± 0.24	0.02 ± 0.05	0.06 ± 0.03	0.02 ± 0.03	0.05	0.02	0.28
220	ST	-	20.6	0.18 ± 0.23	-	-	-	-	-	-
228	E	ethyl Dodecanoate	20.9	-	-	-	-	0.17	0.03 ± 0.07	3.81
220	ST	-	21.1	0.13 ± 0.09	< 0.01	-	0.02 ± 0.03	-	-	0.85
224	ST	-	21.4	3.5 ± 1.66	0.07 ± 0.15	0.09 ± 0.08	0.08 ± 0.06	0.98	0.2 ± 0.44	0.30
222	ST	-	21.5	0.26 ± 0.25	-	-	-	-	-	-
222	ST	-	22.0	0.99 ± 0.48	0.01 ± 0.01	0.01 ± 0	0.05 ± 0.04	0.10	0.02 ± 0.05	0.59
220	ST	-	22.7	2.05 ± 2.1	-	-	-	-	-	-
236	ST	-	22.8	0.27 ± 0.35	-	-	-	-	-	-
236	ST	-	23.3	0.18 ± 0.26	-	-	-	-	-	-
242	E	methyl Tetradecanoate	23.7	-	-	-	-	-	-	0.88
256	E	ethyl Tetradecanoate	25.2	-	-	0.01 ± 0	-	-	-	1.77
236	ST	-	25.2	0.2 ± 0.15	0.02 ± 0.05	-	-	-	-	-
238	AD	Hexadecenal	25.2	-	-	0.01	0.48 ± 0.36	-	-	-
234	ST	-	25.5	0.29 ± 0.16	0.06 ± 0.17	< 0.01	0.02 ± 0.03	-	-	0.67
238	ST	-	26.1	0.35 ± 0.27	0.03 ± 0.08	-	-	-	-	-
268	E	methyl Hexadecenoate	27.3	-	0.17 ± 0.36	-	-	-	-	-
-	U	-	29.1	-	0.03 ± 0.06	0.46 ± 0.63	0.01 ± 0.01	-	0.03 ± 0.03	-
266	AD	Octadecenal	29.1	-	0.13 ± 0.2	-	0.29 ± 0.29	-	-	-
284	E	ethyl Hexadecanoate	29.1	0.02 ± 0.05	-	0.02 ± 0	-	-	-	0.63
296	A	Heneicosane	31.4	0.04 ± 0.03	0.09 ± 0.04	0.04 ± 0.04	0.22 ± 0.32	-	0.06 ± 0.07	0.68
282	AC	Octadecenoic acid	31.7	0.26 ± 0.63	-	-	-	-	-	-
310	E	ethyl Octadecenoate	32.2	0.12 ± 0.34	0.05 ± 0.08	< 0.01	0.06 ± 0.06	-	-	-

Table 1. continued

MW	Class	Substance	RT	*T.collina*	*T.fuscobalteata*	*T.geissleri*	*T.melanocephala*	*P.pendleburyi*	*L.terminata*	*T.melina*
268	AO	Octadecenol	32.6	-	0.32 ± 0.24	-	-	-	-	-
310	A	Docosane	33.1	0.02 ± 0.02	0.04 ± 0.03	0.01 ± 0	0.37 ± 0.8	-	0.02 ± 0.03	0.04
296	AO	Eicosenol	34.0	0.14 ± 0.28	-	-	0.08 ± 0.11	-	-	-
320	ADE	Tricosadiene	34.2	0.02	0.01 ± 0.02	0.01 ± 0	-	-	-	2.15
322	AE	Tricosene	34.3	-	0.57 ± 0.92	-	-	-	-	0.89
322	AE	Tricosene	34.3	0.07 ± 0.06	1.71 ± 1.75	0.01 ± 0.01	0.01 ± 0.01	-	0.08 ± 0.09	1.00
322	AE	Tricosene	34.4	0.02 ± 0.02	3.72 ± 3.28	0.01 ± 0	0.06 ± 0.08	-	0.06 ± 0.09	0.17
324	A	Tricosane	34.8	0.06 ± 0.03	0.69 ± 0.51	0.14 ± 0.14	1.11 ± 1.19	0.09	0.23 ± 0.13	0.94
338	MA	methyl Tricosane	35.5	-	0.32 ± 0.17	-	0.01 ± 0.01	-	-	-
338	A	Tetracosane	36.4	0.02 ± 0.01	0.05 ± 0.02	0.02 ± 0	0.73 ± 1.35	-	0.16 ± 0.1	0.05
350	AE	Pentacosene	37.4	-	0.46 ± 0.55	-	-	-	0.31 ± 0.2	4.23
350	AE	Pentacosene	37.5	-	0.55 ± 0.19	-	0.07 ± 0.02	-	0.31 ± 0.34	-
350	AE	Pentacosene	37.6	-	0.56 ± 0.29	-	0.04 ± 0.03	-	0.22	0.70
352	A	Pentacosane	38.0	0.62 ± 0.2	0.32 ± 0.2	-	3.95 ± 1.45	0.64	9.48 ± 6.01	0.71
366	MA	methyl Pentacosane	39.1	0.01 ± 0.01	0.05 ± 0.03	-	0.03 ± 0.04	-	0.59 ± 0.49	-
366	A	Hexacosane	39.5	0.25 ± 0.08	0.07 ± 0.02	0.04 ± 0.01	0.61 ± 0.92	0.23	0.39 ± 0.19	0.36
376	ADE	Heptacosadiene	40.0	-	-	-	-	-	-	1.02
376	ADE	Heptacosadiene	40.1	-	0.01 ± 0.01	-	-	-	0.36 ± 0.46	0.40
376	ADE	Heptacosadiene	40.2	-	-	-	-	-	0.17	0.41
378	AE	Heptacosene	40.5	0.01 ± 0.01	0.08 ± 0.05	0.05	0.15 ± 0.06	0.11	2.72 ± 2.26	6.98
378	AE	Heptacosene	40.5	< 0.01	0.58 ± 0.43	0.03 ± 0.01	0.02 ± 0.03	-	1.77 ± 1.6	1.20
378	AE	Heptacosene	40.6	0.04 ± 0.02	0.19 ± 0.15	-	-	-	0.69	1.31
380	A	Heptacosane	40.9	8.62 ± 2.05	1.95 ± 2.25	0.15 ± 0.04	2.18 ± 0.85	8.32	8.25 ± 2.94	9.93
379	U	-	41.4	0.01 ± 0.01	0.43 ± 0.2	-	0.03 ± 0.04	-	0.96 ± 0.74	0.08
379	U	-	41.6	-	0.05 ± 0.03	-	-	-	0.34 ± 0.33	-
394	-	-	41.6	-	-	0.34 ± 0.25	0.44 ± 0.83	-	-	-
396	E	tetradecyl Dodecanoate	41.6	1.33 ± 0.3	-	-	-	0.18	0.04 ± 0.08	-
-	U	-	41.9	-	-	-	-	-	1.37 ± 1.05	0.21
394	A	Octacosane	42.3	0.18 ± 0.06	0.12 ± 0.06	0.07 ± 0.02	0.38 ± 0.32	0.29	0.18 ± 0.12	0.12
410	UT	Squalene	42.4	0.05 ± 0.08	0.21 ± 0.22	0.21 ± 0.12	0.74 ± 0.61	0.21	0.07 ± 0.09	0.06
404	ADE	Nonacosadiene	42.9	-	< 0.01	-	-	-	1.03 ± 0.82	0.31
404	ADE	Nonacosadiene	43.0	-	-	-	-	-	3.03 ± 2.28	0.30
406	AE	Nonacosene	43.2	-	0.11 ± 0.08	0.12 ± 0.06	0.15 ± 0.11	-	0.66 ± 0.65	0.14
406	AE	Nonacosene	43.2	0.01 ± 0.02	0.18 ± 0.07	-	0.72 ± 0.57	-	-	2.32
406	AE	Nonacosene	43.3	0.03 ± 0.01	0.05 ± 0.04	0.42 ± 0.22	0.01	-	26.6 ± 15.37	0.78
406	AE	Nonacosene	43.5	0.01 ± 0.01	0.37 ± 0.28	0.49 ± 0.13	-	-	-	-
408	A	Nonacosane	43.6	1.87 ± 0.53	3.58 ± 1.85	1 ± 0.35	4.35 ± 2.92	5.59	3.77 ± 1.29	1.60
422	MA	methyl Nonacosane	44.1	0.04 ± 0.01	0.35 ± 0.17	-	0.15 ± 0.09	-	0.07 ± 0.08	-
424	E	tetradecyl Tetradecanoate	44.3	1.15 ± 0.25	-	-	-	-	-	-
-	U	-	44.4	-	-	-	-	2.88	0.58 ± 1.29	-
432	ADE	Hentriacontadiene	45.7	-	-	1.66 ± 0.81	-	-	-	-
434	AE	Hentriacontene	45.7	-	-	-	0.41 ± 0.16	-	0.13 ± 0.13	-
434	AE	Hentriacontene	45.8	-	-	-	0.52 ± 0.29	-	-	-
434	AE	Hentriacontene	45.9	-	-	1.89 ± 0.58	-	-	-	-
434	AE	Hentriacontene	46.0	-	0.29 ± 0.2	4.16 ± 1.38	-	-	0.26	-
434	AE	Hentriacontene	46.1	-	1.34 ± 1.18	1.27 ± 0.27	0.05	-	-	-
436	A	Hentriacontane	46.2	0.12 ± 0.04	1.84 ± 0.53	1.5 ± 0.23	0.93 ± 0.66	0.69	0.38 ± 0.3	-
-	UT	-	46.6	-	0.1 ± 0.04	0.11 ± 0.01	0.09 ± 0.06	-	0.26	0.44
450	E	dodecyl Octadecenoate	46.6	2.05 ± 0.76	-	-	-	-	-	-
-	U	-	46.7	-	0.42 ± 1.19	-	-	8.56	1.71 ± 3.83	-

Table 1. continued

MW	Class	Substance	RT	*T.collina*	*T.fuscobalteata*	*T.geissleri*	*T.melanocephala*	*P.pendleburyi*	*L.terminata*	*T.melina*
452	E	tetradecyl Hexadecanoate	46.8	2.41 ± 0.57	0.1 ± 0.12	-	-	-	-	-
-	U	-	46.9	-	-	-	-	43.13	8.63 ± 19.29	-
-	U	-	46.9	-	-	-	0.11	-	-	-
-	U	-	47.1	-	0.06 ± 0.04	0.12 ± 0	0.06 ± 0.06	-	0.17	0.21
-	U	-	47.2	-	-	-	-	-	0.30	0.66
-	U	-	47.2	-	0.19 ± 0.22	0.17 ± 0.01	0.09 ± 0.08	-	-	-
-	U	-	47.4	-	0.03 ± 0.06	-	0.12	-	-	-
424	TT	-	47.5	-	0.46 ± 0.85	-	0.02	-	-	-
426	TT	-	47.6	-	-	-	-	-	0.23	-
426	TT	-	47.7	-	-	-	-	-	0.26 ± 0.52	-
424	TT	-	47.8	0.15 ± 0.07	1.71 ± 1.09	0.6 ± 0.04	0.89 ± 0.51	-	-	-
440	TT	-	48.0	0.28 ± 0.13	-	-	-	-	-	0.38
440	TT	-	48.0	-	0.22 ± 0.06	-	0.28 ± 0.22	-	-	-
460	ADE	Tritriacontadiene	48.1	-	-	1.51 ± 0.35	-	-	-	-
424	TT	-	48.2	1.57 ± 0.99	14.8 ± 1.95	7.7 ± 0.03	13.77 ± 1.48	2.51	3.33 ± 3.28	1.27
424	TT	-	48.3	-	-	-	0.71	-	-	-
-	U	-	48.3	-	-	0.87 ± 0.17	-	-	-	-
424	TT	-	48.4	0.8 ± 0.77	6.51 ± 2.67	3.33 ± 0.46	6.24 ± 1.06	0.84	1.89 ± 2.13	0.50
426	TT	-	48.5	0.16 ± 0.34	3.36 ± 0.7	2.41 ± 0.21	2.73 ± 1.33	1.83	0.94 ± 1.21	-
426	TT	-	48.7	-	-	-	0.37 ± 0.56	-	-	-
426	TT	-	48.8	0.43 ± 0.54	2.32 ± 0.56	1.22 ± 0.1	2.89 ± 1.08	0.80	0.41	-
424	TT	-	48.9	1.52 ± 0.9	15.65 ± 2.6	6.17 ± 0.62	13.41 ± 1.62	1.10	3.17 ± 2.8	2.10
478	E	tetradecyl Octadecenoate	49.1	0.56 ± 1.68	-	-	-	2.46	0.49 ± 1.1	-
424	TT	-	49.1	-	11.54 ± 3.49	5.61 ± 0.22	12.87 ± 2.2	-	2.46 ± 2.79	1.11
478	E	tetradecyl Octadecenoate	49.1	18.17 ± 4.6	-	-	-	-	-	-
-	U	-	49.3	-	-	-	-	4.91	0.98 ± 2.2	-
426	TT	-	49.4	-	9.91 ± 3.83	5.46 ± 0.16	9.11 ± 2.77	-	2.83 ± 3.84	2.23
-	U	-	49.3	2.76 ± 0.65	-	-	-	-	-	2.10
440	TT	-	49.5	0.78 ± 0.5	-	-	-	-	-	-
468	TT	-	49.7	-	-	-	1.07 ± 0.64	-	-	-
468	TT	-	49.9	-	-	-	1.88 ± 2.17	-	-	1.10
-	U	-	49.9	-	0.58 ± 0.13	-	-	-	-	-
-	U	-	50.1	-	0.86 ± 0.29	-	0.87 ± 0.07	-	0.59 ± 0.87	-
-	U	-	50.1	1.15 ± 0.56	-	1.39 ± 0.11	-	0.48	0.1 ± 0.22	0.89
468	TT	-	50.2	0.03	1.26 ± 0.73	0.63 ± 0	1.85 ± 1.14	-	-	0.47
468	TT	-	50.9	-	1.97 ± 1.87	0.25	5.1 ± 4.61	-	-	2.07
-	U	-	51.0	6.19 ± 2.92	0.14 ± 0.27	9.21 ± 0.07	0.12 ± 0.17	1.48	0.3 ± 0.66	4.24
-	U	-	51.2	5.16 ± 1.4	2.08 ± 0.32	15.63 ± 0.83	2.31 ± 1.61	3.31	0.82 ± 1.42	6.25
-	U	-	51.3	0.21 ± 0.33	0.09 ± 0.24	-	-	-	-	-
-	U	-	51.6	0.39 ± 0.22	-	0.82 ± 0.13	-	-	-	-
-	U	-	51.7	0.44 ± 0.67	0.17 ± 0.22	4.44 ± 0.14	0.2 ± 0.28	-	-	-
-	U	-	51.8	1.07 ± 0.41	-	3.18 ± 0.64	-	-	-	0.61
-	U	-	51.9	-	-	-	1.44 ± 0.9	-	-	0.87
-	U	-	51.9	-	0.18 ± 0.21	-	-	-	-	-
-	E	-	52.0	0.86 ± 0.38	-	-	-	-	-	-
-	E	-	52.1	2.24 ± 0.6	-	-	-	-	-	-
-	U	-	52.7	-	-	-	-	-	2.02 ± 4.2	-
-	U	-	52.7	-	0.09 ± 0.2	-	-	-	-	0.29
-	U	-	52.9	0.14 ± 0.23	-	-	-	-	-	-
-	U	-	53.1	-	0.11 ± 0.2	-	0.04 ± 0.07	-	-	-
-	U	-	53.6	0.19 ± 0.21	0.03 ± 0.08	0.64 ± 0.05	-	-	-	-
-	U	-	55.2	-	-	0.85 ± 0.29	-	-	-	-
-	E	-	56.3	0.15 ± 0.1	-	0.78 ± 0.3	-	-	-	-

Intraspecific differences

The NMDS analysis of the six *T. collina* colonies showed significant discrimination of their chemical profiles (Table 2; Fig. 4a). The five *T. melanocephala* colonies also differed significantly in the chemical composition of compounds found on their body surface (Table 2; Fig. 4b).

Table 2. Results of the discriminant analyses (*df* = degrees of freedom, % assigned gives percentages of correctly assigned samples in classification matrix).

Bee species tested	Chemical compounds	Wilk's λ	*df*	% assigned
all species	all compounds	0.003	8/44	**86**
all species	terpenoids	0.043	8/44	**64**
all species	non-terpenoids	0.001	8/44	**100**
T. collina	all compounds	0.330	10/82	**54**
T. melanocephala	all compounds	0.044	8/62	**76**

VI. 5 Discussion

Species-specific terpenoids in the chemical profiles of Southeast-Asian meliponines

Seven Southeast-Asian meliponine species differed substantially in their chemical profiles. They contained a variety of compounds with and without functional groups (including n-alkanes, alkenes, alkadienes, methylbranched alkanes, aldehydes, alcohols, esters and carboxylic acids), but, most notably, their chemical profiles contained considerable amounts of mono-, sesqui- and triterpenes. This is the first report of terpenoids found in the surface extracts of social insects. Moreover, the composition of these terpenoids in the bee species studied was highly species-specific, with one group of terpenoids (e.g., sesquiterpenes) being present in one but completely absent in another species. Given these species-specific terpene profiles, it is unlikely that the terpenoids result from contamination of e.g., previously collected resin. Moreover, terpene profiles sampled from body extracts were similar to those from wing extracts, and wings are considered to be the body part least prone to contamination (McDaniel et al. 1984).

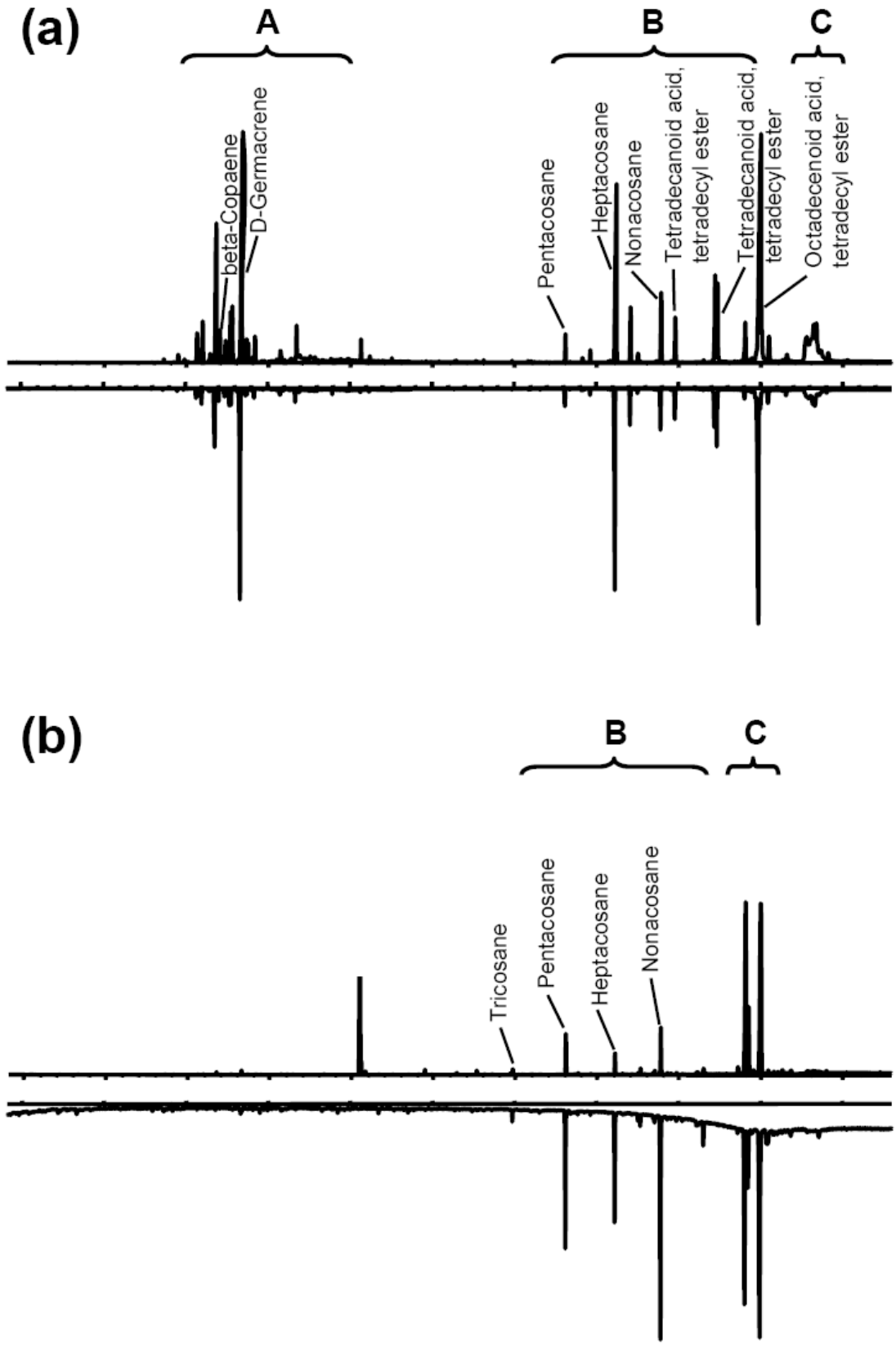


Figure 3. Chromatograms of body (above) and wing (below) surface extracts from (a) *T. collina* and (b) *T. melanocephala* (A = mainly sesquiterpenes, B = non-terpenoid compounds, C = triterpenes and non-terpenoid compounds).

Potential functions of cuticular terpenoids

The presence of terpenes on the cuticle of paleotropical stingless bees contrasts with findings in neotropical stingless bees whose chemical profiles apparently lack terpenes (Abdalla et al. 2003; Jungnickel et al. 2004; Kerr et al. 2004; Nunes et al. 2008). However, terpenoid compounds were frequently found in secretions from cephalic and abdominal glands of neotropical stingless bee species (Francke et al. 2000; Cruz-Lopez et al. 2001; Patricio et al. 2003; Cruz-Lopez et al. 2005). Such glandular terpenoids are likely to function in defensive behaviour (Cruz-Lopez et al. 2005). The defensive use of glandular terpenoids, either obtained from host plants or synthesized de novo (Laurent et al. 2003), is also known from other insects, such as termites (Bagnères et al. 1990), ants (Blum and Brand 1972; Morgan et al. 2003), larvae of the sawfly *Neodiprion sertifer* (Eisner et al. 1974), and other bees (Wheeler et al. 1977; Cane 1986). In the present case, terpenoids may have a similar function. For *T. melanocephala*, *T. geissleri*, *T. collina* and *T.fuscobalteata*, Lehmberg et al. (2008) showed that unmodified chemical profiles deterred predators such as ants, and that this function was reduced when cuticles were washed with solvents such as hexane or dichloromethane. Moreover, terpenoid compounds have been shown to prevent the growth of fungi and bacteria (Ghisalberti 1979; Messer 1985; Velikova et al. 2000a) which, according to Roubik (1983) and Michener (1974), plays a critical role in the survival of tropical bees. Alternatively, the species specific terpene profiles might point to their function as signals in the communication system of stingless bees. Besides the defensive role of terpenoids, insects frequently use terpenoids as pheromones for interspecific communication, such as sex pheromones (e.g., butterflies, diptera, true bugs, aphids, beetles and mites), trail pheromones (e.g., ants), marking pheromones (e.g., cuckoo bees and bumblebees) and for other forms of intercolonial communication (e.g., the aggregation provoking Nasonov pheromone of honeybees) (reviewed by Hick et al. 1999). All these different functions demonstrate the widespread ecological importance of terpenoids. However, the exact function of terpenoids in the chemical profiles of Southeast-Asian stingless bees remains unclear, as does their origin.

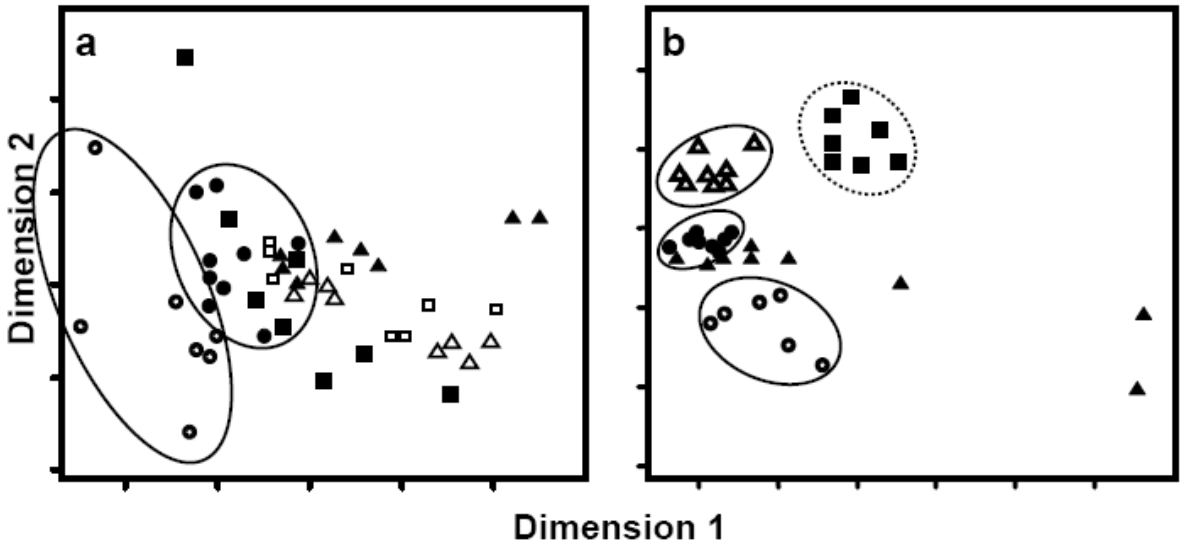


Figure 4. NMDS analysis of the cuticular compounds from hexane extracts of (a) six *T. collina* and (b) five *T. melanocephala* colonies. Different symbols indicate different colonies (each symbol represents one individual): *T. collina* (a): closed squares = KSR Forest 1, closed triangles = KSR Forest 2, open squares = KSR Forest 3, open triangles = KSR Forest 4, open circles = DVC Forest 1; closed circles = DVC Forest 2, closed ellipses indicate colonies from DVC, open ellipses indicate colonies from KSR; *T. melanocephala* (b): squares = KSR Forest, closed triangles = DVC Forest 1 (colony located in human made device to measure water flow), open triangles = DVC Forest 2, closed circles = DVC Forest 3, open circles = DVC Laboratory).

Potential origin of cuticular terpenoids

It is highly likely that terpenoids are originally acquired from plant resins which are known to contain various terpenes and are collected by stingless bees as building material for nest construction (Roubik 1989; 2006). In honeybees and social wasps, exposure to nest material may be a critical step in the development of kin recognition cues ("hive effect"), suggesting that insects acquire environmental cues from the nest environment (Blomquist et al. 1980; Gamboa et al. 1986; Breed et al. 1988a; 1988b). A similar process of compound transfer from nest material to the bee's cuticle might explain the presence of terpenes in the chemical profiles of stingless bees. Resin from Southeast-Asian trees, especially from the dominating dipterocarps, mainly contains mono-, sesqui- and triterpenes (Langenheim 2003) – the same groups of terpenes which we found in the chemical profiles of the seven bee species studied. The similarity between terpenes found in tree resins and terpenes found in chemical profiles of bees as well as the fact that bees collect large amounts of plant resins for nest construction suggest that tree resins may serve as a primary source of terpenoids. It is, however, unclear how paleotropical stingless bees control the composition of terpenoid compounds in their chemical profile, how, for example, some species manage to largely exclude entire terpenoid groups (e.g. mono- and sesquiterpenes in *T. melanocephala* and *T. fuscobalteata*). The

species-specific terpene profiles suggest that different species either selectively collect resin only from specific sources, or that they metabolically change the structure and composition of terpenes derived from plant resins. Roubik (2006) and Howard (1985) found that several neotropical stingless bee species simultaneously collected resin at a given resin source. However, Patricio and colleagues (2002) suggested that different *Frieseomelitta* species used different resin sources because samples of propolis from their hindlegs differed in their chemical composition, indicating at least partly selective resin foraging in *Frieseomelitta*. Our own studies on resin collection in Southeast-Asian meliponines showed that the bees did not use all resin sources available, but seemed to prefer resin of particular trees and to neglect resin of others (Leonhardt and Blüthgen 2009). Selectivity among resin sources might at least partly influence the chemical composition of the bees' nest material (including terpenes) and might thus also influence their chemical profiles. It cannot be ruled out, however, that in addition to a potential behavioural adaptation, bees metabolically alter terpenoids (e.g., sesquiterpenes in *T. melanocephala* or *T. fuscobalteata*).

The high similarity of terpenoids across colonies of the same species even from different regions, and the consistent interspecific variation may suggest a genetic basis of terpene acquisition (e.g., by a species-specific metabolic alteration of terpenoids derived from plant resins). Although different colonies of *T. melanocephala* could be discriminated by their quantitative chemical profiles, their profiles were highly similar qualitatively. *T. collina* colonies were even less differentiable, especially the four *T. collina* colonies from KSR which could only be discriminated based on non-terpenoid compounds. These colonies may represent sister colonies because they were all located at the same tree and thus in close proximity. Stingless bee colonies replicate by colony fission with a virgin queen leaving and the old queen staying in the mother nest (Inoue et al. 1984; Roubik 1989). In contrast to honeybees in which the old queen leaves the nest and mother and daughter colony immediately become independent, daughter colonies in stingless bees are mostly founded in close proximity and exhibit a species-specific time of dependence on the mother colony during which workers and resources are frequently exchanged (Inoue et al. 1984).

Summarising our results, we found that the chemical profiles of seven Southeast-Asian meliponine species contained terpenoids. These terpenoids were highly species-specific, suggesting a potential function in the bees' communication system. However, the role of these terpenoids remains to be investigated, as do the mechanisms which enable paleotropical stingless bees to influence their composition. It also remains unclear why terpenes have not been found in the chemical profiles of those neotropical stingless bees studied so far. Further

studies of the body surface chemistry of meliponine species and genera in both the New and the Old World would be useful in order to draw conclusions on the phylogenetic origin of terpenes in chemical profiles as well as the ecological relationship between bees and their various uses of tree resins.

VIII. Comparing chemical profiles of bee surfaces and nest material

Because stingless bees collect plant resins primarily as nesting substrate, one would presume that they simply acquired cuticular terpenes from their nest material which would result in chemically similar surface and nest profiles. To examine whether stingless bees from Borneo simply transfer terpenes from their nesting substrate to their body surfaces or whether bee surface and nest profiles differ, the chemical profiles of nests from six species were additionally analyzed and compared with the bees' surfaces.

This chapter has been submitted as:
Leonhardt SD, Blüthgen N & Schmitt T – Chemical profiles of body surfaces and nests from six Bornean stingless bee species.

VIII. 1 Summary

Stingless bees (Apidae: Meliponini) are the most diverse group of Apid bees and represent common pollinators in tropical ecosystems. Like honeybees they live in large eusocial colonies and rely on a complex chemical recognition and communication system. In contrast to honeybees, their ecology and especially their chemical ecology have received only little attention, particularly in the Old World. We have previously analyzed the chemical profiles of six paleotropical stingless bee species from Borneo and revealed the presence of species-specific cuticular terpenes – an environmentally derived compound class so far unique among social insects. Here, we compare the bees' surface profiles to the chemistry of their nest material. Terpenes, alkanes and alkenes were the dominant compound groups on both body surfaces and nest material. However, bee profiles and nests strongly differed in their chemical composition. Body surface did thus not merely mirror nests, rendering a passive compound transfer from nests to bees highly unlikely. The difference between nests and bees was particularly pronounced when all resin-derived compounds (terpenes) were excluded and only genetically determined compounds were considered. When terpenes were included, bee profiles and nest material still differed, because whole groups of terpenes (e.g. sesquiterpenes) were found in nest material of some species, but lacked in their chemical profiles, indicating that bees are able to influence the terpene composition both in their nests and on their surfaces.

VIII. 2 Introduction

Social insects – such as ants, termites, wasps and bees – live in large colonies with up to several thousands workers per colony. In order to communicate within colonies, they rely on a sophisticated communication system based on chemical cues (Blum et al. 1970b). In addition to pheromones, the chemical composition of waxy lipids on the cuticle (frequently referred to as cuticular profile or chemical profile) plays an important role in their communication system (Blum et al. 1970b; Buckner 1993) – besides preserving insects from desiccation, cuticle abrasion and infection (Lockey 1988; St. Leger 1995). Cuticular compounds comprise various chemical classes, with non-polar aliphatic compounds, such as methyl-branched alkanes, n-alkanes and n-alkenes, dominating in ants (Hölldobler 1995; Endler et al. 2004), termites (Howard et al. 1982; Kaib et al. 2004), and social wasps (Espelie et al. 1994). In addition to these non-polar compounds, polar substances (with functional groups), such as alcohols, aldehydes, esters and carboxylic acids, are found in various bee species (Ayasse et al. 1999; Paulmier et al. 1999; Fröhlich et al. 2000b; Abdalla et al. 2003; Jungnickel et al. 2004; Kerr et al. 2004; Mant et al. 2005; Nunes et al. 2008; Sramkova et al. 2008; Nunes et al. 2009b). Moreover, we recently reported on yet another class of compounds in stingless bees from Borneo: besides non-polar aliphatic hydrocarbons, their cuticular profiles comprise large amounts of terpenes (Leonhardt et al. 2009) which are most likely derived from plant resins collected (chapter IX). The bees are able to filter these resin-derived compounds, acquiring only a subset of the vast amount of terpenes found in plant resins (chapter IX).

In contrast to the environmentally derived terpenes in the chemical profiles of stingless bees, honeybees acquire compounds from self-produced comb wax (Breed et al. 1995; 1998; 2004b). Therefore, the same compound classes can be found in comb wax and in the chemical profiles of honeybees, albeit in varying quantities (Blomquist et al. 1980; Fröhlich et al. 2000b; Breed et al. 2004b). However, direct comparisons between bee profiles and nest material (such as comb wax) have rarely been made (but see Blomquist et al. 1980; Fröhlich et al. 2000b), which is surprising given that interactions between the bees and their nest material are supposed to play an important role in the origin of recognition cues in bees (Breed et al. 1995; 1998; 2004b). Bees are even able to distinguish between wax of their own and a foreign colony (Fröhlich et al. 2000a; Hepburn et al. 2010).

Here, we compare nest material and chemical profiles of six stingless bee species from Borneo. The chemical profiles of these bees strongly differ between species, with regard to both resin-derived terpenoid compounds and genetically determined non-terpenoid compounds (aliphatic compounds) (Leonhardt et al. 2009). Resin is the main nest building

material in stingless bees, but is frequently mixed with self-produced wax (Roubik 1989; 2006). Because stingless bees have both resin-derived compounds and genetically determined aliphatic hydrocarbons on their cuticle (Leonhardt et al. 2009), we ask whether they simply acquire their cuticular compounds from their nesting substrate which would result in similar bee and nest profiles. The bees might alternatively be able to exclude or modify nest compounds before acquiring them on their body surface or produce cuticular profiles independent of their nest profiles. In this case, the chemical composition of the bees' body surfaces and their nest material might differ in the composition of resin-derived and/or genetically determined compounds.

VIII. 3Methods

Study sites and bees

Bee specimens and nest material were collected at the Danum Valley Conservation Area (DVC: Sabah, 4°55' N 117°40' E, 100 m asl) and the Kabili Sepilok Reserve (KSR: Sabah, 5°54' N, 118°04' E, 20-120 m asl) in Sabah, Borneo (Malaysia). Sampling was performed in February and March 2007. KSR covers an area of 4294 ha of coastal dipterocarp and mangrove forest (Fox 1973) surrounded by oil palm plantations. DVC represents one of the major remaining patches of Sabah's primary lowland dipterocarp rainforest (43 800 ha) (Marsh and Greer 1992). The two sites have a typical equatorial rainforest climate with a mean annual temperature of 26 - 30°C and yearly rainfall of 2600 - 3000 mm (Fox 1973).

Eltz (2004) and Dworschak and Blüthgen (2010) found 15 stingless bee species (species and genus names as in Moure 1961) in DVC, whereas 15 to 20 species can be found in KSR according to specimen collections held by the Forestry Research Centre in Sepilok and our own studies (Leonhardt and Blüthgen 2009).

Table 1. Proportion of substance classes in hexane extracts of bee profiles (profile) and nest material (nest) from six stingless bee species. N1 gives number of compounds per species, N2 gives total number of compounds found for a given substance class across all species. Proportions are obtained by dividing the peak area of a substance class by the total peak area of all compounds in a species (colonies pooled).

	T. collina		*T. fuscobalteata*		*T. geissleri*		*T. melanocephala*		*P. pendleburyi*		*L. terminata*		
	Profile	Nest	Profile	Nest	Profile	Nest	Profile	Nest	Profile	Nest	Profile	Nest	N2
Monoterpenes	0.03%	-	0.03%	-	6.49%	14.84%	0.06%	-	0.06%	-	0.02%	0.03%	8
Sesquiterpenes	34.61%	65.41%	3.36%	0.06%	6.14%	14.03%	1.90%	50.30%	9.17%	24.20%	0.87%	3.81%	104
Triterpenes	5.33%	18.45%	66.36%	78.05%	32.14%	14.35%	70.56%	33.14%	7.01%	48.87%	17.52%	64.21%	59
Alkanes	10.74%	1.12%	8.41%	11.28%	2.92%	0.31%	14.31%	0.69%	15.38%	2.66%	23.63%	16.96%	13
Methyl alkanes	0.04%	-	0.67%	-	-	-	0.18%	-	-	-	0.79%	-	3
Alkenes	0.17%	-	9.56%	0.52%	7.95%	0.93%	2.06%	0.05%	0.10%	-	40.45%	2.17%	19
Alkadienes	-	-	0.01%	-	2.98%	0.21%	-	-	-	-	5.47%	0.28%	6
Ester	26.37%	0.78%	0.30%	-	0.73%	-	0.05%	-	2.54%	-	-	-	11
Carboxylic acids	0.23%	-	-	-	-	-	-	-	-	-	-	-	1
Alcohols	0.13%	-	0.31%	-	-	-	0.08%	0.08%	-	-	-	-	3
Aldehydes	-	-	0.12%	-	0.01%	-	0.72%	-	-	-	-	-	2
Lactones	-	-	-	0.26%	-	-	-	-	-	-	-	-	3
Unidentified substances	16.87%	10.81%	5.63%	8.01%	36.51%	51.12%	5.82%	9.70%	62.31%	16.91%	7.19%	9.29%	76
N1	103	118	72	62	88	109	71	139	32	80	47	80	

Sampling of bees and nest material

We caught departing workers from 29 colonies belonging to six species. Sixteen colonies were located in DVC and 23 in KSR (see also Leonhardt et al. 2009, note that *Tetragonula melina* was excluded from this study because nest material could not be obtained from this species). Bees were caught at their colonies' nest entrances by putting a clean transparent plastic bag over the nest entrance tube.

Nest material was obtained from 15 of the 16 colonies from DVC (comprising all six species), by breaking of small pieces of the nest entrance tube (max. ~1 mg). To test whether old and new nest material had the same chemical composition, we returned to six nests (including colonies from all species except *Tetragonula fuscobalteata*) after 1-9 days and additionally collected fresh, recently added nest material. Only fresh nest material was collected from two *Tetragonula geissleri* colonies.

Extraction and chemical analysis

The bees were killed in a freezer. Dead bees and nest material collected were transferred into 2 ml sample vials containing hexane for extraction. Bees were extracted for 10 minutes and then discarded; nest material was kept in hexane for the rest of the analysis. Extracts from bees and nest materials were analyzed by a Hewlett Packard HP 6890 Series GC System coupled to a Hewlett Packard HP 5973 Mass Selective Detector (Agilent Technologies, Böblingen, Germany) as described in Leonhardt et al. (2009).

Compounds found in the extracts were characterized by their mass spectra and retention times. Compounds with identical mass spectra and retention times were regarded as the same substance. Compound classes characterized comprised alkanes, aldehydes, alcohols, esters as well as mono-, sesqui-, and triterpenes. They were (tentatively) identified by comparison with three commercially available mass spectra libraries (Wiley 275, NIST 98 and Adams EO library 2205) and with compounds from dipterocarp tree resins known to comprise mono-, sesqui-, and triterpenes (see also Leonhardt et al. 2009). Compounds were confirmed by synthetic standards if standards were available (Sigma-Aldrich, Munich, Germany).

Statistical analysis

Prior to analyses, we condensed the dataset by removing trace compounds (for which mass spectra could not be characterized) as well as compounds that accounted for less than 5% of the total peak area across all samples. The analysis is based on a total of 309 compounds, with 253 found in nest material and 195 in bee profiles. The compounds were quantified as

proportions by dividing the peak area of each single compound by the total area of all peaks remaining after having condensed the dataset.

First, we analyzed species specific differences in the chemical composition of nests. Then, we compared nest materials and bee profiles to investigate whether bees chemically resemble their nests. Analyses were performed for all compounds, only terpenoid compounds, and only non-terpenoid compounds to distinguish between the contributions of resin-derived terpenoids and genetically determined compounds. Two-dimensional NMDS (non-metric dimensional scaling) based on Bray-Curtis distance of the proportions of each compound (start configuration: PCoA, 1000 iterations) was used to produce an ordination figure. Groups were compared by an "Adonis" test (R Statistical software 2.9.2, vegan package; command for a randomization-based analysis of dissimilarities), based on the Bray-Curtis distance matrix of the proportions of each compound.

If surface profiles of bees merely mirrored their nest profiles, chemical differences between bees and their nest of origin should be less pronounced than between bees and nests of different colonies. To test whether chemical differences were more pronounced within nests and/or bee profiles or between nests and the equivalent bee profiles, we compared the mean Bray-Curtis distances for the two bee species with the largest sample sizes (*T. fuscobalteata* and *T. melanocephala*).

VIII. 4Results

Nest material of the six stingless bee species analyzed contained large amounts of resin-derived terpenes (particularly sesqui- and triterpenes), alkanes, and alkenes (Table 1). The chemical composition of nests from different species strongly differed both qualitatively and particularly quantitatively (Adonis: $R^2 = 0.90$, $p < 0.0001$; Fig. 1a). Differences between species were similarly pronounced when only terpenoids were included in the analyses (Adonis: $R^2 = 0.90$, $p < 0.0001$; Fig. 1c) and slightly less for non-terpenoids (Adonis: $R^2 = 0.86$, $p < 0.0001$; Fig. 1b).

One hundred and forty compounds were shared by both nest material and bee profiles, whereas 114 compounds were exclusively found in nest material and 55 exclusively in bee profiles. When nest material and bee profiles were combined in one analysis, both nests and bee profiles could still be discriminated (Adonis: all compounds: $R^2 = 0.90$, $p < 0.0001$; Fig. 2a; non-terpenoids: $R^2 = 0.88$, $p < 0.0001$; Fig. 2b; terpenoids: $R^2 = 0.86$, $p < 0.0001$; Fig. 2c). Nest and bee profiles were chemically more similar to one another than nests and their equivalent bee profiles, in both *T. fuscobalteata* (mean Bray-Curtis distances ± SD

between bee profiles: 0.20 ± 0.13; between nest profiles: 0.19 ± 0.06; between nest and equivalent bee profiles: 0.32 ± 0.07) and *T. melanocephala* (between bee profiles: 0.22 ± 0.03; between nest profiles: 0.33 ± 0.07; between nest and equivalent bee profiles: 0.74 ± 0.08). The same trend was observed for the Bray-Curtis distance matrices of only terpenoid compounds (*T. fuscobalteat*a: between bee profiles: 0.18 ± 0.14; between nest profiles: 0.15 ± 0.07; between nest and equivalent bee profiles: 0.22 ± 0.10; *T. melanocephala*: between bee profiles: 0.16 ± 0.05; between nest profiles: 0.31 ± 0.07; between nest and equivalent bee profiles: 0.75 ± 0.10) and only non-terpenoid compounds (*T. fuscobalteat*a: between bee profiles: 0.27 ± 0.12; between nest profiles: 0.32 ± 0.09; between nest and equivalent bee profiles: 0.62 ± 0.04; *T. melanocephala*: between bee profiles: 0.32 ± 0.10; between nest profiles: 0.38 ± 0.15; between nest and equivalent bee profiles: 0.72 ± 0.10).

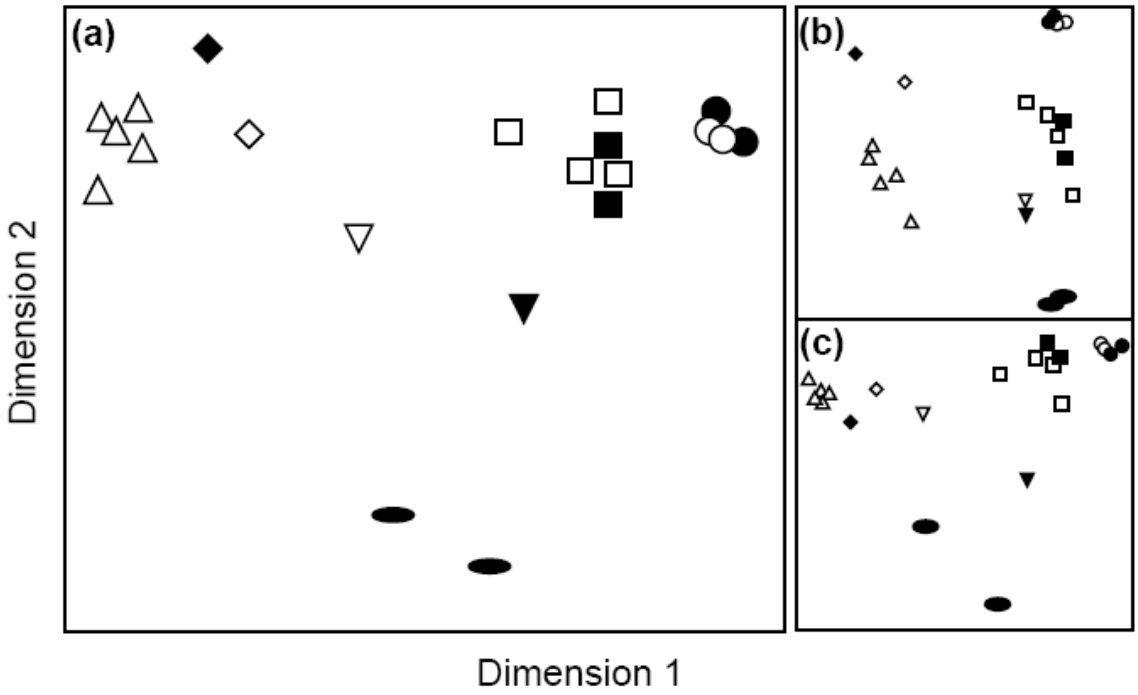


Figure 1. Similarity in the chemical composition of nest material from six stingless bee species including (a) all compounds, (b) only non-terpenoid compounds, and (c) only terpenoid compounds (each symbol represents one colony). NMDS ordination based on Bray-Curtis distances: triangles = *T. fuscobalteata*, diamonds = *L. terminata*, triangles = upside down = *P. pendleburyi*, squares = *T. melanocephala*, circles = *T. collina*, ellipses = *T. geissleri*; open symbols indicate old nest material and filled symbols indicate fresh nest material.

For terpenoids, bee and nest profiles with sesquiterpenes and without sesquiterpenes were clearly separated (Fig. 2c), but discrimination was less pronounced for bee and nest profiles that solely contained triterpenes (Adonis: $R^2 = 0.67$, $p < 0.0001$; Fig. 2c). Notably, sesquiterpenes were present in hexane extracts of both nest material and bee surfaces of some

species (e.g.; *T. collina*) (Fig. 3a and 3c), whereas they were lacking on the body surfaces of other species (e.g.; *T. melanocephala*), while being present in their nest material (Fig. 3b and 3d).

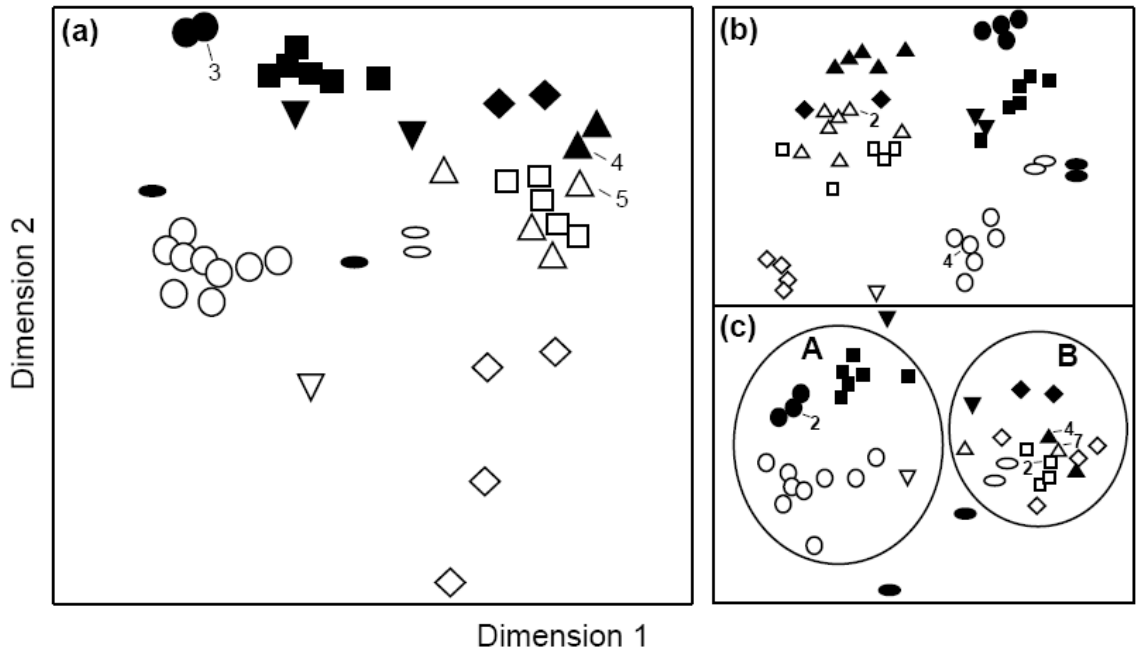


Figure 2. NMDS of chemical compounds from hexane extracts of nest material and bee profiles combined of six bee species including (a) all compounds, (b) only non-terpenoid compounds, and (c) only terpenoid compounds (each symbol represents one colony); symbol codes as in Fig. 1; filled symbols indicate nest material and open symbols indicate bee profiles. Numbers indicate where several symbols were entirely overlapping. The two circles (in 2c) comprise samples with sesquiterpenes (*A*) and without sesquiterpenes (*B*).

VIII. 5Discussion

The chemical composition of nest material from six Bornean stingless bee species strongly differed both qualitatively and quantitatively. Moreover, bee profiles did not merely mirror the chemical profiles of their nests, because bee and nest profiles were clearly separated by their chemical composition. Furthermore, chemical distances were more pronounced between bees and their nests of origin than within bees and/or within nests from different colonies. Discrimination was about equally accurate when analyses were confined to either terpenoid or non-terpenoid compounds. The least discrimination was revealed for surface profiles and nests with triterpenes as sole terpene group, indicating that triterpenes were qualitatively and quantitatively similar across those species' nests and surface profiles.

Besides terpenes, bee surfaces comprised alkanes, alkenes, branched alkanes, alkadienes as well as compound classes with functional groups (such as esters or alcohols) (see also

Leonhardt et al. 2009), whereas nest material mainly contained terpenes, alkanes and alkenes. These findings are in accordance with studies of Milborrow et al. (1987) and Blomquist et al. (1985) that also revealed non-polar hydrocarbons and compounds from exogenous materials (terpenes) as dominant compound groups in the nesting substrate of stingless bees. However, genetically determined non-terpenoid compounds of stingless bees comprise mainly, partly even exclusively, non-polar aliphatic hydrocarbons (n-alkanes, alkenes and branched alkanes) and only few compounds with functional groups (see also: Abdalla et al. 2003; Jungnickel et al. 2004; Kerr et al. 2004; Nunes et al. 2008; Leonhardt et al. 2009; Nunes et al. 2009b). By contrast, honeybees have non-polar compounds and compounds with functional groups in equal quantities both on their body surfaces and in their nest material (comb wax) (Blomquist et al. 1980; Francis et al. 1985; 1989; Fröhlich et al. 2000b). Moreover, honeybees more closely resemble their nest material than do stingless bees (Blomquist et al. 1980; Fröhlich et al. 2000b). Stingless bees further have large amounts of resin-derived terpenes (especially sesqui- and triterpenes) both on their body surfaces and in their nests. This compound group completely lacks in the surface profiles of honeybees (let alone any other social insect).

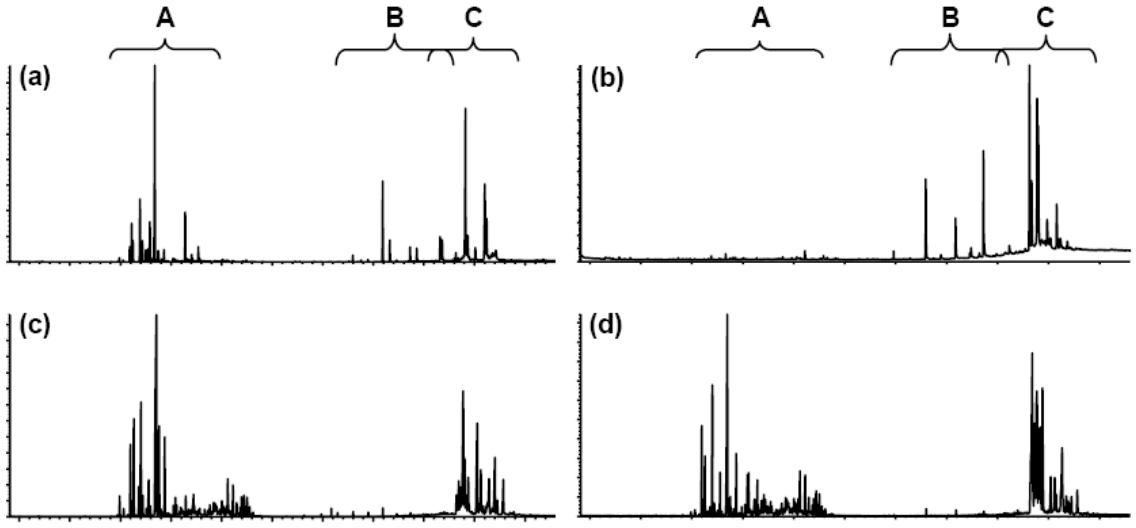


Figure 3. Chromatograms of hexane extracts from (a) the chemical profile of *T. collina*, (b) the chemical profile of *T. melanocephala*, (c) nest material of *T. collina*, and (d) nest material of *T. melanocephala*. Capital letters above chromatograms indicate substance classes of peaks/compounds: *A* = mainly sesquiterpenes, *B* = non-terpenoid compounds, and *C* = triterpenes and non-terpenoid compounds.

Because most substances (e.g.; terpenes) were found in both nest material and surface profiles of stingless bees, compound transfer from nests to surfaces appears likely and would be in accordance with findings in honeybees whose cuticular surfaces are influenced by compounds derived from beeswax (especially fatty acids and esters) (Breed et al. 1995; 1998; 2004b).

However, in stingless bees, resin-derived terpenes are not passively transferred from their nest material to their surface, because, in some species (e.g.; *T. melanocephala*), one group of terpenes (sesquiterpenes) is found in nests, but completely lacking on their body surface. Such a lack of sesquiterpenes indicates that stingless bees are able to actively influence the chemical composition of compounds in nests and on their surface by, for instance, excluding a whole group of terpenes. This idea is further supported by the highly species specific distribution of different groups of terpenes among surface profiles of different bee species (Leonhardt et al. 2009). Moreover, even nest material of different species could be discriminated based on terpenes alone, rendering a purely opportunistic inclusion of resin-derived compounds in nest material unlikely. It appears most likely that resin and thus resin-derived compounds are processed prior to nest building, because resin foragers do not alter the resin collected directly at resin wounds (chapter X). But how stingless bees process plant resins remains as yet to be investigated.

Stingless bees clearly respond to terpenes as they use these compounds to locate and re-recognize resin sources (wounds) at trees (chapter V). Moreover, adding sesquiterpenes to the surface profile of species that lack sesquiterpenes themselves reduces aggression in these species, indicating that terpenes affect the bees' recognition system (chapter X). Whether terpenes are further involved in nestmate recognition remains to be tested, but their species-specific distribution in both the bees' surface profiles and their nests strongly suggests that terpenes represent more than pure contamination by plant resins.

IX. Resin-derived terpenes and the chemical diversity of bee profiles

Where do the terpenes on the body surfaces of stingless bees come from? Their highly species-specific distribution might suggest that bees synthesize them *de novo*; but the intensive use of terpene rich resins as building and defensive material render the sticky plant sap an equally likely source of origin. In this chapter, I compare the composition of terpenes from bee surfaces, bee nests and resins of seven tree species to track terpenes from trees to bee surfaces.

This chapter has been submitted as:
Leonhardt SD, Schmitt T & Blüthgen N – Chemodiversity: tree resin composition, collection behavior and selective filters shape chemical profiles of tropical bees.

IX. 1 Summary

The biodiversity of species is striking, but is far exceeded by the chemical diversity of compounds collected, produced or used by them. Here, we relate biodiversity to chemical diversity using two-dimensional network analyses, considering chemical networks of tree resins, foraging networks of resin collecting bees, and their acquired chemical networks. Stingless bees collect plant resins for nest construction and to deter predators and microbes. Resins also function as environmental source for terpenes that serve as appeasement allomones and protection against predators when accumulated on the bees' body surfaces. To unravel the origin of the bees' complex chemical profiles, we investigated resin collection and the processing of resin-derived terpenes.

We revealed that 113 terpenes in nests of six bee species and 83 on their body surfaces comprised a subset of the 1117 compounds found in resins from seven tree species. Stingless bees showed a generalized collecting behavior among resin sources, and only a hitherto unknown species-specific filtering of resin-derived terpenes can explain the variation in chemical profiles of nests and body surfaces from different species. The tight relationship between bees and tree resins of a large variety of species elucidates why the bees' surfaces contain a much higher chemodiversity than other hymenopterans.

IX. 2 Introduction

Biodiversity is considered a crucial feature of ecosystems worldwide, by, for instance, providing a variety of organisms that maintain ecosystem functioning and services (Loreau et al. 2001). The higher the diversity of species in a habitat, the more interactions occur between them, resulting in complex interaction networks (Jordano 1987; Blüthgen et al. 2007; Olesen et al. 2007). Here, we attempted to reveal the origin of another, rather neglected kind of diversity: chemical diversity – describing the heterogeneity of chemical compounds produced or acquired and used by organisms. The reliance on such chemical compounds is particularly pronounced in plants and insects.

Plants produce secondary metabolites to defend themselves against herbivores (Schoonhoven et al. 1998) or to attract mutualists, such as parasitoids (Baldwin et al. 2006; Dicke and Baldwin 2010) and pollinators (Dötterl et al. 2006; Dudareva and Pichersky 2006; Raguso 2008; Chen et al. 2009). The composition of secondary metabolites may vary across seasons (Hector et al. 1999), developmental states (Hector et al. 1999; Goff and Klee 2006), species (Langenheim 2003; Dudareva and Pichersky 2006), individuals, different plant parts of the same individual (Langenheim et al. 1978; Kainulainen et al. 1998) or in response to herbivore attack (Baldwin et al. 2006; Dicke and Baldwin 2010).

Insects use chemical compounds to recognize potential mates, relatives, nestmates or enemies, but also to mark suitable nesting sites or resources and to defend themselves against predators (Blum and Brand 1972; Pasteels et al. 1983; Ayasse et al. 2001). Qualitative and quantitative differences between chemical mixtures/bouquets usually indicate different species (Said et al. 2005; Terzo et al. 2005; Menzel et al. 2008a; Leonhardt et al. 2009). Within species, quantitative differences between compounds signify different colonies, ages, genders, castes and/or differences in the reproductive status of individuals (Howard et al. 1982; Nielsen et al. 1999; Heinze et al. 2002; Bruschini et al. 2008; Nunes et al. 2009a).

The large number of functions and meanings mediated by chemical compounds is thus associated with a chemical heterogeneity that far exceeds the diversity of plants and insects themselves, because even conspecific individuals may have different chemical profiles due to quantitative variation.

Insects synthesize chemical compounds *de novo* in specialized glands (genetically determined compounds; Breed et al. 1988a; Legendre et al. 2008; Thomas and Simmons 2008; Gleason et al. 2009) and/or acquire compounds from the environment – predominantly from plants. For instance, euglossine bees collect various volatiles from flowers or other plant parts (Vogel 1966; Dressler 1982), and some specialized herbivores sequester defensive compounds from

their host plant (e.g.; alkaloids in butterflies, Edgar and Culvenor 1974; resin terpenoids in sawfly larvae, Eisner et al. 1974). Particularly chemical profiles on the body surfaces of insects often represent a mixture of both genetically determined and plant-derived compounds (Downs et al. 2000; Leonhardt et al. 2009), thereby increasing the diversity and heterogeneity of compounds available for communication and/or defense. The secondary metabolites of plants can thus be tracked along the food chain, in which the specificity of plant-insect interactions mediates the distribution of plant compounds among insects.

We here focus on the origin of plant-derived chemical compounds in tropical stingless bees (Meliponini). Stingless bees have eusocial colonies and are considered crucial pollinators in tropical forests (Roubik 1989; Corlett 2004). Besides pollen and nectar, they also collect large amounts of plant resins for nest construction and defense (Roubik 1989; 2006). Terpenes derived from these resins are transferred to the bees' chemical profiles, where they are mixed with self-produced non-terpenoid compounds (non-polar aliphatic compounds, alcohols, aldehydes and esters) (Leonhardt et al. 2009). Notably, different bee species strongly differ in their terpene profiles, excluding contamination as main reason for the presence of these resin-derived compounds (Leonhardt et al. 2009). The terpenes on the bees' surfaces repel predators (ants, Lehmberg et al. 2008) and reduce interspecific aggression (chapter X).

We attempted to reveal how the bees' foraging behavior and the chemical diversity of tree resins affect the chemical diversity of their surface profiles. We thereby link behavior and chemistry by applying two-dimensional network analyses (Blüthgen et al. 2006) to both species – interaction (foraging) networks and compound – species (chemical) networks. By observing bees at trees (sources of chemical compounds) and nest entrances, we investigated whether different stingless bee species collected resin from different tree species (specialized) or from the same tree species (generalized). If bees merely transferred resin-derived terpenes to their surfaces without filtering or modifying them, we would expect that their species-specificity of resin collection would directly predict the specificity of their chemical profiles. In addition to the behavioral observations, we therefore analyzed and compared the chemical profiles of tree resins, nest and bee profiles with regard to resin derived terpenes and non-terpenoid compounds, in order to track terpenes from tree resins to the bees' profiles. Moreover, we compared the chemical diversity of stingless bees with that of other hymenopterans and discuss the different functions of environmentally derived and genetically determined compounds.

IX. 3 Methods

Study sites and bees

Fieldwork was performed in Borneo (Malaysia), from March 2006 to November 2008. Observations and sample collection took part at the Danum Valley Conservation Area (DVC: Sabah, 4°55' N 117°40' E, 100 m asl), the Kabili Sepilok Reserve (KSR: Sabah, 5°54' N, 118°04' E, 20-120 m asl) and the Rainforest discovery centre (RDC). DVC represents one of the major remaining patches of Sabah's primary lowland dipterocarp rainforest (43 800 ha) (Marsh and Greer 1992). KSR comprises 4294 ha of coastal dipterocarp and mangrove forest (Fox 1973) and the RDC is a small (148.6 ha) education centre about 2 km west of KSR.

About fifteen stingless bee species (species and genus names as in Moure 1961) were reported for DVC (Eltz 2004). In KSR and RDC, 15 to 20 species can be found according to collections of specimens held by the Forestry Research Centre in Sepilok and our own studies (Leonhardt and Blüthgen 2009).

Foraging networks: Observation of resin collection at trees and at nest entrances

To analyze the degree of specialization on resin sources in stingless bees, we observed bees collecting resin from wounds of totally 15 tree species at the RDC, in August 2008. Observations comprised five natural and 55 artificially induced resin wounds (belonging to five tree families, with 75% of the trees representing dipterocarps). Artificial resin wounds were inflicted to trees by either hammering nails in the trunk or cutting the trees' bark with a machete. We noted the number of bee species collecting resin at a given resin wound following wound insertion (artificial wounds) or wound discovery (natural wounds).

To see whether our findings at resin wounds hold true for resin carried into the bees' nests, resin foragers were caught at nest entrances of six *Tetragonilla collina* colonies, three *Tetragonula melanocephala* colonies and two colonies the *Tetragonula geissleri* group, in 2007 and 2008. We recorded the number of resin foragers carrying resin of a particular color. We defined 25 different color patterns for resin (including white, yellow, red, black, brown and opaque resin with different varieties of these colors, e.g. light-brown and dark brown). Each nest was observed at different times of the day and between ten and 40 times in total to ensure that the whole spectrum of daily resin foragers was recorded.

Chemical networks: Collection of bee-, nest- and resin-samples and chemical analysis

Bees from 31 colonies (six species) were collected and their chemical profiles analyzed as described in Leonhardt et al. (2009). For a comparison of terpene composition, we further analyzed the chemical profiles of the bees' nest material from a subset of 18 colonies

(including all six species) as well as of resin samples from 23 trees. Nest material was collected by breaking off small pieces from the bees' nest entrance tubes. Fresh resin samples were obtained directly from natural or artificially induced resin wounds studied in 2007 (see Leonhardt and Blüthgen 2009).

If bees were able to filter or modify resin-derived compounds, they could do so by e.g. adding specific enzymes either directly during resin collection at trees or later inside their nests. We thus additionally collected resin from the hindlegs (corbiculae) of five *T. collina* foragers collecting resin from an *Agathis borneensis* tree. The resin from corbiculae was processed directly or after having been stored in a plastic bag for 1-12 h to see whether its chemical composition changed with time. For comparison with resin not touched by the bee, resin from the same tree was also obtained manually and treated equally.

Nest material and resin samples were transferred into 2 ml sample vials containing pure hexane. We analyzed the solvable components of these materials using a Hewlett Packard HP 6890 Series GC System coupled to a Hewlett Packard HP 5973 Mass Selective Detector (Agilent Technologies, Böblingen, Germany). The components were characterized and identified in the same way as described in Leonhardt et al. (2009) for the components of the bees' chemical profiles: by comparing their mass spectra and retention times with mass spectra from three commercially available libraries (Wiley 275, NIST 98 and Adams EO library 2205), and by comparing them to synthetic standards (Sigma-Aldrich, Munich, Germany) if standards were available. For statistical analyses, we used only compounds that accounted for at least 0.05% of the total peak area in at least one sample. Overall, we analyzed 1177 resin-compounds, 247 nest-compounds and 194 bee-compounds. The following substance classes were determined: non-polar aliphatic compounds (alkanes, alkenes, alkadienes and methylated alkanes/alkenes), oxygenated aliphatic compounds (aldehydes and alcohols), esters, monoterpenes, (methylated) sesquiterpenes, oxygenated sesquiterpenes and putatively identified triterpenes. Across nests, bee profiles and resin samples, we characterized peaks with the same mass spectra and retention times as the same substance.

Fractionation and analysis of resin from bee legs and from A. borneensis

The resin samples from corbiculae of *T. collina* foragers as well as the resin obtained from the bees' collecting tree (*A. borneensis*) were fractionated to test for changes in the chemical composition of polar compounds which have been found in large amounts in tree resins but only in traces on the cuticle of bees, and are more likely to be targeted by enzymes potentially added by the bees. We used 6 ml SiOH polypropylene columns (CHROMABOND®, 500mg,

Macherey-Nagel, Düren, Germany) that were conditioned with pentane before adding about 40 µl of surface extract. Non-polar and polar fractions of extracts were eluted with hexane and subsequently with dichloromethane. Success of fractionation was controlled by GC-MS. We then compared the chemical composition of polar compounds in resin samples collected from bee corbiculae and resin samples collected directly from *A. borneensis* using an "Adonis" test (R Statistical software 2.9.2, vegan package; command for a randomization-based analysis of dissimilarities).

Statistical analyses, profile modelling and chemical diversity

To directly compare behavioral observations (foraging networks) and chemical analyses (chemical networks), we used the quantitative specialization indices d_i' and H_2' (Blüthgen et al. 2006). The index d_i' (species-level specialization) describes the exclusiveness of a species, i.e. its quantitative deviation from the overall distribution of all bees on resin sources or of the overall distribution of compounds on all bees. The related network-level specialization index H_2' characterizes the overall quantitative partitioning of resin sources or chemical compounds across species. Both measures range between 0 (all species use the same resin sources or have identical chemistry) and 1 (species uses a different set of resins or have unique compounds, i.e. complementary specialization). These indices take the observed variation in number of observations per species into account, using a null model approach.

To model hypothetical degrees of specialization (H_2'$_M$) for the bees' chemical profiles, we assumed that for bee species b, p_{bc} is the proportion of chemical substance c on its profile (for each bee, $\sum p_{bc} = 1$). For a complete admixture of substances, p_{bc} is predicted by the proportional distribution of this bee across each resin source r (p_{br}) and the proportion of substance c at each resin source r (p_{cr}). These proportions are summed over all R resin types to yield the expected p_{bc} as

$$E(p_{bc}) = \sum_{r}^{R} p_{cr} \cdot p_{br} \,.$$

The entire chemical profile of b is given as a vector containing a total of C substances.

We compared the chemical diversity of stingless bees with the chemical diversity of (environmentally derived) fragrances collected by euglossine bees and of the (genetically determined) surface profiles from formicine ants and bumblebees. Data for 15 euglossine bees were obtained from Thomas Eltz (pers. comm.), who provided an extended dataset including all compounds detected, which is the basis of the study by Zimmermann et al. (2009). For ant species, we used the table compiled by Martin and Drijfhout (2009) from which only those 29 species were selected that occur in Central Europe. Chemical profiles of bumblebees were

analyzed and characterized by GC-MS using the same methods and criteria as described above for stingless bees. Chemical diversity was simply defined as the total number of different compounds, because concentrations were unavailable for ants and most compounds in euglossines. For a set of species, the cumulative diversity increases with additional species, but the slope saturates depending on the overlap between species. Like in biodiversity studies, we modeled the cumulative diversity curves using rarefaction of the available data (10000 randomisations) using EcoSim 7 (Gotelli and Entsminger 2009).

IX. 4 Results

Foraging networks

Different stingless bee species often collected resins from the same tree species. The quantitative resin – bee interaction network showed a very low degree of complementary specialization (H_2' = 0.20, Fig. 1), suggesting a largely opportunistic collecting behavior. The interaction network did not differ significantly from a random distribution of species (p = 0.06). Ten of the 13 bee species collecting resin at trees showed very low degrees of specialization (all $d_i' \leq 0.18$). Only *Tetrigona binghami*, *Tetrigona apicalis* and *Geniotrigona thoracica* were slightly more specialized resin foragers ($0.31 \leq d_i' \leq 0.37$). *Tetragonilla collina* was most frequently observed at trees and collected resin from overall twelve different tree species (Fig. 1). Among trees, *Shorea xanthophylla* (Dipterocarpaceae) was most commonly found to secrete resin (13 tree individuals) and most frequently visited by bees (Fig. 1).

All species also collected a similar range of resin colors, again yielding a very low degree of complementary specialization (2007: $H_2' = 0.15$; 2008: $H_2' = 0.27$). Within species, colonies did not differ either (2007: *T. collina*: $H_2' = 0.09$, *Tetragonula melanocephala*: $H_2' = 0.18$; 2008: *T. collina*: $H_2' = 0.23$, *T. melanocephala*: $H_2' = 0.12$, *Tetragonula geissleri*: $H_2' = 0.20$).

Chemical source networks

Tree species strongly differed both qualitatively and quantitatively in their resin chemistry ($H_2' = 0.60$). Resin extracts comprised mono-, sesqui-, di- and triterpenes as well as some unknown and very few aliphatic compounds (Fig. 2). Sesqui- and triterpenes represented the most prominent groups of terpenes (Fig. 1, Fig. 2) and were highly characteristic of dipterocarp trees (Langenheim 2003) – the dominant tree family of Southeast Asian forests (Soepadmo et al. 2004).

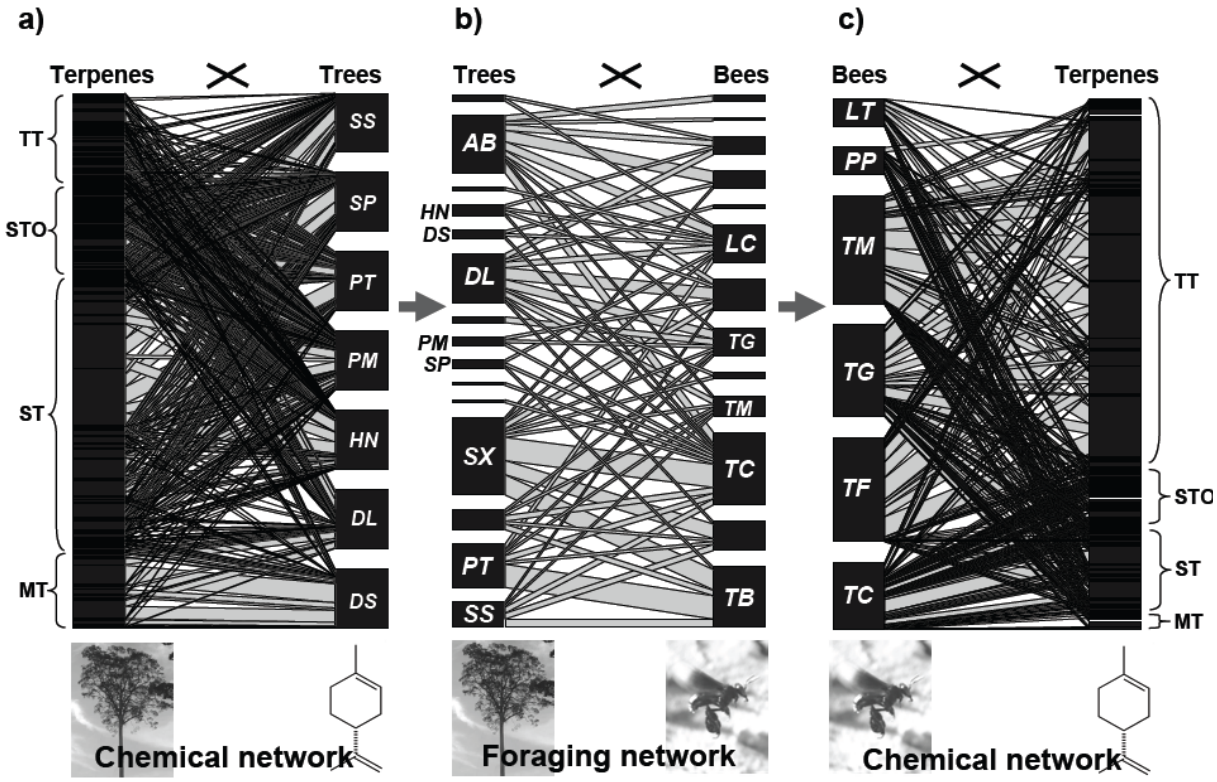


Figure 1. Chemical and foraging networks, representing (a) seven tree species and the terpenes of their resins (MT = monoterpenes, ST = sesquiterpenes without functional groups, STO = sesquiterpenes with functional groups, TT = triterpenes), (b) 15 tree species and 13 bee species collecting resin at these trees, and (c) terpenes found on the body surface of six bee species. Note that resin samples could not be analyzed for all tree species visited by bees and that nests were only found for six bee species, limiting the number of bee species whose chemical profiles were analyzed. Species names as follows: HN = *Hopea nervosa* (Dipterocarpacea), SP = *Shorea parvifolia* (Dipterocarpacea), SS = *Shorea smithiana* (Dipterocarpacea), SX = *Shorea xantophylla* (Dipterocarpacea), PM = *Parashorea melanonaan* (Dipterocarpacea), PT = *Parashorea tomentella* (Dipterocarpacea), AB = *Agathis borneensis* (Araucariaceae), DL = *Dryobalanops lanceolata* (Dipterocarpacea), *Dacryodes* spec. (Burseraceae), LC = *Lophotrigona canifrons*, TC = *Tetragonilla collina*, TB = *Tetrigona binghami*, TM = *Tetragonula melanocephala*, TF = *Tetragonula fuscobalteata*, TG = *Tetragonula geissleri/laeviceps* group, PP = *Pariotrigona pendleburyi*, LT = *Lepidotrigona terminata*.

Acquired chemical networks

Most of the terpenoid compounds in the bees' nests and on their body surfaces were identical with compounds found in one or several of the seven tree resins analyzed (60 – 100% congruence, depending on the species and class of terpenoids), indicating that bees obtain their cuticular terpenes from resin collected. However, only a small subset of the 1117 terpenes from resins was found in nest material (0.4 – 3.7%) and body surface profiles (0.4 – 3.0%) of all bee species studied. Overall, the chemical profiles of bee surfaces and nests were dominated by the most prominent resin terpenes: proportional concentrations of terpenes were significantly correlated between all tree resin samples and all bee surfaces (Spearman rank

correlation: $r_S = 0.31$, $p < 0.0001$, n = 1117 terpenes), as well as between resin and nests ($r_S = 0.32$, $p < 0.0001$). However, this correlation was much less pronounced for the surface profiles of single bee species (*T. collina*: $r_S = 0.26$, $p < 0.0001$; *T. melanocephala*: $r_S = 0.14$, $p < 0.0001$). Moreover, bee species strongly differed in the proportion of terpene classes derived from resin and included in their chemical profiles (as shown for *T. collina* and *T. melanocephala*, Fig. 2). Some species (*T. melanocephala*, *Lepidotrigona terminata*, *Pariotrigona pendleburyi* and *Tetragonula fuscobalteata*) even completely lacked sesquiterpenes, whereas all species had triterpenes (see also Leonhardt et al. 2009).

Terpenes in the chemical profiles of nests and bee surfaces were largely identical, but did not completely overlap. Twelve resin-derived terpenes were found on the bees' surfaces but not in their nest material. The bees' nest material also had a species-specific chemical composition ($H_2' = 0.42$), but differed less with regard to resin-derived compounds (terpenes: $H_2' = 0.38$), whereas wax-derived compounds showed a higher specificity ($H_2' = 0.45$). The same was true for the bees' body surface profiles (all compounds: $H_2' = 0.50$; only non-terpenoids: $H_2' = 0.59$; only terpenes: $H_2' = 0.26$). However, given the generalized resin collecting behaviour of stingless bees ($H_2' = 0.20$), the species-specificity of cuticular terpenes ($H_2' = 0.26$) was substantially higher than would be expected for a simple compound transfer (contamination) from resin to bee surfaces ($0.12 < H_2'_M < 0.18$). Moreover, sesquiterpenes are much reduced in the chemical profile of *T. melanocephala* (8%) compared to their collected resins (from which the mixing model would predict a proportion of sesquiterpenes of 58%).

When the two major terpene classes in the bees' chemical profiles (sesquiterpenes and triterpenes) were analyzed separately, bees appeared more similar (sesquiterpenes: $H_2' = 0.17$; triterpenes: $H_2' = 0.16$), suggesting that all bee species largely filter the same subset of terpenoid compounds within a given class.

Within each species, different colonies showed only small differences in their chemical profiles (all $H_2' \leq 0.19$), independent of whether terpenoid or waxy compounds were considered (Table S1).

Resin samples from corbiculae of *T. collina* did not chemically differ from resin samples directly obtained from the collecting tree (Adonis: $R^2 = 0.15$, $p = 0.28$).

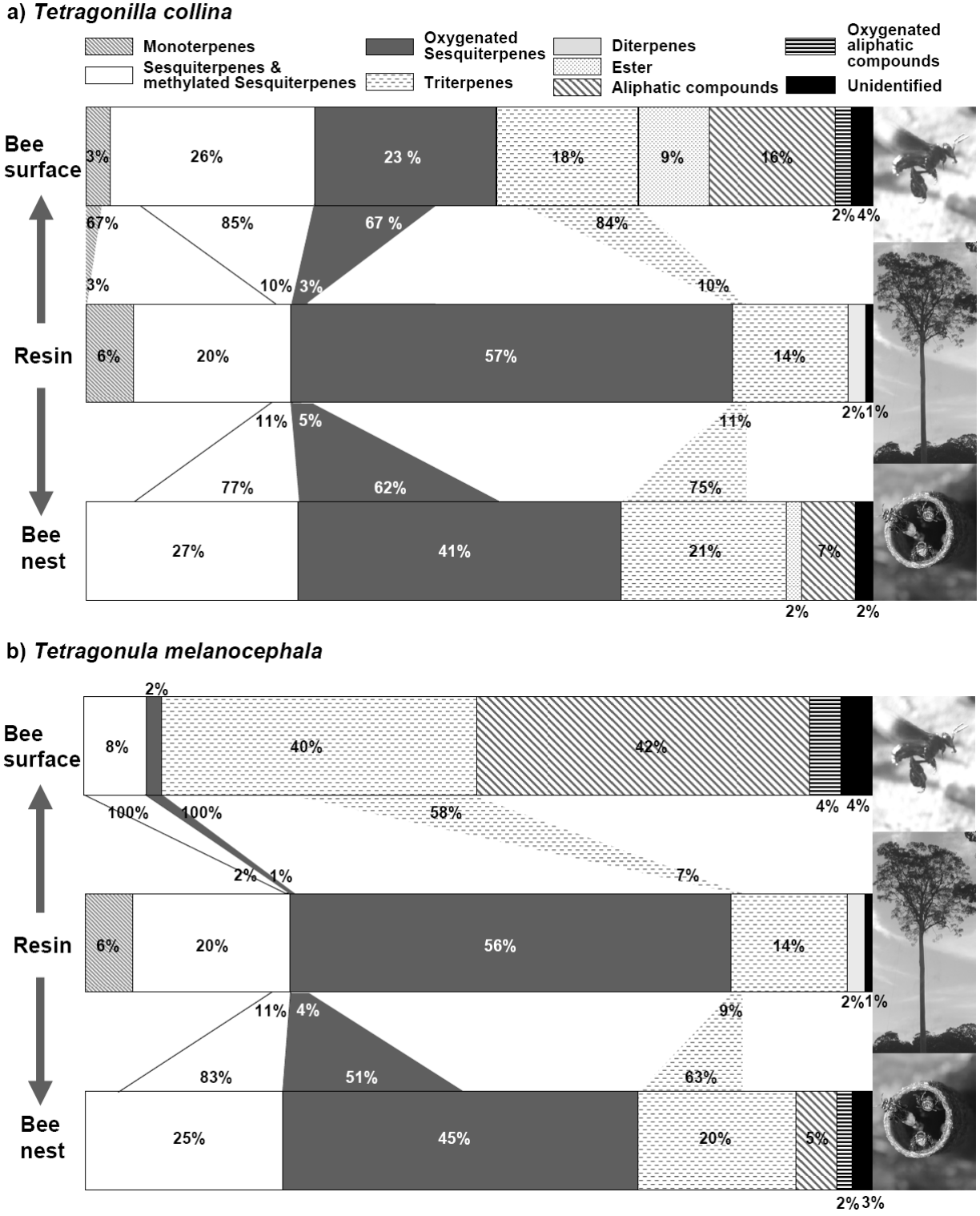


Figure 2. Proportions of compound classes in the chemical profile of all tree resins as well as individuals of two bee species and their nests, and proportions of resin-compounds transferred to the body surface and nest of (a) one *Tetragonilla collina* bees and (b) one *Tetragonula melanocephala* bee.

Chemical diversity in other hymenopterans

If all compounds (including substances that accounted for less than 0.05% of the total peak area) were included in the cumulative diversity analysis, stingless bees showed the highest

diversity of chemical compounds on their body surface (Fig. 3). Moreover, the diversity curve was far from saturation, indicating that the chemical diversity would strongly increase if additional species were included (Fig. 3). By contrast, surface compounds of ants and bumblebees had a relatively low chemical diversity and a lower slope (Fig. 3). Fragrances of euglossine bees showed an intermediate chemical diversity (Fig. 3).

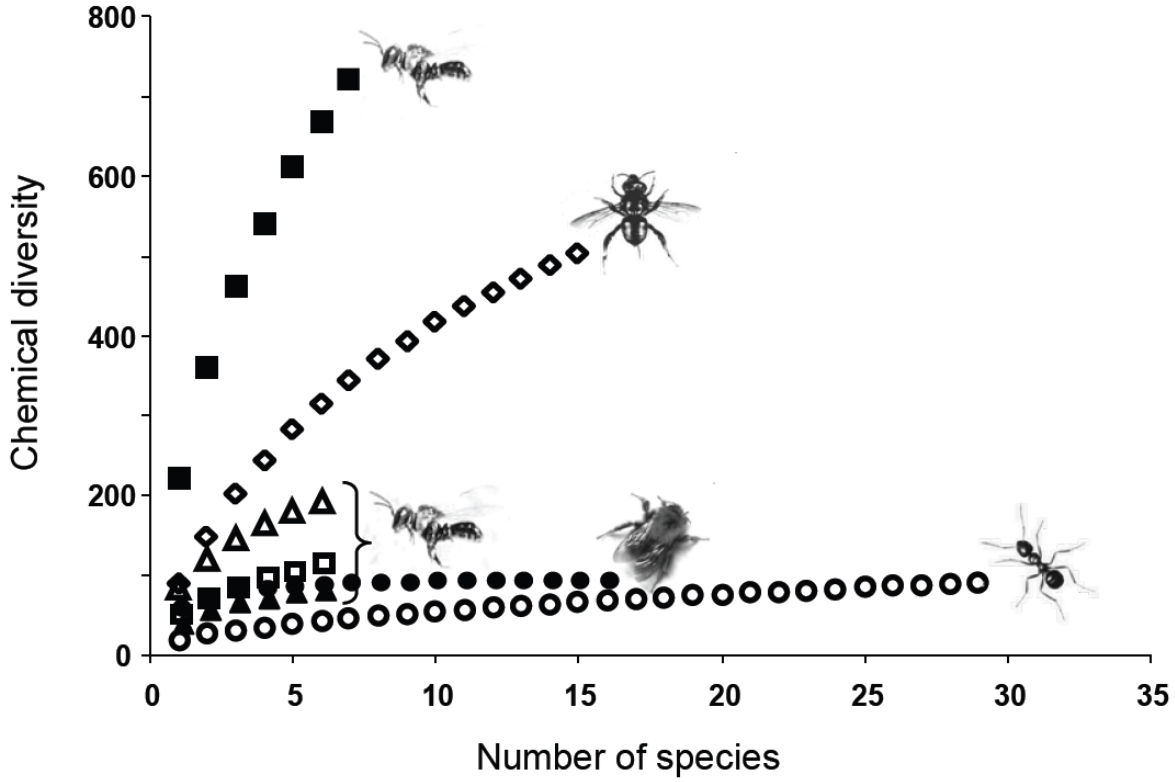


Figure 3. Chemical diversity of the surface profiles from six stingless bee species (squares and triangles), fragrances of 16 euglossine bee species (diamonds) as well as surface profiles of 29 Central European ant species (open circles) and 16 bumblebee species (solid circles). Note that there are two diversity curves for surface profiles of stingless bees, one for all compounds (solid squares) and one for compounds that account for more than 0.05% of the total peak area (open triangles); the reduced compound group is further divided in one curve with only terpenoid compounds (open squares) and one with only non-terpenoid compounds (solid triangles). The bumblebee curve is also based on a reduced dataset using the same threshold and therefore directly comparable to the lower curves of stingless bees, whereas data for ants and euglossine bees have been obtained from other sources (T. Eltz, pers. comm., Martin and Drijfhout 2009).

IX. 5 Discussion

The chemical diversity of insects comprises both genetically determined and environmentally derived compounds with the latter fraction depending on the chemical heterogeneity of environmental sources as well as on how they are collected and selected by the insect. Stingless bees are generalized resin collectors with species-specific compositions of terpenes

derived from plant resins in their cuticular profiles (Leonhardt et al. 2009) and their nest profiles. Most of these terpenes could be directly allocated to resin from trees in their habitat (particularly to resins from the dominant dipterocarp trees) even in the small set of tree species studied, but comprised only a subset of the vast amount of terpenes found in tree resins. Along with their genetically determined non-terpenoid compounds, these cuticular terpenes account for a remarkably high chemical diversity in stingless bees. By contrast, ants and bumblebees – which do not or only rarely include environmentally derived compounds in their chemical profiles (van Zweden et al. 2009) – show a relatively low chemical diversity. The chemical diversity of fragrances from different orchid bee species (Euglossini) is also relatively high because these fragrances comprise a large variety of predominantly plant derived compounds. Male orchid bees show a highly specialized collection behavior when collecting fragrances for their courtship bouquets (Eltz et al. 2005). Here, specialized foraging directly translates into highly specific odor bouquets (H_2' = 0.66; data obtained from Zimmermann et al. 2009), rendering any selective filtering or modification of compounds unnecessary. In contrast to euglossine bees, the cuticular terpenes of stingless bees as well as the slope of their diversity curve cannot be explained by direct or passive compound-transfer from resin to bee surfaces. The restricted number of cuticular terpenes on the bees' body surfaces rather suggests that bees are able to filter and thus limit the number of resin-derived compounds. Moreover, cuticular terpenes of all bee species are deduced from the same small subset of prominent resin-derived terpenes, but can strongly differ in their quantitative and qualitative composition between different bee species (e.g.; sesquiterpenes are present in *T. collina*, but absent in *T. melanocephala*). It is therefore likely that stingless bee species are able to specifically filter resin-derived compounds, with some species excluding whole compound classes, suggesting that filtering of terpenes has a genetic base in these bees. In addition to variation in genetically determined hydrocarbons, bee species-specific terpene profiles due to filtering (H_2' = 0.50) account for a steeper slope of the diversity curve, comparable to the diverse fragrances of euglossine bees, but contrasts with the more similar cuticular profiles of other hymenopterans (e.g.; bumblebees: H_2' = 0.21).

The filtering process appears to take place within the bees' nests. We did not find chemical differences between resin samples collected from bee corbiculae and samples directly collected from the bees' collecting tree, excluding the possibility that bees add specific enzymes during the collecting process. It thus remains to be investigated where and how precisely the bees build up their cuticular terpene profiles (e.g.; by consumption and

subsequent sequestration, as shown for the sawfly larva *Neodiprion sertifer*, (Eisner et al. 1974).

Mixing environmental and genetic compounds does not only result in a higher diversity of compounds, it also increases the amount of functions mediated by them. Genetically determined hydrocarbons are known to play a role in the bees' recognition system (Buchwald and Breed 2005), while resin-derived terpenes in both nest material and chemical profiles protect the bees and their nests against bacteria and fungi (Messer 1985). In a humid and warm environment – like the wet tropics – defense against microbial pathogens and infections of their brood and food storage is crucial for the survival of eusocial bees (Michener 1974; Roubik 1983). Cuticular terpenes also deter predators such as ants and termites (Lehmberg et al. 2008). Therefore, resin-derived terpenes may have primarily functioned as defense against microbes and predators. Due to their species-specific distribution, they could have become involved in intra- and interspecific recognition as has generally been suggested for primarily defensive compounds in arthropods (Blum and Brand 1972).

Overall, resin and resin-derived terpenes play a fundamental and hitherto largely neglected role in the ecology of tropical stingless bees, directly linking the chemical ecology of trees and bees. Resin-derived compounds increase the chemical diversity of stingless bee profiles – which exceeds levels found in other hymenopterans – and simultaneously expand the functional diversity mediated by them.

X. A surprising function of cuticular terpenoids: reduction of interspecific aggression

I have shown that stingless bees from Borneo enrich their surface profiles by terpenes acquired from tree resins. Moreover, the composition of terpenes strongly varies between different bee species, suggesting that these terpenes do not only protect their bearers against predators and microbes, but might further play a role in the inter- and/or intraspecific communication of stingless bees.

This chapter is in press:
Leonhardt SD, Jung L-M, Schmitt T & Blüthgen N – Terpenoids tame aggressors: role of chemicals in stingless bee communal nesting. ***Behavioral Ecology and Sociobiology****.*

X. 1 Abstract

Social insects aggressively defend their nest and surrounding against non-nestmates, which they recognize by an unfamiliar profile of aliphatic hydrocarbons on the cuticle. Prominent exceptions are communal nest aggregations of stingless bees. Stingless bees (Apidae: Meliponini) are also unique in possessing cuticular terpenes which are derived from tree resins and have not yet been reported for any other insect.

We showed experimentally that sesquiterpenes from the body surface of the communal nesting bee *Tetragonilla collina* reduced aggression in otherwise aggressive bees which did not have sesquiterpenes themselves. In the field, bee species nesting in aggregations with *T. collina* often lack sesquiterpenes in their own cuticular profiles. These species show little aggression towards *T. collina*, whereas it can be heavily attacked by non-aggregated species that also possess cuticular sesquiterpenes.

We conclude that appeasement by sesquiterpenes represents a novel mechanism to achieve interspecific tolerance in social insects.

X. 2 Introduction

Territorial animals are rarely found in close proximity to each other, and encounters usually result in aggressive behavior. Colonies of social insects show high intra- as well as inter-specific aggression and may engage in mortal combat when different colonies meet (Wilson 1971; Hölldobler and Wilson 1990; Gloag et al. 2008). This high level of aggression is

triggered by differences in the composition of chemical compounds on the insects' body surfaces which translate into a colony-specific odor or chemical profile (Crozier and Dix 1979; Getz and Chapman 1987; Hölldobler 1995; Vander Meer and Morel 1998). In most social insects studied to date, these chemical compounds comprise hydrocarbons (e.g. non-polar long-chain linear n-alkanes, methyl-branched alkanes or alkenes) and polar compounds with functional groups (e.g. esters, alcohols) (Buckner 1993; Howard 1993). The latter are particularly prominent in social bees (Ayasse et al. 1999; Paulmier et al. 1999; Fröhlich et al. 2000b; Abdalla et al. 2003; Jungnickel et al. 2004; Kerr et al. 2004; Mant et al. 2005; Nunes et al. 2008) and are known to be used as nestmate recognition cues in honeybees (Breed and Stiller 1992). Whereas differences in the cuticular profile trigger aggression among social insects, tolerance is achieved by similar profiles or profiles without compounds involved in recognition (Lacy and Sherman 1983; Vander Meer and Morel 1998). Therefore, chemical mimicry (i.e. a similar profile) or chemical insignificance (no profile) are common strategies of parasites or predators that exploit social insect colonies and thus need to avoid a defensive response (Howard et al. 2001; Lenoir et al. 2001; Cervo et al. 2008; Strohm et al. 2008). Alternatively, glandular compounds are used to appease aggressive behavior in potential host or prey, as e.g. in the slave-making ant *Polyergus rufescens* (Mori et al. 2000; Visicchio et al. 2000). In the Neotropics, the stingless bee *Lestrimelitta limao* uses the monoterpene aldehyde citral in its mandibular glands (Blum 1966) to confuse heterospecifics: they rob colonies of other bee species from the genera *Melipona* and *Trigona* which are disoriented by this terpene (Blum et al. 1970a). In general, terpenes are mainly known as glandular products in several insects, such as termites (Bagnères et al. 1990), ants (Blum and Brand 1972; Morgan et al. 2003), larvae of the sawfly *Neodiprion sertifer* (Eisner et al. 1974), stingless bees (Blum and Brand 1972; Francke et al. 2000; Cruz-Lopez et al. 2001; Patricio et al. 2003; Cruz-Lopez et al. 2005) and other bees (Wheeler et al. 1977; Cane 1986). However, terpenes were also found on the body surface of seven stingless bee species from Borneo (Leonhardt et al. 2009) – a unique feature among social insects studied so far. These terpenes are derived from tree resins regularly collected by the bees to construct and defend their nests (Roubik 1989; Souza et al. 2006; Leonhardt and Blüthgen 2009). The species-specific composition of terpenes on the bees' body surfaces as well as their presence on the bees' wings (Leonhardt et al. 2009) indicates that they do not merely represent contamination by resins. Although each bee species un-specifically collects resin from many different tree species and often from the same wound as other species (Leonhardt and Blüthgen 2009), the species-specific composition of

terpenes is consistent across colonies from different regions, suggesting that bees are able to modify their terpene profiles.

Terpene profiles comprise monoterpenes, sesquiterpenes, and compounds that have been tentatively identified as triterpenes (Leonhardt et al. 2009). The highest variation between species is found for sesquiterpenes, with some bee species possessing multiple sesquiterpenes and others lacking them entirely (Leonhardt et al. 2009). The terpenes appear to have a defensive function (e.g. against ants, Lehmberg et al. 2008), but their species-specific distribution further suggests a role in the bees' communication or recognition system (Leonhardt et al. 2009). In our study, we therefore investigated the role of terpenes (particularly sesquiterpenes) as appeasers of interspecific aggression in stingless bees.

Stingless bees frequently build their nests at or in the base of tree trunks (Roubik 1979; Wille 1983; Roubik 1989; Souza et al. 2006). Communal nesting seems to be particularly common in the Paleotropics (Starr and Sakagami 1987; Salmah et al. 1990; Roubik 1996; Nagamitsu and Inoue 1997; Eltz et al. 2001; 2002) where species can aggregate at particular trees, nesting in association with colonies of the same or of up to three different species (Starr and Sakagami 1987; Salmah et al. 1990; Roubik 1996; Nagamitsu and Inoue 1997; Eltz et al. 2001; 2002; Cameron et al. 2004). Among South-East Asian meliponine species, aggregated nesting is particularly pronounced in *Pariotrigona pendleburyi* and *Tetragonilla collina* (Roubik 1996; Eltz et al. 2001) and can persist for at least ten years (T Eltz, personal communication). Ecological benefits which promote nest aggregation are currently unclear. Limited availability of suitable nest sites could be one possible factor, particularly in disturbed areas where large trees with natural cavities are rare (Eltz et al. 2001). Alternatively, associated nesting between conspecific but unrelated colonies could help virgin queens to quickly locate a large number of unrelated males on their mating flight, thereby significantly decreasing the effects of inbreeding (Cameron et al. 2004) which is particularly pronounced in stingless bees and honeybees due to their sex determination mechanism (Cook and Crozier 1995).

Aggressive encounters between stingless bees are common at different resources and baits (Hubbell and Johnson 1977; Nagamitsu and Inoue 1997), particularly at resin sources (Leonhardt and Blüthgen 2009) – contrasting with the apparent tolerance within nest associations. Our study thus examined whether bees from aggregated colonies differed in their aggressive behavior against other species and colonies from their own aggregation, a different aggregation, or non-aggregated nests. We further tested whether sesquiterpenes may be one

factor enabling communal nesting in paleotropical stingless bees by appeasing aggressive behavior.

X. 3 Methods

Study sites and bees

Data on nest density and the number of bee nests in aggregations were collected at three different field sites in Borneo (Malaysia) from August to November 2008: (1) Danum Valley Conservation Area (DVC: Sabah, 4°55' N 117°40' E, 100 m asl), (2) Kabili Sepilok Reserve (KSR: Sabah, 5°54' N, 118°04' E, 20-120 m asl) with the Rainforest discovery centre (RDC) attached, and (3) Lambir Hills National Park (LHN: Sarawak, 4°20' N and 113°50' E, 150 m asl). Aggression in bees was studied at DVC and KSR/RDC only. All field sites have a typical equatorial rainforest climate with a mean annual temperature of 26 - 30°C and a yearly rainfall of 2600 - 3000 mm (Fox 1973; Sakai et al. 1997). DVC comprises one of the major remaining patches of Sabah's primary lowland dipterocarp rainforest (43 800 ha) (Marsh and Greer 1992). KSR covers an area of 4294 ha of coastal dipterocarp and mangrove forest (Fox 1973) and is surrounded by oil palm plantations. The RDC is a small (148.6 ha) education centre about 2 km west of KSR with secondary and planted vegetation. LHN (Sarawak, 4°20' N and 113°50' E, 150 m asl) comprises 6952 ha of intact mixed-dipterocarp forest.

Eltz (2004) recorded 15 stingless bee species (species and genus names as in Moure 1961) in DVC. Collections of specimens held by the Forestry Research Centre in Sepilok, as well as our own studies (Leonhardt and Blüthgen 2009) suggest 15 to 20 species can be found in KSR and RDC. For LHN, 21 stingless bee species have been described by Inoue and colleagues (1994).

All nest aggregations selected for this study were characterized by a high nest density: nests had at least one neighboring nest less than 50 cm away, and all nests were ≤ 3 m apart from the most distant nests. Aggregations included nests in or beneath tree trunks, but also in walls, posts and along buildings.

Bee sampling

Bees used for behavioral tests were directly caught at their nest entrances by putting a clean transparent plastic bag or container above the nest entrance tube. Ten of the bees caught were immediately used for the behavioral experiments, whereas the other bees were killed and stored in a freezer until needed. We used only bees that had been stored for less than 12 hours.

Behavioral tests

Behavioral experiments were performed in an arena: a plastic Petri dish was placed inversely on gauze spanned over large plastic box, allowing for air exchange from below to minimize accumulation of any pheromones emitted by the bees. Petri dishes and gauze were cleaned after each trial using soapy water, ethanol, and hexane and air-dried to ensure complete volatilization of cleansing solvents.

To test for aggression between colonies, we observed the behavioral responses of one focal living worker towards another worker that had recently been killed in the freezer ('alive against dead experiment'). This design facilitated a detailed record of behavioral responses. In another study with the same bee community (Dworschak and Blüthgen 2010), responses to alive and to dead bees were similar, rendering a merely hygienic responses to carcasses unlikely. The focal bee was directly transferred from a plastic bag or container to the arena and remained undisturbed for about 1 min for habituation. The dead bee was then inserted into the arena and 3 min observation trials started immediately. Ten replicates were performed per colony per trial, using ten different individuals. Further ten individuals of all colonies were tested against bees of their own colony (nestmates) to control for intracolonial aggression (control trials), resulting in overall 620 bees (21 colonies) tested (four bees that died during the testing procedure were excluded from the analysis, Table 1). Both focal and dead bees were tested only once.

We assigned each behavioral response towards the dead bee to one of the following four aggression levels: '1' neutral response (investigation of dead bee with antennae, mandibles closed), '2' slight aggression (open mandibles), and '3' high aggression (biting in extremities, biting off body parts, dragging dead bee around arena). We calculated the proportion of aggressive responses towards dead bees for each focal bee individual i to obtain the aggression level $A_i = (L_2 + L_3) / (L_1 + L_2 + L_3)$, where L_1, L_2 and L_3 are the number of behavioral responses of each aggression level. For all control trials of a colony, the median aggression level A_C was obtained across ten workers. We then assigned each focal bee worker i either as non-aggressive (when $A_i \leq A_C$) or as aggressive (when $A_i > A_C$). We tested for differences in the proportion of aggressive bees between intercolonial trials with associated colonies and intercolonial trials with non-associated colonies using Fisher's exact test. To test whether aggression shown towards dead *T. collina* bees corresponded to the presence of cuticular sesquiterpenes in the species' chemical profiles, we further compared the number of bees showing aggression towards dead *T. collina* between bees with sesquiterpenes (other

than *T. collina*) versus bees without sesquiterpenes using Fisher's exact test. All statistical analyses were performed in R (R-Development-Core-Team 2009).

Effect of sesquiterpenes on intraspecific aggression

To investigate whether terpenes influence aggression in stingless bees, we focused on the behavioral response of *Tetragonula melanocephala*, a bee that can be highly aggressive against conspecific workers from other colonies (non-nestmates, personal observation). We compared their reaction towards dead non-nestmates whose chemical profiles had been modified, versus their response towards non-nestmates with unmodified profiles. While *T. melanocephala* mainly has non-polar hydrocarbons, esters and putative triterpenes in its chemical profile, the chemical profile of *Tetragonilla collina* has large amounts of sesquiterpenes in addition to these three compound classes (Leonhardt et al. 2009). Modifying the chemical profiles of dead *T. melanocephala* bees by coating the bees' surface with (1) *T. collina* extract should thus reveal possible effects of sesquiterpenes on nestmate recognition in *T. melanocephala*. To ensure that these effects were due to sesquiterpenes and not to other compounds in the *T. collina* surface extract, dead *T. melanocephala* bees were also treated with (2) a mixture of commercially available sesquiterpenes and with (3) *Pariotrigona pendleburyi* hexane extract, whose chemical profile comprises the same groups of chemical compounds as *T. melanocephala* and no sesquiterpenes (Leonhardt et al. 2009). *P. pendleburyi* extract thus enabled the modification of the *T. melanocephala* profile by changing the relative amounts of chemical compounds potentially familiar to *T. melanocephala*. Bee extracts were prepared by washing workers in hexane, but only for a maximum of 3-5 min to minimize the compounds of glands being dissolved in hexane. The number of bees per extract was 20 per 100 µl for *T. collina* and 80 per 100 µl for the smaller *P. pendleburyi*. Dead *T. melanocephala* bees were coated with chemical compounds equivalent to the body surface one *T. collina* and four *P. pendleburyi* bees (both ~ 5 µl). The sesquiterpene mixture (2) contained ~0.11% of each of the following terpenes: pure *trans*-caryophyllene, pure α-humulene, pure (-)α -copaene and germacrene (containing trace amounts of further sesquiterpene impurities) (all pure substances were obtained from Sigma-Aldrich, Munich, Germany; germacrene was obtained from the Department of Chemistry at the University of Würzburg); 3µl of the sesquiterpene mixture (equivalent to half of the amount found on a *T. collina* worker) were applied per dead bee. All sesquiterpenes in the mixture were also identified on the body surface of *T. collina* (Leonhardt et al. 2009), but note that *trans*-caryophyllene was only tentatively identified and is therefore not listed in our previous study. Behavioral tests were similar to those described above, except that the same

'focal' bee was tested twice: against a dead bee coated with one of the three extracts (extract trial) and against a dead bee coated only with the same amount of the solvent hexane (control trial). We performed 18 trials for each extract. The order of control and extract trials was randomized, as were the extracts used for each trial. Wilcoxon matched pairs tests were used to test for differences in the aggressive responses shown by 'focal' *T. melanocephala* bees towards dead conspecifics with unmodified chemical profiles (control trials) and dead bees with modified profiles (extract trials).

All dead bees used for testing were extracted in hexane afterwards (5 min). We analyzed the resulting extracts using gas chromatography-mass spectrometry (GC-MS), following the procedure described in Leonhardt et al (2009), to check whether the bees' chemical profiles had effectively been modified by the coating. We further analyzed the chemical profiles of those species that were not collected and analyzed in our previous study (Leonhardt et al. 2009).

X. 4 Results

Nest aggregations

Altogether, we found twelve nest aggregations at our three field sites (five in DVC, six in KSR, and one in LHN), comprising 35 nests (2-12 nests per aggregation). Between two and four different bee species could be found in one aggregation (Fig. 1). Six aggregations were located at the base of large trees in mature (5) or secondary (1) forest, whereas the other six aggregations were found in stone walls, posts or along buildings. Within aggregations, *Tetragonilla collina* was the most common species (Fig. 1) found in 50% of the aggregations (24 nests, Fig. 1). They were associated with other *T. collina* colonies or with up to four different species. Thirteen associated colonies (at three aggregations in total) as well as eight non-associated colonies were tested in the aggression experiments (Table 1).

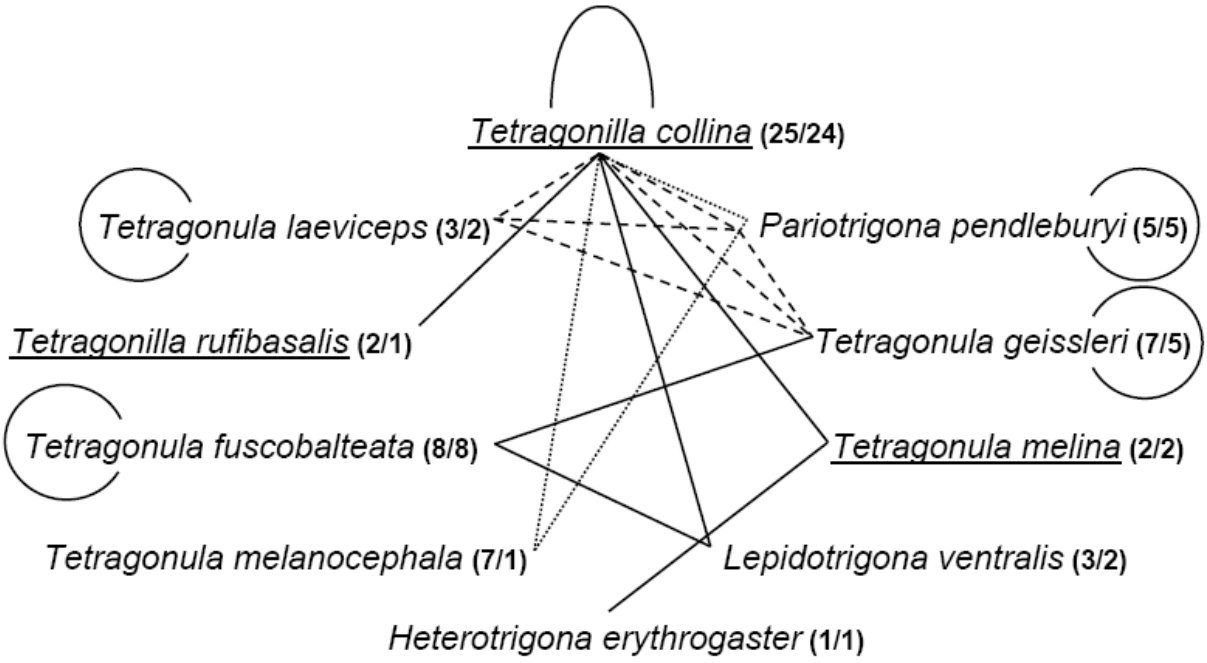


Figure 1. Nest associations between different stingless bee species (lines) or the same species (loops). Numbers in parentheses behind species names provide the number of bee nests found in total / aggregated. Note that one aggregation may comprise several conspecific colonies. Moreover, one aggregation (dotted lines) involved three species, another one (dashed lines) four species. Underlined species possess sesquiterpenes in their chemical profile.

Tolerance within aggregations

Within aggregations, we found very little or no aggression between colonies of both the same and different species (Table 1). A maximum of three (out of ten) 'focal' bees responded aggressively towards dead association-members, but this level of aggression was similar to aggression observed in respective control trials (Table 1). Aggression was significantly higher between all non-associated colonies pooled (62:196 bees were aggressive/non-aggressive; Table 2) than between all associated colonies (14:146, Fisher's exact test: $P < 0.0001$; Table 1). Notably, *T. collina* colonies showed no or only very low aggression against any other colony, irrespective of whether these colonies were from their own, or a foreign aggregation, or solitary nesting (Table 1 & Table 2).

Effect of sesquiterpenes on inter- and intraspecific aggression

Five out of twelve species have considerable concentrations of sesquiterpenes and putative triterpenes in their chemical profiles (besides alkanes, alkenes, methylated alkanes and esters) (Fig. 1 & Table 1 & Table 2, see also Leonhardt et al. 2009). *Lepidotrigona ventralis* only has putative triterpenes in its chemical profile. Therefore, the only two species that were not

found in any aggregation (*O. haematoptera* and *T. binghami*) have sesquiterpenes in their chemical profiles, as does *T. collina*. Notably, bees with sesquiterpenes (other than *T. collina*) showed significantly more aggression (26:24) towards *T. collina* than bees without sesquiterpenes (11:69, Fisher's exact test: $P < 0.0001$).

Table 1. Percent aggressive bees (rows) towards dead bees (columns, indicated by abbreviated species names) for each species combination within associations: numbers in parentheses give number of individuals/colonies tested per species combination; N gives total numbers of individuals/colonies tested. Bold names mark species with sesquiterpenes.

		Aggression against					
	N	Control	*Pp*	***Tc***	***Tr***	*Tg*	*Tm*
Pariotrigona pendleburyi	30/1	0% (10/1)	-	0% (10/1)	-	-	20% (10/1)
Tetragonilla collina	178/8	13% (78/8)	0% (10/1)	15% (60/6)	20% (10/1)	0% (10/1)	0%(10/1)
Tetragonilla rufibasalis	20/1	0% (10/1)	-	10% (10/1)	-	-	-
Tetragonula geissleri group	30/2	0% (20/2)	-	0% (10/1)	-	-	-
Tetragonula melanocephala	30/1	0% (10/1)	0% (10/1)	0% (10/1)	-	-	-

Table 2. Percent aggressive bees (rows) towards dead bees (columns, indicated by abbreviated species names) for each species combination between associations: numbers in parentheses give number of individuals/colonies tested per species combination; N gives total numbers of individuals/colonies tested. Bold names mark species with sesquiterpenes.

		Aggression against							
	N	Control	*Lv*	***Oh***	***Tc***	***Tr***	*Tg*	*Tm*	***Tb***
Lepidotrigona ventralis	20/1	50% (10/1)	-	-	60% (10/1)	-	-	-	-
Odontotrigona haematoptera	20/1	60% (10/1)	-	-	50% (10/1)	-	-	-	-
Tetragonilla collina	206/8	13% (78/8)	0% (10/1)	0% (10/1)	5% (38/4)	0% (20/2)	5% (20/2)	0% (20/2)	0% (10/1)
Tetragonilla rufibasalis	30/2	30% (10/1)	-	-	75% (20/2)	-	-	-	-
Tetragonula geissleri group	80/4	15% (20/2)	-	-	20% (20/2)	-	55% (40/4)	-	-
Tetragonula melanocephala	50/2	35% (20/2)	-	-	10% (20/2)	-	-	90% (10/1)	-
Tetrigona binghami	20/1	50% (10/1)	-	-	90% (10/1)	-	-	-	-

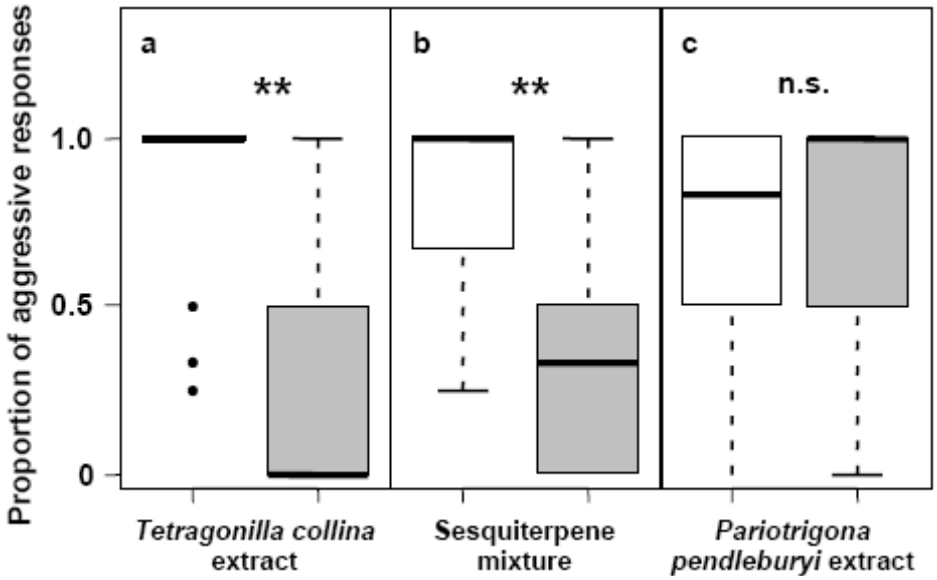


Figure 2. Proportion of aggressive responses of *Tetragonula melanocephala* bees towards non-nestmates. Dead target bees were treated with the solvent hexane for control (white bars) and the following treatments (grey bars): (a) *T. collina* extract, (b) a sesquiterpene mixture, and (c) *Pariotrigona pendleburyi* extract. Significance levels: ** $p < 0.01$, n.s. = not significant ($p > 0.05$).

When dead *T. melanocephala* were coated with either *T. collina* extract or a mixture of sesquiterpenes, 'focal' non-nestmates showed significantly less aggression towards these bees than towards control bees treated with hexane (*T. collina* extract, Wilcoxon matched pairs: $Z = 2.803$, $N_1 = N_2 = 18$, $P = 0.005$, Fig. 2a; sesquiterpene mixture: $Z = 2.803$, $N_1 = N_2 = 18$, $P = 0.005$, Fig. 2b). In most trials, control bees were strongly attacked, whereas bees treated with *T. collina* extract or sesquiterpenes were only antennated. By contrast, dead *T. melanocephala* bees coated with *Pariotrigona pendleburyi* extract were attacked by non-nestmates as forcefully as were control bees (Wilcoxon matched pairs: $Z = 0.700$, $N_1 = N_2 = 18$, $P = 0.48$, Fig. 2c). GC-MS analyses confirmed that the body surfaces of *T. melanocephala* (Fig. 3a) were either supplemented with sesquiterpenes, esters, additional alkanes and additional putative triterpenes when treated with *T. collina* extract (Fig. 3b), with sesquiterpenes when treated with the applied sesquiterpene mixture (Fig. 3c), or with additional alkanes and unknown compounds when treated with the *P. pendleburyi* extract (Fig. 3d).

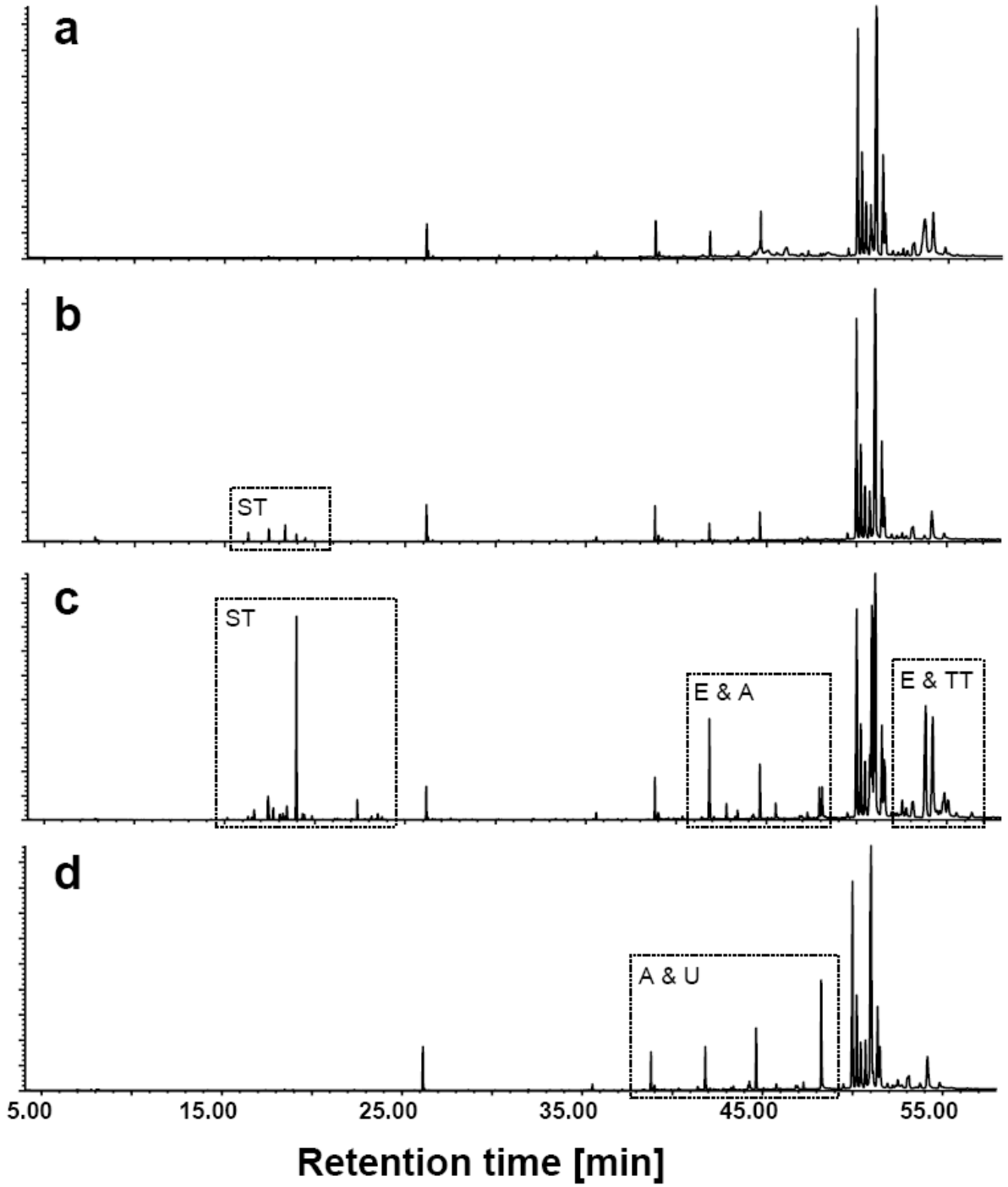


Figure 3. Chromatograms of dead *T. melanocephala* bees used for the coating experiment (each chromatogram represents a single bee individual). The dead bees were treated with (a) the solvent hexane for control, (b) a mixture of sesquiterpenes, (c) *T. collina* extract, and (d) *P. pendleburyi* extract. The treatment successfully altered the chemical profile of *T. melanocephala* by additionally adding compounds or groups of compounds to the compounds specific to *T. melanocephala* (a): ST = sesquiterpenes, TT = putative triterpenes, E = esters, A = alkanes, U = unknown compound(s)

X. 5 Discussion

Stingless bee colonies differ substantially in their aggressiveness. Little aggression was found between colonies nesting in aggregation, while aggressive interactions prevailed between non-associated colonies. Such nest aggregations frequently included one or several *Tetragonilla collina* colonies, the only bee in our study that completely lacked both intra- and interspecific aggression. While being "peaceful" itself, it may control the tolerance of its associated counterparts chemically. Sesquiterpenes in the profile of this bee species were found to significantly reduce aggression in a species without sesquiterpenes and may play a key role in the tolerance within such associations. This is the first report of terpenes as appeasement allomones in social insects.

Most bee species that tolerated *T. collina* lack sesquiterpenes in their profiles, while species that possess sesquiterpenes – like *T. collina* itself – tended to be more aggressive towards *T. collina* (except for *T. collina*). For a potential aggressor, lacking sesquiterpenes on its own, the presence of such compounds may decrease its readiness to attack, perhaps by masking the carrier's chemical profile and, therefore, by failure to recognize this bee as a competitor. Appeasement may explain why *T. collina* can nest communally and is tolerated by other bees such as *Pariotrigona pendleburyi* or *Tetragonula melanocephala*. Associations including several colonies of the same species or more than two different species remain unexplained by our findings and suggest that further mechanisms are involved in the formation and sustenance of stingless bee nest associations. Moreover, *Tetragonilla rufibasalis* possesses sesquiterpenes and was also found in association with *T. collina*. Interestingly, bees of this colony responded less aggressively against its associated *T. collina* colony than towards an unfamiliar colony (Table 1 & Table 2). Such reduced aggression towards a familiar heterospecific colony has been described as "dear enemy effect" and was also observed in vertebrates (Temeles 1994) and ants (Hölldobler and Wilson 2009), where this tolerance may even sustain parabioses, i.e. two species sharing a nest (Menzel et al. 2008b). However, a larger sample size is needed before any conclusions on interspecific tolerance of familiar heterospecifics can be drawn for stingless bees.

Modifying the chemical profile of social insects by adding additional compounds usually provokes aggression among nestmates, as shown in experiments with stingless bees (Couvillon and Ratnieks 2009), honeybees (Breed and Stiller 1992; Wood and Ratnieks 2004), and ants (but see Martin et al. 2008; Guerrieri et al. 2009). One function of chemical compounds on the body surface of social insects is to convey information about the insects' identity to others (Howard 1993; Howard and Blomquist 2005). Insects appear to learn their

current colony odor and use it as a 'template' to which they compare the chemical cues perceived during encounters with other individuals (Crozier and Dix 1979; Lacy and Sherman 1983; Getz and Chapman 1987; Crozier and Pamilo 1996; Hauber and Sherman 2001). If these cues do not match their expected colony odor or 'template' – as is the case for modified chemical profiles – the perceivers will respond aggressively (Lacy and Sherman 1983), although not all compounds used for modifications provoke aggression (Guerrieri et al. 2009). In order to mask themselves, e.g. in the case of social parasites that exploit host colonies, insects can rely on "chemical insignificance" either by simply being odorless when usurping their host (Lenoir et al. 2001), or by expressing only compounds that are less meaningful to their hosts (Cervo et al. 2008). Masking can also be achieved by acquiring a chemical profile that matches the profile of a parasite's host (Strohm et al. 2008). Alternatively or additionally, they can use specific appeasement allomones which reduce aggression in their hosts, as it is the case in the slave-making ant *Polyergus rufescens* that relies on an ester (decyl butyrate) as appeasement allomone (Mori et al. 2000; Visicchio et al. 2000). The honeybee queen mandibular pheromone, a complex blend of substances (4-hydroxy-3-methoxyphenylethanol and methyl *p*-hydroxybenzoate among others) is also known to reduce aggression in young worker bees (Vergoz et al. 2007) by binding to dopamine receptors (Beggs et al. 2007). The use of terpenes as appeasement allomones located on the body surface appears to be unique to stingless bees. Our results fit the appeasement model because the addition of sesquiterpenes, compounds not found in an aggressor species, significantly reduced aggression towards dead non-nestmates of the same species. Thus, sesquiterpenes may act as appeasement allomones, but it is also possible that they led attackers to mistakenly identify treated bees as the peaceful *T. collina* which they may have learned to tolerate. Reduced aggression might then extend to any bee bearing the same or similar sesquiterpenes. Moreover, *T. melanocephala* showed the same response towards a mixture of four sesquiterpenes as it did towards *T. collina*. These sesquiterpenes differed both qualitatively and quantitatively from the natural bouquet of *T. collina*, indicating that appeasement or recognition can be achieved by few such compounds alone.

The key function of terpenes in regulating tolerance among certain bee colonies supplements other functions, unrelated to communication or recognition. It is known that terpenes have strong antimicrobial properties, successfully preventing the growth of bacteria and fungi (Messer 1985; Gershenzon and Dudareva 2007). Defense against microbial pathogens and infections of their brood and food storage is important for the survival of tropical eusocial bees (Michener 1974; Roubik 1983). Moreover, terpenes may protect bees against predators

such as ants (Lehmberg et al. 2008). It is thus possible that the acquisition of terpenes by stingless bees (most likely from tree resins gathered) primarily helped to protect their nests and individuals, while their role in recognition (masking) evolved secondarily.

Summarizing our results, we found that the chemical profile of certain Bornean stingless bees contains sesquiterpenes and that species with sesquiterpenes tended to exhibit more aggression towards *T. collina*, a species that also has sesquiterpenes. Moreover, experimental treatment with sesquiterpenes appeased aggressive bees which lack sesquiterpenes in their own chemical profile. Appeasement by sesquiterpenes thus represents a hitherto unknown mechanism to achieve interspecific tolerance in social insects.

XI. Resin collection and cuticular terpenes in Australian stingless bees

Stingless bees are found in tropical and subtropical regions across four continents, which makes them ideal subjects to examine the environment's influence on resource allocation in these insects as well as the factors involved. In this and the following chapter, I investigate resin collection and the occurrence of cuticular terpenes in another group of paleotropical bees from Australia as well as in neotropical bees from Central America (Costa Rica).

This chapter has been submitted as:
Leonhardt SD & Schmitt T – The cuticular profiles of Australian stingless bees mirror the unusual resin of their resin source (Corymbia torelliana).

XI. 1 Summary

Bees are known to collect pollen and nectar to provide their larvae and themselves with food. That bees, especially the tropical stingless bees (Apidae: Meliponini), also collect plant resins is however often neglected. Resins are used for nest construction, nest maintenance, and nest defense. Some Southeast Asian bee species further transfer resin-derived terpenes to their cuticular profiles. The bees' need for resin is in turn "exploited" by certain plant species which attract bees either for pollination by providing resin in their inflorescences, or for seed dispersal by providing resin in their seed capsules (melittochory). Melittochory is found in the eucalypt tree *Corymbia torelliana*, the resin of which is heavily collected by Australian stingless bees. We analyzed the chemical profiles of eight Australian stingless bee species, comprising two genera (*Tetragonula* and *Austroplebeia*), which are known to collect resin from *C. torelliana* and other tree species. We additionally investigated the chemical composition of resin from *C. torelliana* seed capsules. The surface profiles of the eight bee species analyzed differed significantly in their chemical composition. Similar to Southeast Asian stingless bees, 51% of all compounds on the body surfaces of the five *Tetragonula* species were most likely derived from plant resins. Up to 32 compounds on their body surfaces were identical with compounds from *C. torelliana* resin, suggesting that they directly include *C. torelliana* compounds in their chemical profiles. By contrast, no or only few resinous compounds were found on the body surfaces of the three *Austroplebeia* species sampled. However, one prominent but as yet unknown substance was found in both *C. torelliana* resin and the chemical profiles of all *Tetragonula* and five *Austroplebeia* colonies

sampled, revealing that most colonies (76%) collected resin from *C. torelliana*. *C. torelliana* should thus be considered a common resin source of Australian stingless bees.

XI. 2 Introduction

Plants have evolved highly efficient mechanisms to defend themselves against all kind of potential enemies, such as herbivores, parasites or microbes. One mechanism is the secretion of a highly sticky and often aromatic resin which effectively seals wounds and deters attackers (Langenheim 2003). The deterrent property of resin was assigned to the release of terpenes, some of which were found to be detrimental to insects and other organisms (Gershenzon and Dudareva 2007). However, a considerable number of insects evolved the ability to deal with resin and even utilize it for their own purpose. A well known example are sawfly larvae (*Neodiprion sertifer*) that sequester terpenes from resin of their host plant *Pinus sylvestris* to deter predators (Eisner et al. 1974). *Formica paralugubris* ants use conifer resin pieces to repel pathogens (Christe et al. 2003; Chapuisat et al. 2007) and *Vollenhovia* ants even build their whole nests out of resin (Brühl 2003). To deter ants, the assassin bug *Apiomerus flaviventris* coats its eggs with *Encelia farinosa* resin (Choe and Krust 2007). Tropical stingless bees (Apidae: Meliponini) also exploit the chemical and physiological properties of resin to build their nests and defend their colonies (Roubik 1989; Souza et al. 2006). Resin therefore represents an important factor in the bees' (chemical) ecology. It is even considered a limiting (Howard 1985) plant resource for bees that is crucial for their survival because its antimicrobial properties protect brood and food storage (Michener 1974; Roubik 1983). In turn, several plant species exploit the bees' need for resin. They secrete resin not only to defend themselves against predators, but also to attract bees. For instance, some species of *Dalechampia* (Euphorbiaceae) (Armbruster 1984; Armbruster et al. 2009) and *Clusia* (Clusiaceae) (Mesquita and Franciscon 1995; Lopes and Machado 1998) provide resin as a reward to pollinating bees. Bees are further exploited as seed dispersers (melittochory) by the rainforest eucalypt *Corymbia torelliana* (Wallace and Trueman 1995; Wallace et al. 2008; Wallace and Lee 2009), the tree legume *Zygia racemosa* (Bacelar-Lima et al. 2006), and the epiphyte *Coussapoa asperifolia* (Garcia et al. 1992; Nunez et al. 2008). All these plants have evolved fruits with seeds embedded in resin or resin-like substances which are intensively gathered by bees. While foraging on resin, the seeds get attached to the bees' bodies and are dispersed when they return to their nests.

The use of resin as pollinator reward and the occurrence of melittochory demonstrate that bees and their resin sources can be closely linked. In Borneo, bees use resin and resin-derived

compounds not only to build and defend their nests, but also to enrich their cuticular/chemical profiles (Leonhardt et al. 2009). They collect and directly transfer sesqui- and triterpenes from resin of various dipterocarp trees (and few other tree families) to their body surfaces (chapter IX). Here, sesquiterpenes, which are present in some and absent in other bee species, mediate interspecific tolerance by reducing aggression in those species that lack this particular group of terpenes (chapter X). In Australia, wounds of eucalypt trees appear to be the major resin sources of stingless bees (Helen Wallace, personal communication). Between November and February, they further collect resin from fruits of *C. torelliana* in northern Australia where the tree occurs naturally as well as on the Australian East Coast where it has been introduced (Wallace and Trueman 1995; Wallace et al. 2008; Wallace and Lee 2009).

By analyzing and comparing extracts of cuticular profiles from eight Australian bee species and of resin from *C. torelliana* fruits, we attempted to reveal (a) whether Australian stingless bees have species-specific chemical surface profiles, (b) whether they transfer resin-derived compounds to their body surfaces and (c) whether resin of *C. torelliana* fruits represents one source of such compounds.

XI. 3 Methods

Study sites and sampling

Bees were sampled at different sites all along the Australian East coast (from Sydney in the South to Shiptons flat in the North, Fig. 1). Sampling took place during the early fruiting season of *Corymbia torelliana*, in November and December 2008. Our study sites comprised both sites in subtropical (Sydney – Brisbane) and in tropical (savanna) (Townsville – Kuranda) climate as well as disturbed (cities) and relatively undisturbed (Kuranda and Shiptons flat) areas.

Overall, we sampled 42 colonies, comprising eight different species of two genera (*Austroplebeia* and *Tetragonula*, Fig. 1). Bees were collected from bee hives in the backyards of local beekeepers in Brisbane (6 colonies, 153°2´E, 27°28´S), Elonora (11, 153°27´E, 28°07´S) and Dalby (4, 151°16´E, 27°11´S). In Sydney, bees were collected from hives that belong to the University of Sydney (2, 151°12´E, 33°51´S). In Kuranda (5, 145° 38´E, 16°49´S) and in Shiptons flat (14, 145°13´E, 15°48´S), they were collected from natural nests built in walls of buildings, soil or crevices of tree trunks. We caught only departing foragers by placing a clean clear plastic bag over the bees' nest entrances. In addition to bee samples, resin samples were obtained from *C. torelliana* fruits of four different tree individuals near Walkamin on the Atherton Tableland (145°25´E, 17°07´S).

Extract preparation and fractionation

Foragers caught were killed in a freezer, then transferred to 2 ml sample vials, and washed in hexane, for 3 minutes, to extract surface compounds (chemical profiles). Resin samples were completely dissolved in hexane.

To test whether resin-derived compounds (e.g.; terpenoids) were non-polar or comprised functional groups, we fractionated pooled extracts using 6ml SiOH polypropylene columns (CHROMABOND®, 500mg, Macherey-Nagel, Düren, Germany). Columns were conditioned with pentane before 40µl of bee/resin extract were added. Non-polar and polar fractions of extracts were eluted with three column equivalents of pentane (non-polar) and two column equivalents of dichloromethane (polar), respectively.

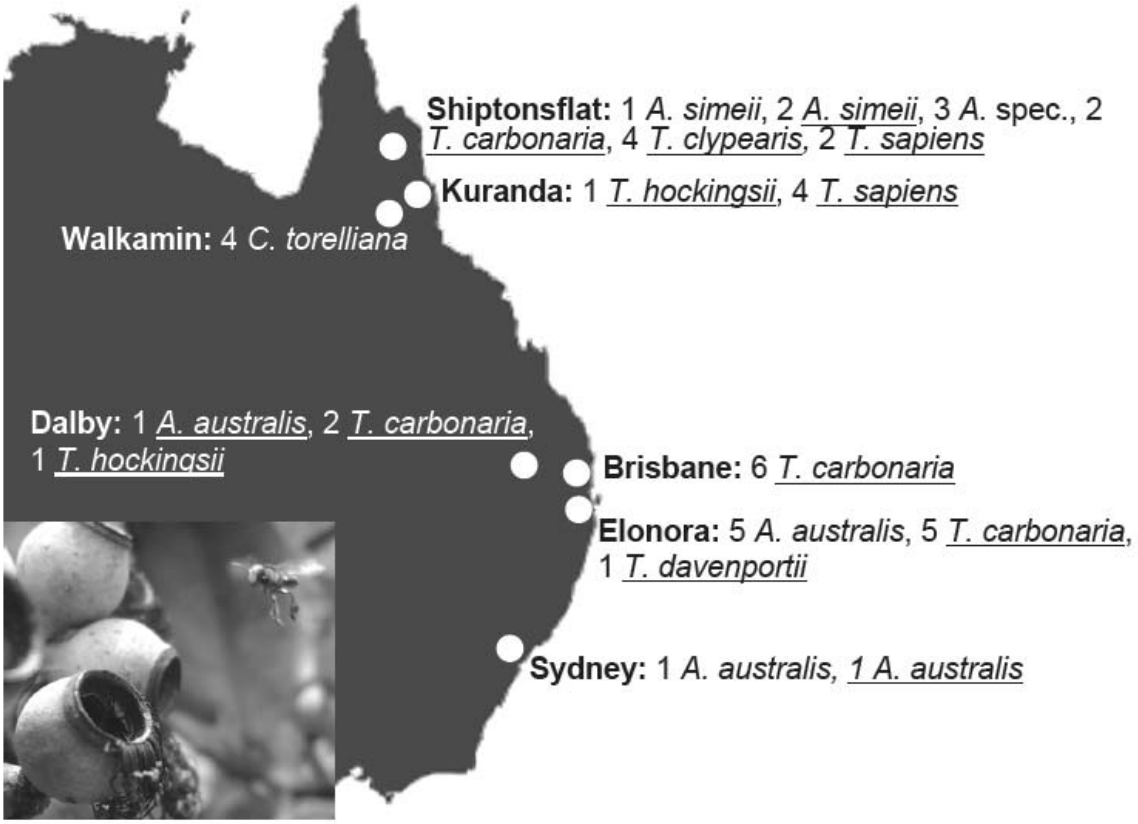


Figure 1. Sites of bee and resin sampling along the Australian East coast: numbers of colonies sampled per species per site are given, underlined species mark species with compounds derived from *Corymbia torelliana* resin in their cuticular profile. Photograph shows *Tetragonula carbonaria* resin forager approaching fruit of *Corymbia torelliana* tree.

Chemical analyses

Compounds found in extracts of bee surfaces and resin samples were characterized by their mass spectra and retention times. Compounds with identical mass spectra and retention times were regarded as the same substance. Three commercially available mass spectra libraries

(Wiley 275, NIST 98 and Adams EO library 2205) were used to determine substance classes with regard to mass spectra and retention times. Identification of alkanes was further confirmed by comparison with synthetic standards and retention indices (Sigma-Aldrick, Munich, Germany). Based on diagnostic ions, aldehydes, alcohols, carboxylic acids and esters were tentatively identified by comparing their mass spectra with mass spectra from libraries. Mono- and sesquiterpenes were identified based on mass spectra and retention indices. If standards were available we additionally compared substances with synthetic standards (Sigma-Aldrich, Munich, Germany). Di- and triterpenes were tentatively determined by their molecular mass, typical diagnostic ions and the range of retention times where they normally elute.

For characterization of compounds in bee and resin extracts we used a Hewlett Packard HP 6890 Series GC System coupled to a Hewlett Packard HP 5973 Mass Selective Detector (Agilent Technologies, Böblingen, Germany). The GC was equipped with a J & W, DB-1 fused silica capillary column (30m x 0.25 mm ID; df = 0.25 µm; J & W, Folsom, CA, USA). Temperature was programmed from 60°C to 300°C with a 5°C/min heating rate. It was held for 10 min at 300°C. Helium was used as carrier gas (constant flow of 1 ml/min). Injection was carried out at 250°C in the splitless mode for 1 min. Electron impact mass spectra (EI-MS) were recorded at an ionization voltage of 70 eV and a source temperature of 230°C. We used the Windows version of the ChemStation software package (Agilent Technologies, Böblingen, Germany) for data acquisition.

Statistical analysis

Comparisons and statistical analyses were based on 197 compounds for bee profiles. Resin of *C. torelliana* seed capsules comprised 59 compounds. We used two-dimensional meta NMDS (non-metric dimensional scaling) based on Bray-Curtis distances on the proportion of each compound that accounted for more than 0.5 % of the total peak area in all samples to produce ordination figures (start configuration: PCoA, 1000 iterations). Proportions of compounds were calculated by dividing the peak area of each compound by the total area of all peaks included in the analysis. “Adonis” tests (R Statistical software 2.9.2, vegan package; command for a randomization-based analysis of dissimilarities) based on the Bray-Curtis distance-matrix were used to reveal chemical differences between different bee species and genera. Separate “Adonis” were performed on the basis of all compounds, only resin-derived compounds (e.g.; terpenes) and only non-resin-derived compounds (e.g.; alkanes, alkenes). Statistical analyses were performed in R (R-Development-Core-Team 2009).

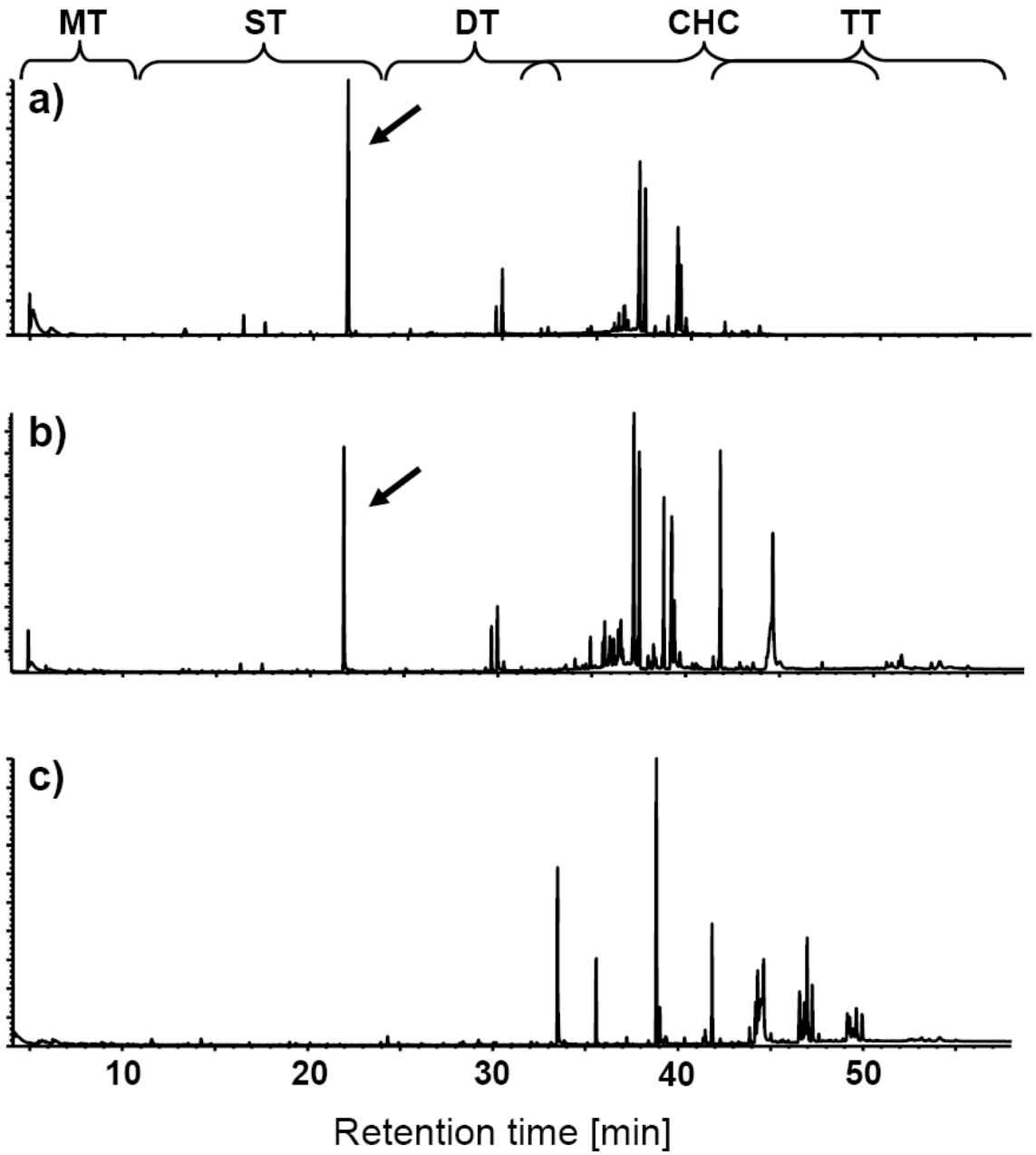


Figure 2. Chromatograms of (a) resin from seed capsules of a *Corymbia torelliana* tree, (b) the body surface of *Tetragonula carbonaria* and (c) the body surface of *Austroplebeia australis*. Time ranges where monoterpenes (MT), sesquiterpenes (ST), diterpenes (DT), most long-chained cuticular hydrocarbons (CHC) and triterpenes (TT) normally elute are indicated. Arrows mark the prominent unknown polar substance ($C_{15}H_{22}O_3$) found in *C. torelliana* resin and in most bee profiles.

XI. 4 Results

Resin chemistry

Overall, 58 compounds were found in resin sampled from *Corymbia torelliana* seed capsules, comprising seven monoterpenes, six sesquiterpenes, 14 potential diterpenes, three potential triterpenes, one ester and several unknown compounds (Fig. 2). The most prominent compound was an unknown polar substance with the molecular formula $C_{15}H_{22}O_3$ (Fig. 2).

This substance is most likely composed of ring-structures which were determined by high resolution mass spectrometry (Till Beuerle, personal communication).

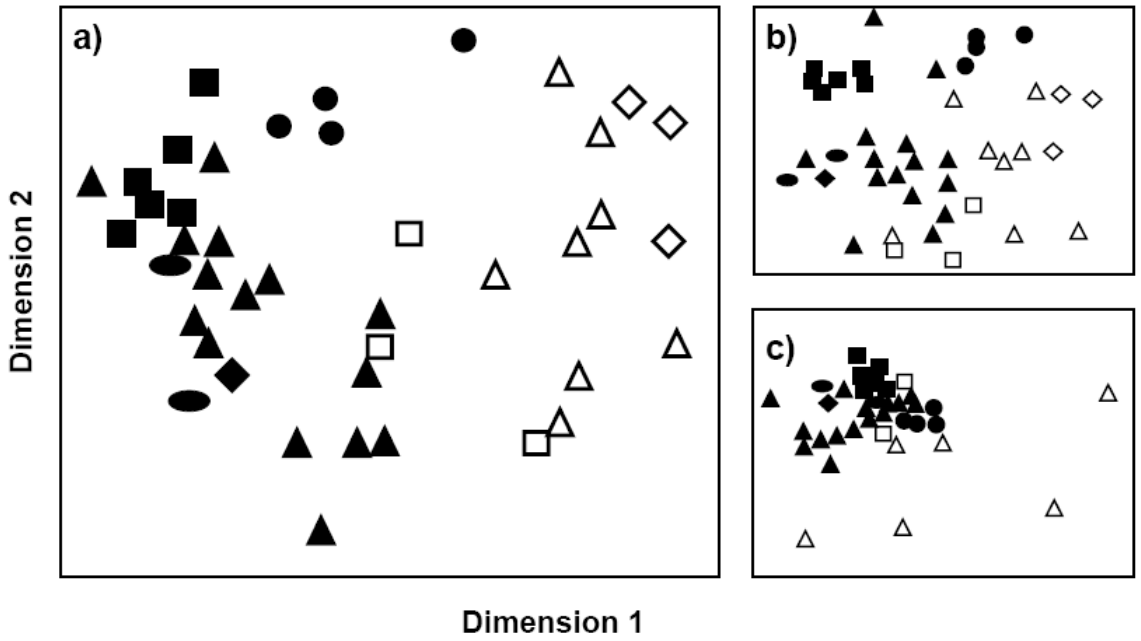


Figure 3. Similarity in the chemical composition of (a) all chemical compounds, (b) only compounds that are not derived from plant resins (genetically determined compounds) and (c) only resin-derived compounds from eight Australian stingless bee species. Different symbols represent different species (each symbol represents one colony): *Tetragonula* species are represented by: closed circles = *T. clypearis*, closed squares = *T. sapiens*, closed triangles = *T. carbonaria*, closed ellipses = *T. hockingsii*, closed diamond = *T. davenportii*; *Austroplebeia* species are represented by: open triangles = *A. australis*, open square = *A. simeii*, open diamond = *A. spec.*

Bee chemistry

Compounds of cuticular profiles from the two Australian stingless bee genera comprised *n*-alkanes, alkenes, alkadienes, methyl-branched alkanes, alcohols, esters, carboxylic acids, as well as mono-, sesqui-, di- and triterpenes and some unknown compounds which are most likely derived from plant resins (Table S2, Fig. 2). Potentially resin-derived compounds made up for 50% of the total number of compounds (Table S2). They were particularly prominent in *Tetragonula*, whereas only three out of 15 *Austroplebeia* colonies (1 *A. australis* and 2 *A. simeii*) had substantial amounts of resin-derived compounds in their cuticular profiles. Moreover, 32 resin-derived compounds (16% of all compounds) could be directly allocated to resin from *C. torelliana* seed capsules (Table S1, Fig. 1, and Fig. 2). All 27 bees from colonies with resin-derived compounds in their chemical profiles had the prominent unknown polar substance ($C_{15}H_{22}O_3$) – mentioned above for *C. torelliana* seed capsule resin (Table S2).

Chemical differences between bee species and genera

Different species could be clearly discriminated based on differences in their chemical profiles (Adonis: all $R^2 = 0.56$, $p < 0.001$, Fig. 3). Discrimination was equally pronounced when the analysis was confined to compounds that could not be allocated to resin and are thus most likely produced by the bees themselves (Adonis: $R^2 = 0.56$, $p < 0.001$, Fig. 3). Discrimination was less pronounced when only resin-derived compounds were included (Adonis: $R^2 = 0.41$, $p < 0.001$, Fig. 3). Notably, when only *C. torelliana* resin-derived compounds were considered, different species still differed in their chemical profiles (Adonis: $R^2 = 0.43$, $p < 0.001$).

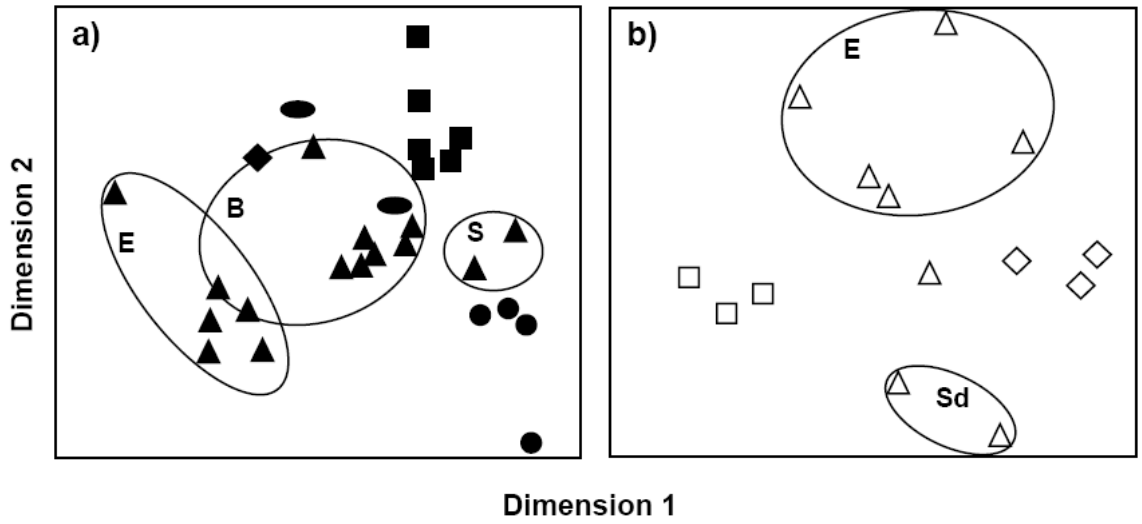


Figure 4. Similarity in the chemical composition of compounds from (a) five *Tetragonula* and (b) three *Austroplebeia* species. Different symbols represent different species (each symbol represents one colony): symbol codes as in Fig. 3. Circles comprise *T. carbonaria* and *A. australis* colonies from the same regions: B = Brisbane, E = Elonora, S = Shiptons flat and Sd = Sydney.

Chemical profiles of the different *Tetragonula* species also varied significantly (Adonis: $R^2 = 0.48$, $p < 0.001$, Fig. 4). Discrimination was slightly more pronounced when resin-derived compounds were excluded (Adonis: $R^2 = 0.54$, $p < 0.001$) than when the analysis was confined to resin-derived compounds (Adonis: $R^2 = 0.40$, $p < 0.001$). *T. davenportii* and *T. hockingsii* were chemically highly similar to *T. carbonaria* (Fig. 4) and the three species could not be distinguished based on their chemical profiles (Adonis: $R^2 = 0.15$, $p = 0.18$).

Chemical profiles of the three *Austroplebeia* species were also significantly different (Adonis: all compounds: $R^2 = 0.38$, $p < 0.001$, Fig. 4; only non resin-derived compounds: $R^2 = 0.38$, $p < 0.001$). Because only three *A. australis* colonies had substantial amounts of resin-derived compounds in their chemical profiles, we did not perform a separate analysis on terpenes.

Note that the chemical profiles of both *T. carbonaria* and *A. australis* colonies from particular regions were in general more similar within than between regions (Adonis: *T. carbonaria*: $R^2 = 0.59$, $p < 0.001$, mean Bray Curtis distance within regions: 0.39 ± 0.17, between regions: 0.56 ± 0.17; *A. australis*: $R^2 = 0.42$, $p = 0.03$, mean Bray Curtis distance within regions: 0.58 ± 0.16, between regions: 0.65 ± 0.11; Fig. 4), indicating that location also impairs the chemical profiles of Australian stingless bees.

XI. 5 Discussion

Similar to their Bornean relatives (Leonhardt et al. 2009), the chemical profiles of Australian stingless bees comprise both self-produced (genetically determined) compounds and compounds derived from plant resins (environmentally derived). The prominence of environmentally derived compounds on the bees' body surfaces is so far unique to stingless bees. It underlines their remarkable ability to handle a highly sticky and toxic plant product and even selectively filter compounds derived from this product to enrich their own chemical ecology. Particularly *Tetragonula* species include compounds of resin in their chemical profiles, thereby increasing the number of chemical compounds (chemical diversity) on their body surface. By contrast, resin-derived compounds were comparatively rare (and partly absent) in *Austroplebeia* species (except *A. simeii*), revealing a strong difference in the chemical ecology of these two genera of stingless bees. The chemical difference may be linked to differences in foraging behavior: Whereas *Tetragonula* species are known to collect resin in relatively large quantities with up to 10 % of resin foragers in a given colony (Wallace and Trueman 1995; Wallace and Lee 2009), resin collection is comparatively rare in *Austroplebeia* (T. Heard personal communication). Moreover, while Australian *Tetragonula* species and all species from Borneo genetically fall within the Indo-Malayan/Austral-Asian stingless bee clade, *Austroplebeia* species more closely resemble their Neotropical/Afrotropical sister clade (Rasmussen and Cameron 2007; 2010). The observed behavioral differences (with regard to resin and resin-derived compounds) therefore conform to the bees' phylogeny.

When the composition of chemical compounds on the body surface of different species were compared, they could be clearly discriminated based on their chemical profiles, especially when analyses were confined to genetically determined compounds. When only resin-derived compounds were included in the analyses, bees could however still be discriminated, indicating that Australian bees are able to influence the composition of environmentally derived compounds on their body surface. This ability was first described for stingless bees

from Borneo (Leonhardt et al. 2009). It results in a species-specific distribution of resin-derived compounds (Leonhardt et al. 2009) as well as in an increased chemical heterogeneity of bee profiles, thereby likely extending the number of potential functions mediated by them. Notably, statistical discrimination remained even when the analysis was confined to compounds derived from *C. torelliana* resin, suggesting that Australian stingless bees (particularly *Tetragonula* species) qualitatively and/or quantitatively differ in the amount of compounds acquired from a single eucalypt tree.

Hexane extracts of resin from *C. torelliana* seed capsules were dominated by one prominent, but hitherto unknown, polar substance ($C_{15}H_{22}O_3$). This substance was also found in the chemical profiles of 32 bee colonies comprising all (but one *Austroplebeia*) species. Because of its unique chemical structure this substance can be used as a "chemical indicator" for bees that collect resin from *C. torelliana* seed capsules. However, the chemical profiles of Australian stingless bees comprised further compounds that are most likely derived from plant resins other than *C. torelliana* (e.g.; triterpenes), suggesting that Australian bees collect resin from a broad range of plant species. Moreover, resin from *C. torelliana* is only available for a relatively short period of up to 4 months while the trees are fruiting. It thus remains to be investigated which tree species Australian stingless bees visit for resin collection, besides *C. torelliana*, and how they compensate for the seasonal fluctuation of this chemically prominent resin source.

XII. Resin collection and cuticular terpenes in neotropical stingless bees

XII. 1 Summary

The diversity of stingless bees in the Neotropics is at least three times higher than in the Paleotropics. Although several studies have analyzed the chemical profiles of stingless bees from Central and South America, they revealed no cuticular terpenes, suggesting that cuticular terpenes are unique to paleotropical bees. To investigate whether cuticular terpenes were actually absent in neotropical bees, I collected bees from 78 colonies (27 species) in Costa Rica. To compare the resin foraging behavior between neotropical and paleotropical bees, I further observed resin foraging at nest entrances of seven neotropical bee species. I found that, in Costa Rica, fewer foragers were engaged in resin collection and that fewer species had cuticular terpenes compared to species from Borneo. However, similar to Borneo, Costa Rican bees were highly opportunistic resin foragers and collected resin from a broad set of trees. Moreover, species with cuticular terpenes varied in the quantity and quality of groups of terpenes present in their chemical profiles, suggesting that they are also able to influence their terpene profiles. Although triterpenes represented the prominent terpene group on both sides of the world, the diversity of resin-derived compounds was overall higher in Costa Rica than in Borneo, comprising diterpenes and several unknown compounds groups in addition to mono-, sesqui-, and triterpenes. Likewise, the diversity of cuticular terpenes in chemical profiles of Costa Rican bees exceeded those of bees from Borneo.

XII. 2 Introduction

The species diversity of paleotropical stingless bees is far exceeded by the number of species that occur in the Neotropics (Michener 2000). Likewise, the number of studies performed on paleotropical stingless bees is far exceeded by the number of studies on neotropical species. Although neotropical stingless bees have been investigated more thoroughly than their paleotropical sister group, studies on resin collection are equally rare on both sides of the globe. However, multiple studies analyzed the chemical composition of cuticular profiles and gland contents in neotropical stingless bees (Da Cruz Landim 1967; Engels et al. 1990; Breed and Page 1991; Suka and Inoue 1993; Suka et al. 1994; Francke et al. 2000; Cruz-Lopez et al. 2001; Abdalla et al. 2003; Patricio et al. 2003; Jungnickel et al. 2004; Buchwald and Breed

2005; Abdalla 2006; Schorkopf et al. 2009), disclosing that terpenoid compounds are commonly found in glands of the species analyzed, but absent from their body surfaces.

To reveal whether neotropical stingless bees also have cuticular terpenes, I collected bee specimens from eight different field sites in Costa Rica and analyzed their surface profiles using the same method and criteria as described for paleotropical species. I further observed resin intake at nest entrances of seven bee species to see whether these species showed a resin collection behavior similar to or different from paleotropical bees. To find out which tree species are visited by neotropical bees I observed bees collecting resin from tree wounds in the forest. In addition to my own observations, I received information on the identity of tree resins visited by bees from Costa Rican beekeepers. I collected and analyzed resin samples from those tree species as well as from trees where I observed resin foragers myself.

XII. 3. Methods

Study sites

Specimen collections and observations were performed in June 2009. Bees were collected at eight sites in Costa Rica: Atenas (Valle Central: 9°58’ N, 84°22’ W, 700 m asl), Santa Elena and Santa Fe as well as the Finca de Bosques Verdes (close to San Vito (Puntarenas): 8°49’ N, 82°58’ W, 980 m asl), La Gamba (field station (Puntarenas): 8°42’ N, W 83°12’ W, 70 m asl), Parque Nacional Manuel Antonio (Puntarenas: 9°22’ N, 84°08’ W), Reserva Natural Monte Alto close to Hojancha (Guanacaste: 10°00’ N, 85°24’ W, 480 – 833 m asl) and Santa Cruz (Guanacaste: 10°49’ N, 85°35’ W, 63 m asl). Observations of resource intake at nests of overall seven species were performed in Atenas and La Gamba.

Observations at nest entrances

One colony of each of the following species, *Cephalotrigona zexmeniae*, *Melipona beechei*, *Paratrigona opaca*, *Scaptotrigona pectoralis*, *Tetragonisca angustula*, *Tetragona ziegleri* and *Trigona fulviventris*, was observed 2-3 times a day for at least 4 consecutive days. I recorded the number of foragers returning with resin, pollen, nectar as well as both nectar and pollen and compared the proportion of resin foragers between different species. To analyze the degree of specialization across species, I further noted the color spectra of resins collected.

Chemical analyses

Between one and 14 individuals were collected from overall 78 colonies, comprising 27 species (Table 1). Except for bees from two colonies which were collected at foraging sites (*Partamona orizabaensis* and *Trigona fuscipennis*) all individuals were caught at their nests,

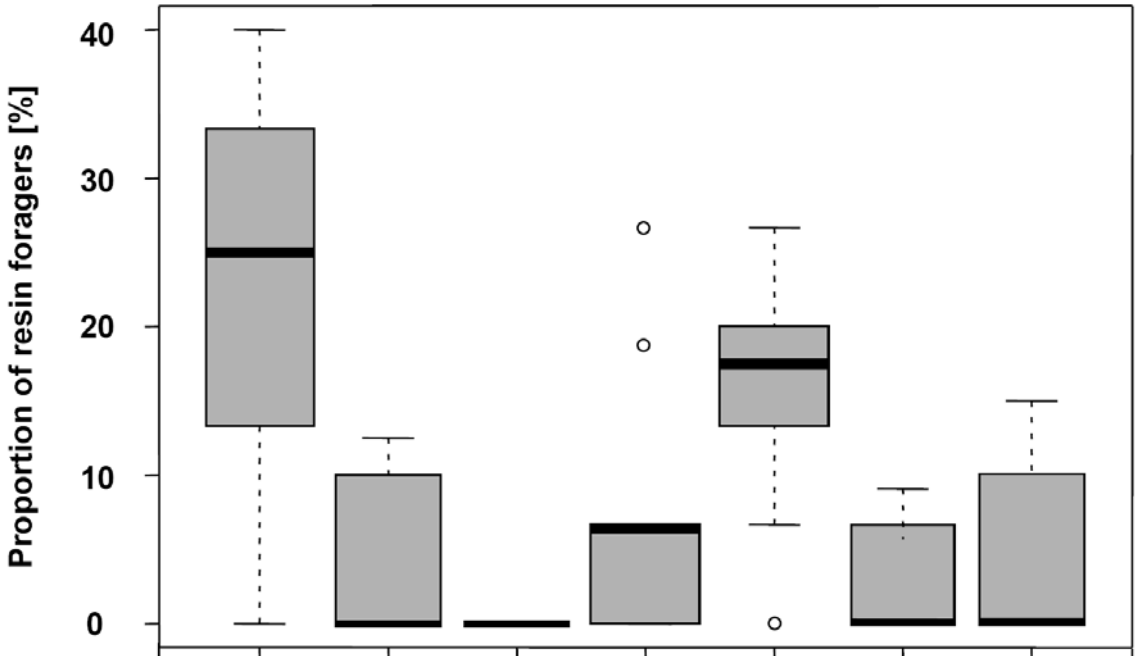
using a clean clear plastic bag attached to the nests' entrances. Bees were killed in a freezer and washed in pure hexane for a maximum of 3 min to prevent extraction of gland compounds. In addition to bees, nest material from each colony was obtained from nest entrances. I further collected samples of tree resins from resin wounds. Bee, nest and resin hexane extracts were analyzed by GC-MS using the same method and criteria as for paleotropical and Australian samples (see chapter VI). Compounds were characterized by their mass spectra and retention times using the same libraries as described in chapter VI and – wherever possible – by comparison with purchased standards.

XII. 4 Results

Resin collection at nest entrances

Across all seven bee species, resin intake was relatively low (0-40 % resin foragers, Fig. 1) compared with the number of pollen (0-67 %) or nectar (13-100 %) foragers. Different species differed significantly in their resin intake ($\chi^2 = 29.46$, $p < 0.001$; Fig. 1). The highest proportions of resin foragers were observed at nest entrances of *Cephalotrigona zexmeniae* and *Tetragonisca angustula*, whereas not a single resin forager was caught at the nest entrance of *Paratrigona opaca* (Fig. 1). All species tended to collect more resin in the morning (6-10 am) than around noon (10 am - 2 pm) ($\chi^2 = 2.84$, $p = 0.09$). Afternoon and evening resin collection could barely be observed due to frequent rainfall.

The seven bee species collected a similar range of resin colors, indicating a generalistic resin collection behavior ($H_2' = 0.32$). Only *T. ziegleri* showed a more specialist collection behavior with regard to resin colors (Fig. 1).



	C. zexmeniae	*M. beechei*	*P. opaca*	*S. pectoralis*	*T. angustula*	*T. fulviventris*	*T. ziegleri*
N resin foragers	40	3	0	10	23	4	6
N foragers	115	97	55	117	150	129	115
N resin colors	9	3	0	3	8	2	5
di' (resin)	0.28	0.30	-	0.39	0.34	0.30	0.54

Figure 1. Percentages of resin forages from seven neotropical stingless bee species. Total numbers (N) of foragers and resin foragers as well as of resin colors collected by each colony during the observation period and their degree of specialization with regard to these resin colors (*di'*) are given below. Underlined species mark species with cuticular terpenes.

Resin collection at trees

Neotropical stingless bees visit a large variety of different tree species and families for resin collection. Resin foragers were observed at wounds of two fig trees (*Ficus* spec., Moraceae), one *Spondias purpurea* tree (Anacardiaceae), one unidentified legume (Fabaceae) as well as one unidentified conifer tree. Besides, stingless bees are known to collect resin from *Hymenea coubaril* (Fabaceae), *Roton draco* (Euphorbiaceae), *Bursera simaruba* (Burseraceae), *Ocotea veraguensis* (Lauraceae), mango (*Mangifera* spec.) trees (Anacardiaceae) as well as various fig trees (Moraceae).

Chemical profiles of bees, nests and tree resins

Eleven out of 27 (41 %) Costa Rican stingless bee species had terpenoid compounds on their body surface (Table 1). Here, triterpenes represented the dominant terpene group, whereas

mono-, sesqui- and diterpenes are found less frequently (Table 1). All species had non-polar long-chained aliphatic hydrocarbons (mainly alkanes and alkenes). Three species further had hydrocarbons with functional groups (esters, aldehydes and/or alcohols) which were unlikely to be derived from glands (Table 1). Bees from different colonies of the same species occasionally varied in their surface profiles, particularly with regard to the amount of mono- and sesquiterpenes (Table 1).

Bee species with terpenes on their body surface tended to have terpenes in their nest material (Table 1). Here, triterpenes also represented the dominant compound group and were found in nests of 19 bee species sampled. Nest material of nearly all species further comprised non-polar aliphatic hydrocarbons and occasionally hydrocarbons with functional groups, but the amount of non-terpenoid hydrocarbons was mostly exceeded by the amount of triterpenes (Table 1). Mono- and sesquiterpenes were particularly prominent in nests of *Ptilotrigona occidentalis* and *Plebeia jatiformis* (Table 1).

Tree resins known or observed to be collected by bees also comprised mono-, sesqui-, di- and triterpenes (Fig. 2) as well as some unknown compounds. However, quality and quantity of different terpene groups strongly differed across resins from different tree species. For instance, triterpenes were the prominent (and often only) terpene group in *Ficus* trees, whereas resin of a conifer tree comprised monoterpenes, sesquiterpenes and diterpene acids in about equal quantities (Fig. 2). Moreover, resins of *Hymenea coubaril* and *Ocotea veraguensis* comprised only sesqui- and diterpenes or solely sesquiterpenes, respectively.

Table 1. Compound classes in chemical profiles of body surfaces and nests from 80 colonies of 27 neotropical species: N = number of bees sampled per colony, MT = monoterpenes, ST = sesquiterpenes, DT = diterpenes, TT = triterpenes, A = non-polar aliphatic compounds, AO = aliphatic compounds with functional groups. Bold signs indicate compounds that are most likely from cephalic glands; an asterisk marks species/colonies that also have flavonoid compounds in their surface profiles.

	Species	**Surface profiles**							**Nest profiles**					
		N	**MT**	**ST**	**DT**	**TT**	**A**	**AO**	**Nest part**	**MT**	**ST**	**DT**	**TT**	**A**	**AO**
1	*Cephalotrigona zexmeniae*	4	0	0	0	++	+	(+)	entrance	0	0	0	++	+	(+)
2	*Cephalotrigona zexmeniae*	3	0	0	0	++	+	(+)		-	-	-	-	-	-
3	*Cephalotrigona zexmeniae*	5	0	0	0	++	+	(+)		-	-	-	-	-	-
4	*Dolichotrigona schultessi*	7	0	0	0	0	++	0		-	-	-	-	-	-
5	*Dolichotrigona schultessi*	7	0	0	0	0	++	0		-	-	-	-	-	-
6	*Frieseomelitta paupera*	8	+	+	+	++	+	0	entrance	+	(+)	0	++	+	0
7	*Geotrigona spec.*	5	0	(+)	+	++	+	0		-	-	-	-	-	-
8	*Geotrigona lutzi*	5	(+)	(+)	+	(+)	+	0		-	-	-	-	-	-
9	*Geotrigona lutzi*	5	0	(+)	+	0	++	0		-	-	-	-	-	-
10	*Lestrimelitta spec.*	8	**+**	0	0	0	+	0	entrance	0	0	(+)	++	+	+
11	*Melipona beechei*	1	0	0	0	0	+	0		-	-	-	-	-	-
12	*Melipona beechei*	2	0	0	0	0	+	0		-	-	-	-	-	-
13	*Melipona beechei*	2	0	0	0	0	+	0		-	-	-	-	-	-
14	*Melipona costaricasensis*	2	0	0	0	0	+	0	pillar	0	+	0	+	+	0
15	*Melipona costaricasensis*	3	+	(+)	(+)	+	+	0	pillar	0	0	0	+	+	0
16	*Nannotrigona spec.*	6	0	0	+	0	+	0	entrance	0	0	0	++	+	(+)
17	*Nannotrigona perilampoides*	7	0	0	0	0	+	0	entrance	0	0	0	+	+	0
18	*Nannotrigona perilampoides*	9	0	0	0	0	+	0	entrance	0	0	0	++	+	0
19	*Oxytrigona mellicolor*	6	0	0	0	0	+	**+**		-	-	-	-	-	-
20	*Oxytrigona mellicolor*	6	0	0	0	0	+	**+**		-	-	-	-	-	-
21	*Oxytrigona mellicolor*	7	0	0	0	0	+	**+**		-	-	-	-	-	-
22	*Oxytrigona mellicolor*	7	0	0	0	0	+	**(+)**		-	-	-	-	-	-
23	*Paratrigona opaca*	6	0	0	0	0	+	0		-	-	-	-	-	-
24	*Paratrigona opaca*	7	0	0	0	0	+	0		-	-	-	-	-	-
25	*Paratrigona opaca*	6	0	0	0	0	+	0		-	-	-	-	-	-
26	*Partamona orizabaensis*	12	0	0	0	(+)	+	0		-	-	-	-	-	-
27	*Partamona orizabaensis*	5	0	0	0	0	+	0		-	-	-	-	-	-
28	*Partamona orizabaensis*	6	0	0	0	0	+	0		-	-	-	-	-	-
29	*Partamona orizabaensis*	7	0	0	0	0	+	0		-	-	-	-	-	-
30	*Partamona orizabaensis*	8	0	0	0	0	+	0		-	-	-	-	-	-
31	*Partamona orizabaensis*	8	0	0	0	(+)	+	**+**		-	-	-	-	-	-
32	*Partamona orizabaensis*	3	0	0	0	0	+	0		-	-	-	-	-	-
33	*Partamona orizabaensis*	4	0	0	0	0	+	0	entrance	0	0	0	+	+	(+)
34	*Partamona orizabaensis*	6	0	0	0	0	+	0	entrance	0	0	(+)	+	+	0
35	*Partamona spec.*	6	0	++	0	0	+	0		-	-	-	-	-	-
36	*Plebeia jatiformis*	11	0	+	0	0	+	0	pillar	+	++	+	+	+	0
									brood cell	0	0	0	(+)	+	0

0 no compounds detected
(+) trace amounts of compounds
+ several compounds detected
++ dominant compound group

Table 1. continued.

	Species	Surface profiles							Nest profiles					
		N	MT	ST	DT	TT	A	AO	Nest part	MT	ST	DT	TT	A	AO
37	*Plebeia pulchra*	5	0	0	0	(+)	+	0	entrance	0	0	0	++	+	0
38	*Ptilotrigona occidentalis*	6	+	+	+	+	+	0	entrance	+	+	(+)	++	(+)	0
39	*Ptilotrigona occidentalis**	7	+	+	+	+	+	0	entrance	+	+	+	+	+	0
40	*Ptilotrigona occidentalis*	8	+	+	+	0	+	0	entrance	+	+	+	+	+	0
41	*Scaptotrigona pectoralis*	10	0	0	0	0	+	0		-	-	-	-	-	-
42	*Scaptotrigona pectoralis*	5	0	0	0	0	+	0		-	-	-	-	-	-
43	*Scaptotrigona pectoralis*	6	0	0	0	0	+	0	entrance	0	0	0	++	+	(+)
44	*Scaptotrigona pectoralis*	8	0	0	0	0	+	**+**	entrance	0	0	0	++	+	(+)
45	*Scaptotrigona pectoralis*	8	0	0	0	0	+	**(+)**		-	-	-	-	-	-
46	*Scaptotrigona pectoralis*	12	0	0	0	0	+	**+**	entrance	0	0	0	++	+	(+)
47	*Scaptotrigona pectoralis*	5	0	0	0	0	+	0		-	-	-	-	-	-
48	*Scaptotrigona pectoralis*	4	0	0	0	0	+	**+**	entrance	0	(+)	0	++	+	(+)
49	*Scaptotrigona pectoralis*	6	0	0	0	+	+	0	entrance	0	0	0	++	+	0
50	*Scaptotrigona subobscuripennis*	4	0	0	0	0	+	**+**	entrance	0	0	0	++	+	(+)
51	*Scaptotrigona subobscuripennis*	4	0	0	0	0	+	**+**	entrance	0	+	0	++	+	(+)
52	*Scaptotrigona subobscuripennis*	5	0	0	0	0	+	**+**		-	-	-	-	-	-
54	*Scaura spec.*	5	0	0	0	0	+	0	entrance	0	(+)	0	+	+	0
55	*Tetragona ziegleri**	10	+	+	+	++	+	+	entrance	0	+	+	+	+	0
56	*Tetragona ziegleri**	5	+	+	+	++	+	+		-	-	-	-	-	-
57	*Tetragona ziegleri**	6	(+)	+	+	++	+	+		-	-	-	-	-	-
58	*Tetragona ziegleri**	6	(+)	+	+	++	+	+	entrance	0	0	0	+	+	(+)
59	*Tetragonisca angustula*	10	0	(+)	+	+	+	0		-	-	-	-	-	-
60	*Tetragonisca angustula*	10	0	0	0	++	+	0		-	-	-	-	-	-
61	*Tetragonisca angustula*	7	0	0	0	+	+	0	entrance	0	0	0	+	++	0
62	*Tetragonisca angustula*	8	0	0	0	++	+	0	entrance	0	0	(+)	(+)	++	0
63	*Tetragonisca angustula*	13	(+)	(+)	+	+	+	0		-	-	-	-	-	-
64	*Tetragonisca angustula*	9	0	(+)	+	+	+	0	entrance	0	0	0	+	++	0
65	*Tetragonisca angustula**	14	0	0	+	++	+	0	entrance	0	0	(+)	(+)	++	0
66	*Tetragonisca angustula*	8	0	(+)	+	++	+	0		-	-	-	-	-	-
67	*Tetragonisca buchwaldi**	10	0	(+)	0	+	+	0	entrance	0	0	0	+	+	0
68	*Tetragonula perangulata*	3	+	+	(+)	+	+	+	entrance	(+)	+	(+)	++	+	0
69	*Tetragonula perangulata*	3	+	+	(+)	+	+	+	entrance	(+)	+	(+)	++	+	0
70	*Trigona corvina*	4	0	0	0	0	+	0		-	-	-	-	-	-
71	*Trigona fulviventris**	2	**+**	+	+	+	+	0		-	-	-	-	-	-
72	*Trigona fulviventris*	7	**+**	(+)	+	+	+	**+**		-	-	-	-	-	-
73	*Trigona fulviventris*	6	**(+)**	(+)	0	+	+	**+**	entrance	0	++	+	+	+	(+)
74	*Trigona fulviventris**	6	**(+)**	(+)	0	+	+	**+**	entrance	+	+	0	++	(+)	0
75	*Trigona fulviventris*	6	**+**	(+)	+	+	+	0		-	-	-	-	-	-
76	*Trigona fulviventris**	8	**+**	(+)	(+)	+	+	0		-	-	-	-	-	-
77	*Trigona fulviventris**	3	**+**	+	+	+	+	0	entrance	(+)	(+)	0	+	+	+
78	*Trigona fuscipennis**	10	0	0	0	++	+	0		-	-	-	-	-	-
79	*Trigona fuscipennis**	9	0	0	0	++	+	0	entrance	0	0	(+)	++	+	0
80	*Trigona sylvestriana*	2	0	0	0	+	+	0		-	-	-	-	-	-

0 no compounds detected
(+) trace amounts of compounds
+ several compunds detected
++ dominant compound group

XII. 5 Discussion

Similar to Bornean stingless bees, different bee species in Costa Rica strongly differed in their resin intake. However, none of the colonies investigated collected such high amounts of resin as was observed in some *Tetragonilla collina* colonies of Borneo (Leonhardt and Blüthgen 2009). Resin intake tended to be highest in the morning which contrasts with observations in Borneo where resin collection was most pronounced in the afternoon (Leonhardt and Blüthgen 2009). In Costa Rica, however, frequent afternoon rainfall prevented colonies from foraging. Foraging patterns may thus be different at other times of the year with lower precipitation.

Analysis of specialization with regard to resin colors revealed a generalist collection behavior which corresponds to findings in Bornean stingless bees (Leonhardt and Blüthgen 2009). Similar to their paleotropical sisters, Costa Rican bees showed no species-specific preference for a particular resin color, but collected a relatively broad range of resin (color)s.

Eleven of the 27 Costa Rican stingless bee species studied had cuticular terpenoids. This finding contrasts with previous studies on chemical profiles of neotropical stingless bees which revealed non-polar aliphatic hydrocarbons as main substance class, but listed no or only few terpenes (Abdalla et al. 2003; Jungnickel et al. 2004; Kerr et al. 2004; Nunes et al. 2008; 2009a; 2009b; but see Pianaro et al. 2009). We found triterpenes to be the most prominent terpene group in chemical profiles of both the bees' surfaces and their nests. Such dominance of triterpenes was also found in paleotropical stingless bees (Leonhardt et al. 2009). However, compared to paleotropical stingless bees – particularly from Borneo where nearly 100% of the species analyzed had terpenes on their body surfaces (Leonhardt et al. 2009) – only 41 % of the neotropical species studied had cuticular terpenes. Moreover, their body surfaces did not only comprise mono-, sesqui- and triterpenes, but occasionally also diterpenes – a group of terpenes that was not detected in Bornean bees. The larger variety of terpenoid groups in the chemical profiles of neotropical bees correlates with the chemical diversity of resins from Costa Rican tree species. In contrast to study sites sampled in Costa Rica where no particular tree family was overly abundant, rainforests in Borneo are dominated by dipterocarp trees (Soepadmo et al. 2004). Dipterocarps have a specific resin profile comprising particularly sesqui- and triterpenes (Langenheim 2003) and they are predominantly visited by Bornean stingless bees for resin collection (Leonhardt and Blüthgen 2009). In Costa Rica, tree resins analyzed from various tree species visited by resin foragers comprise a broader variety of compounds (including mono-, sesqui-, di- and triterpenes as well as potential flavonoids and unknown compound classes) as do bee profiles. It is therefore

highly likely that some neotropical stingless bee species also include resin-derived terpenes in their chemical profiles. Whether they are further able to filter resin-derived compounds and produce species-specific terpene profiles will be revealed by a more detailed analysis and characterization of their cuticular profiles.

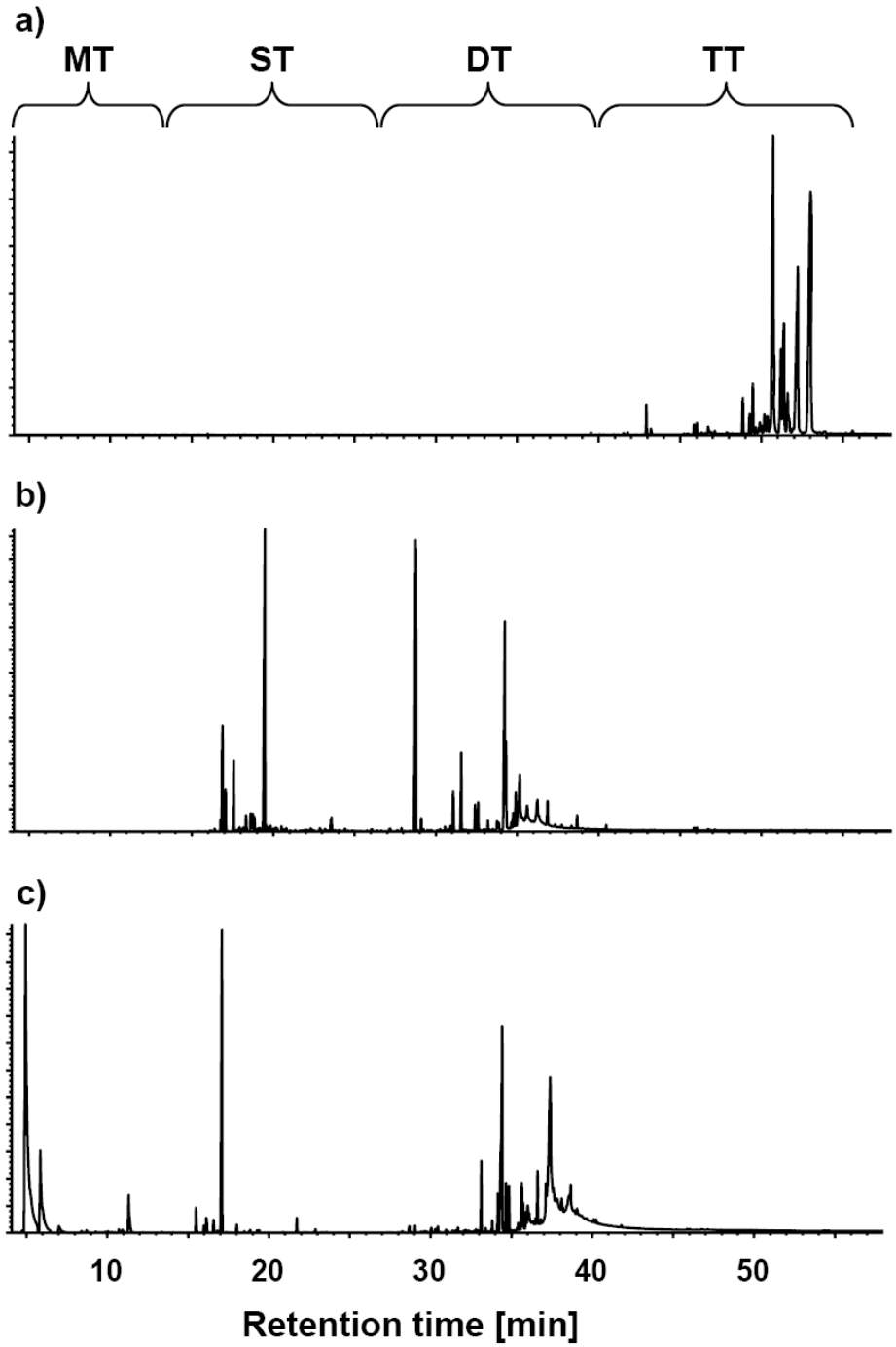


Figure 2. Chromatograms of resins from a *Ficus* tree (a), *Hymenea coubaril* (b) and a conifer tree (c) collected from different sites in Costa Rica: MT = monoterpenes, ST = sesquiterpenes, DT = diterpenes, TT = triterpenes.

XIII. Literature

Abdalla FC. 2006. Morphological, chemical and developmental aspects of the Dufour gland in some eusocial bees (Hymenoptera, Apidae): a review. *Revista Brasileira De Entomologia* 50:153-162.

Abdalla FC, Jones GR, Morgan ED, Da Cruz-Landim C. 2003. Comparative study of the cuticular hydrocarbon composition of *Melipona bicolor* Lepeletier, 1836 (Hymenoptera, Meliponini) workers and queens. *Genet. Molec. Res.* 2:191-199.

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

Armbruster WS, Lee J, Baldwin BG. 2009. Macroevolutionary patterns of defense and pollination in *Dalechampia* vines: Adaptation, exaptation, and evolutionary novelty. *Proc. Natl. Acad. Sci. U. S. A.* 106:18085-18090.

Ayasse M, Engels W, Lübke G, Taghizadeh T, Francke W. 1999. Mating expenditures reduced via female sex pheromone modulation in the primitively eusocial halictine bee, *Lasioglossum* (*Evylaeus*) *malachurum* (Hymenoptera : Halictidae). *Behav. Ecol. Sociobiol.* 45:95-106.

Ayasse M, Marlovits T, Tengö J, Taghizadeh T, Francke W. 1995. Are there pheromonal dominance signals in the bumblebee *Bombus hypnorum* L (Hymenoptera, Apidae). *Apidologie* 26:163-180.

Ayasse M, Paxton RJ, Tengo J. 2001. Mating behavior and chemical communication in the order hymenoptera. *Annu. Rev. Entomol.* 46:31-78.

Bacelar-Lima CG, Brito Freire DdC, Coletto-Silva A, da Costa KB, Barros Laray JP, Vilas-Boas HC, Carvalho-Zilse GA. 2006. Melitocory of *Zygia racemosa* (Ducke) Barneby & Grimes by *Melipona seminigra merrillae* Cockerell 1919 and *Melipona compressipes manaosensis* Schwarz 1932 (Hymenoptera, Meliponina) in Central Amazon, Brazil. *Acta Amazonica* 36:343-348.

Bagnères AG, Clement JL, Blum MS, Severson RF, Joulie C, Lange C. 1990. Cuticular hydrocarbons and defensive compounds of *Reticulitermes flavipes* (Kollar) and *R. santonensis* (Feytaud) - polymorphism and chemotaxonomy. *J. Chem. Ecol.* 16:3213-3244.

Baldwin IT, Halitschke R, Paschold A, von Dahl CC, Preston CA. 2006. Volatile signaling in plant-plant interactions: "Talking trees" in the genomics era. *Science* 311:812-815.

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

Barth FG, Hrncir M, Jarau S. 2008. Signals and cues in the recruitment behavior of stingless bees (Meliponini). *Journal of Comparative Physiology a-Neuroethology Sensory Neural and Behavioral Physiology* 194:313-327.

Bassindale R. 1955. The biology of the stingless bee *Trigona* (*Hypotrigona*) *gribodoi* Magretti. *Proc. Zool. Soc. Lond.* 125:49-62.

Beggs KT, Glendining KA, Marechal NM, Vergoz V, Nakamura I, Slessor KN, Mercer AR. 2007. Queen pheromone modulates brain dopamine function in worker honey bees. *Proc. Natl. Acad. Sci. U. S. A.* 104:2460-2464.

Biesmeijer JC, Slaa EJ. 2004. Information flow and organization of stingless bee foraging. *Apidologie* 35:143-157.

Biesmeijer JC, Toth E. 1998. Individual foraging, activity level and longevity in the stingless bee Melipona beecheii in Costa Rica (Hymenoptera, Apidae, Meliponinae). *Insect. Soc.* 45:427-443.

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.

Blomquist GJ, Chu AJ, Remaley S. 1980. Biosynthesis of wax in the honeybee, *Apis mellifera* L. *Insect Biochemistry* 10:313-321.
Blomquist GJ, Roubik DW, Buchmann SL. 1985. Wax chemistry of 2 stingless bees of the *Trigonisca* group (Apididae, Meliponinae). *Comp. Biochem. Physiol. B-Biochem. Mol. Biol.* 82:137-142.
Blum MS. 1966. Chemical releasers of social behavior - VIII. Citral in the mandibular gland secretion of *Lestrimelitta limao* (Hymenoptera: Apoidea: Melittidae). *Ann. Entomol. Soc.* 59:962-964.
Blum MS, Brand JM. 1972. Social insect pheromones: their chemistry and function. *Am. Zool.* 12:553-576.
Blum MS, Crewe RM, Kerr WE, Keith LH, Garrison AW, Walker MM. 1970a. Citral in stingless bees: isolation and functions in trail-laying and robbing. *J. Insect Physiol.* 16:1637-1648.
Blum MS, Kerr WE, Fales HM. 1970b. The chemical basis of insect sociality. In: Beroza M, ed. *Chemicals controlling Insect Behavior*. New York: Academic Press. p. 61-94.
Blüthgen N, Menzel F, Blüthgen N. 2006. Measuring specialization in species interaction networks. *BMC Ecology* 6.
Blüthgen N, Menzel F, Hovestadt T, Fiala B, Blüthgen N. 2007. Specialization, constraints, and conflicting interests in mutualistic networks. *Curr. Biol.* 17:341-346.
Bowden RM, Garry MF, Breed MD. 1994. Discrimination of conspecific and heterospecific bees by *Trigona* (*Tetragonisca*) *angustula* guards. *J. Kans. Entomol. Soc.* 67:137-139.
Breed MD. 1983. Nestmate recognition in honey bees. *Anim. Behav.* 31:86-91.
Breed MD. 1998. Recognition pheromones of the honey bee. *Bioscience* 48:463-470.
Breed MD, Bennett B. 1987. Kin recognition in highly eusocial insects. In: Fletcher DJC, Michener CD, ed. *Kin recognition in animals*. Chichester, NY: J. Wiley & Sons. p. 243-285.
Breed MD, Diaz PH, Lucero KD. 2004a. Olfactory information processing in honeybee, Apis mellifera, nestmate recognition. *Anim. Behav.* 68:921-928.
Breed MD, Garry MF, Pearce AN, Hibbard BE, Bjostad LB, Page RE. 1995. The role of wax comb in honeybee nestmate recognition. *Anim. Behav.* 50:489-496.
Breed MD, Leger EA, Pearce AN, Wang YJ. 1998. Comb wax effects on the ontogeny of honey bee nestmate recognition. *Anim. Behav.* 55:13-20.
Breed MD, Page RE. 1991. Intraspecific and interspecific nestmate recognition in *Melipona* workers (Hymenoptera, Apidae). *J. Insect Behav.* 4:463-469.
Breed MD, Perry S, Bjostad LB. 2004b. Testing the blank slate hypothesis: why honeybee colonies accept young bees. *Insect. Soc.* 51:12-16.
Breed MD, Smith TA, Torres A. 1992. Role of guard honey bees (Hymenoptera, Apidae) in nestmate discrimination and replacement of removed guards. *Ann. Entomol. Soc.* 85:633-637.
Breed MD, Stiller TM. 1992. Honey bee, *Apis mellifera*, nestmate discrimination - hydrocarbon effects and the evolutionary implications of comb choice. *Anim. Behav.* 43:875-883.
Breed MD, Stiller TM, Moor MJ. 1988a. The ontogeny of kin discrimination cues in the honey bee, *Apis mellifera*. *Behav. Genet.* 18:439-448.
Breed MD, Williams KR, Fewell JH. 1988b. Comb wax mediates the acquisition of nest-mate recognition cues in honey bees. *Proc. Natl. Acad. Sci. U. S. A.* 85:8766-8769.
Brühl C. 2003. *Vollenhovia* sp. aff. *acanthina*: an ant species that build nests using resin. *ANeT Newsletter* 6:21-22.
Bruschini C, Cervo R, Protti I, Turillazzi S. 2008. Caste differences in venom volatiles and their effect on alarm behaviour in the paper wasp *Polistes dominulus* (Christ). *J. Exp. Biol.* 211:2442-2449.

Buchwald R, Breed MD. 2005. Nestmate recognition cues in a stingless bee, *Trigona fulviventris*. *Anim. Behav.* 70:1331-1337.
Buckner JL. 1993. Cuticular polar lipids in insects. In: Stanley-Samuelson DW, Nelson DR, ed. *Insect Lipids: Chemistry, Biochemistry and Biology*. Lincoln, Nebraska: University of Nebraska Press. p. 227-270.
Bultman JD, Chen S-L, Schloman Jr. WW. 1998. Anti-termitic efficacy of the resin and rubber in fractionator overheads from guayule extraction process. *Industrial Crops and Products* 8:133-143.
Bultman JD, Gilbertson RL, Adaskaveg J, Amburgey TL, Parikh SV, Bailey CA. 1991. The efficacy of guayule resin as a pesticide. *Bioresour. Technol.* 35:197-201.
Butler CG, Calam DH. 1969. Pheromones of the honey bee - the secretion of the Nassanoff gland of the worker. *J. Insect Physiol.* 15:237-244.
Cameron EC, Franck P, Oldroyd BP. 2004. Genetic structure of nest aggregations and drone congregations of the southeast Asian stingless bee *Trigona collina*. *Molecular Ecology* 13:2357-2364.
Cane JH. 1986. Predator deterrence by mandibular gland secretions of bees (Hymenoptera, Apoidea). *J. Chem. Ecol.* 12:1295-1309.
Castella G, Chapuisat M, Christe P. 2008a. Prophylaxis with resin in wood ants. *Anim. Behav.* 75:1591-1596.
Castella G, Chapuisat M, Moret Y, Christe P. 2008b. The presence of conifer resin decreases the use of the immune system in wood ants. *Ecological Entomology* 33:408-412.
Cervo R, Dani FR, Cotoneschi C, Scala C, Lotti I, Strassmann JE, Queller DC, Turillazzi S. 2008. Why are larvae of the social parasite wasp *Polistes sulcifer* not removed from the host nest? *Behav. Ecol. Sociobiol.* 62:1319-1331.
Chapuisat M, Oppliger A, Magliano P, Christe P. 2007. Wood ants use resin to protect themselves against pathogens. *Proc. R. Soc. Lond. B* 274:2013-2017.
Chen C, Song QS, Proffit M, Bessiere JM, Li ZB, Hossaert-McKey M. 2009. Private channel: a single unusual compound assures specific pollinator attraction in *Ficus semicordata*. *Functional Ecology* 23: 941-950.
Choe D-H, Krust MK. 2007. Use of plant resin by a bee assassin bug, *Apiomerus flaviventris* (Hemiptera: Reduviidae). *Ann. Entomol. Soc.* 100:320-326.
Christe P, Oppliger A, Bancala F, Castella G, Chapuisat M. 2003. Evidence for collective medication in ants. *Ecol. Letters* 6:19-22.
Codella SG, Raffa KF. 1995. Host plant influence on chemical defense in conifer sawflies (Hymenoptera: Diprionidae). *Oecologia* 104:1-11.
Cook JM, Crozier RH. 1995. Sex determination and population biology in the Hymenoptera. *Trends in Ecology and Evolution* 10:281-286.
Corlett RT. 2004. Flower visitors and pollination in the Oriental (Indomalayan) Region. *Biological Reviews* 79:497-532.
Couvillon MJ, Ratnieks FLW. 2009. Odour transfer in stingless bee marmelada (*Frieseomelitta varia*) demonstrates that entrance guards use an "undesirable-absent" recognition system. *Behav. Ecol. Sociobiol.* 62:1099-1105.
Crozier RH, Dix MW. 1979. Analysis of two genetic models for the innate components of colony odor in social Hymenoptera. *Behav. Ecol. Sociobiol.* 4:217-224.
Crozier RH, Pamilo P. 1996. *Evolution of social insect colonies*. Oxford: Oxford University Press.
Cruz-Lopez L, Malo EA, Morgan ED, Rincon M, Guzman M, Rojas JC. 2005. Mandibular gland secretion of *Melipona beecheii*: chemistry and behavior. *J. Chem. Ecol.* 31:1621-1632.
Cruz-Lopez L, Patricio E, Morgan ED. 2001. Secretions of stingless bees: The Dufour gland of *Nannotrigona testaceicornis*. *J. Chem. Ecol.* 27:69-80.

Da Cruz-Landim C, Abdalla FC, Gracioli-Vitti LF. 2005. Morphological and functional aspects of volatile-producing glands in bees (Hymenoptera: Apidae). *Insect Science* 12:467-480.
Da Cruz Landim C. 1967. Estudo comparative de algumas glandulas das abelhas (Hymenoptera, Apoidea) e respectivas implicacoes evolutivas. *Archos Zool. S. Paulo* 15:177-290.
Dicke M, Baldwin IT. 2010. The evolutionary context for herbivore-induced plant volatiles: beyond the 'cry for help'. *Trends in Plant Science* 15:167-75.
Dötterl S, Jürgens A, Seifert K, Laube T, Weißbecker B, Schütz S. 2006. Nursery pollination by a moth *Silene latifolia*: the role of odours in eliciting antennal and behavioural responses. *New Phytologist* 169: 707-718.
Downs SG, Ratnieks FLW, Jefferies SL, Rigby HE. 2000. The role of floral oils in the nestmate recognition system of honey bees (*Apis mellifera* L.). *Apidologie* 31:357-365.
Dressler RL. 1982. Biology of the orchid bees (Euglossini). *Annual Review of Ecology and Systematics* 13:373-394.
Duangphakdee O, Koeniger N, Deowanish S, Hepburn HR, Wongsiri S. 2009. Ant repellent resins of honeybees and stingless bees. *Insect. Soc.* preprint.
Dudareva N, Pichersky E. 2006. *Biology of floral scent*. Boca Raton, FL: CRC Press.
Duffey SS. 1980. Sequestration of plant natural-products by insects. *Annu. Rev. Entomol.* 25:447-477.
Dworschak K, Blüthgen N. 2010. Networks and dominance hierarchies: does interspecific aggression explain flower partitioning among stingless bees? *Ecological Entomology* 34.
Edgar JA, Culvenor CCJ. 1974. Pyrrolizidine ester alkaloid in danaid butterflies. *Nature* 248: 614-616.
Eisner T. 1970. Chemical defense against predation in arthropods. In: Sondheimer E, Simeone JB, ed. *Chemical Ecology*. New York: Academic Press. p. 157-217.
Eisner T, Deyrup M, Jacobs R, Meinwald J. 1986. Defense mechanisms of anthropods. Necrodols - anti-insectan terpenes from defensive secretion of carrion beetle (*Necrodes surinamensis*). *J. Chem. Ecol.* 12:1407-1415.
Eisner T, Johnesse J, Carrel J, Hendry LB, Meinwald J. 1974. Defensive use by an insect of a plant resin. *Science* 184:996-999.
Eisner T, Kriston I, Aneshansley DJ. 1976. Defensive behavior of a termite (Nasutitermis exitiosus). *Behav. Ecol. Sociobiol.* 1:83-125.
Eltz T. 2004. Spatio-temporal variation of apine bee attraction to honeybaits in Bornean forests. *J. Trop. Ecol.* 20:317-324.
Eltz T, Brühl CA, Imiyabir Z, Linsenmair KE. 2003. Nesting and nest trees of stingless bees (Apidae: Meliponini) in lowland dipterocarp forests in Sabah, Malaysia, with implications for forest management. *Forest Ecology and Management* 172:pp. 301-313.
Eltz T, Brühl CA, Kaars Svd, Linsenmair KE. 2002. Determinants of stingless bee nest density in lowland dipterocarp forests of Sabah, Malaysia. *Oecologia* 131:27-34.
Eltz T, Brühl CA, Zamrie I, Linsenmair KE. 2001. Nesting and nest trees of stingless bees (Apidae: Meliponini) in lowland dipterocarp forests in Sabah, Malaysia, with implications for forest management. *Forest Ecology and Management* 5833:1-13.
Eltz T, Roubik DW, Lunau K. 2005. Experience-dependent choices ensure species-specific fragrance accumulation in male orchid bees. *Behav. Ecol. Sociobiol.* 59:149-156.
Endler A, Liebig J, Schmitt T, Parker JE, Jones GR, Schreier P, Holldobler B. 2004. Surface hydrocarbons of queen eggs regulate worker reproduction in a social insect. *Proc. Natl. Acad. Sci. U. S. A.* 101:2945-2950.

Engels W, Engels E, Lubke G, Schroder W, Francke W. 1990. Volatile Cephalic Secretions of Drones, Queens and Workers in Relation to Reproduction in the Stingless Bee, *Scaptotrigon postica* (Hymenoptera, Apidae, Trigonini). *Entomol. Gen.* 15:91-101.
Espelie KE, Gamboa GJ, Grudzien TA, Bura EA. 1994. Cuticular hydrocarbons of the paper wasp, *Polistes fuscatus* - a search for recognition pheromones. *J. Chem. Ecol.* 20:1677-1687.
Fletcher DJC, Michener CD. 1987. *Kin Recognition in Animals*. Chichester: John Wiley & Sons.
Fordyce JA, Marion ZH, Shapiro AM. 2005. Phenological variation in chemical defense of the pipevine swallowtail, *Battus philenor*. *J. Chem. Ecol.* 31:2835-2846.
Fordyce JA, Nice CC. 2008. Antagonistic, stage-specific selection on defensive chemical sequestration in a toxic butterfly. *Evolution* 62:1610-1617.
Fox JD. 1973. *A Handbook to Kabili-Sepilok Forest Reserve*. Sandakan, Malaysia.
Francis BR, Blanton WE, Littlefield JL, Nunamaker RA. 1989. Hydrocarbons of the cuticle and hemolymph of the adult honey bee (Hymenoptera, Apidae). *Ann. Entomol. Soc.* 82:486-494.
Francis BR, Blanton WE, Nunamaker RA. 1985. Extractable surface hydrocarbons of workers and drones of the genus *Apis*. *J. Apic. Res.* 24:13-26.
Francke W, Lübke G, Schröder W, Reckziegel A, Imperatriz-Fonseca A, Kleinert A, Hartfelder K, Radtke R, Engels W. 2000. Identification of oxygen containing volatiles in cephalic secretions of workers of Brazilian stingless bees. *J. Braz. Chem. Soc.* 11:562-571.
Fröhlich B, Riederer M, Tautz J. 2000a. Comb-wax discrimination by honeybees tested with the proboscis extension reflex. *J. Exp. Biol.* 203:1581-1587.
Fröhlich B, Riederer M, Tautz J. 2001. Honeybees discriminate cuticular waxes based on esters and polar components. *Apidologie* 32:265-274.
Fröhlich B, Tautz J, Riederer M. 2000b. Chemometric classification of comb and cuticular waxes of the honeybee *Apis mellifera carnica*. *J. Chem. Ecol.* 26:123-137.
Gamboa GJ, Reeve HK, Ferguson ID, Wacker TL. 1986. Nestmate recognition in social wasps - the origin and acquisition of recognition odors. *Anim. Behav.* 34:685-695.
Garcia MVB, Deoliveira ML, Campos LAD. 1992. Use of seeds of *Coussapoa asperifolia magnifolia* (Cecropiaceae) by stingless bees in the central Amazonian forest (Hymenoptera, Apidae, Meliponinae). *Entomol. Gen.* 17:255-258.
Gershenzon J, Dudareva N. 2007. The function of terpene natural products in the natural world. *Nature Chemical Biology* 3:408-414.
Getz WM, Chapman RF. 1987. An odor discrimination model with application to kin recognition in social insects. *Int. J. Neurosci.* 32:963-978.
Ghisalberti EL. 1979. Propolis: a review. *Bee World* 60:59-84.
Gleason JM, James RA, Wicker-Thomas C, Ritchie MG. 2009. Identification of quantitative trait loci function through analysis of multiple cuticular hydrocarbons differing between *Drosophila simulans* and *Drosophila sechellia* females. *Heredity* 103:416-424.
Gloag RS, Oldroyd BP, Heard TA, Beekman M. 2008. Nest defence in a stingless bee: What causes fighting swarms in *Trigona carbonaria* (Hymenoptera, Meliponini)? *Insect. Soc.* 55:387-391.
Goff SA, Klee HJ. 2006. Plant volatile compounds: Sensory cues for health and nutritional value? *Science* 311:815-819.
Gotelli NJ, Entsminger GL, 2009. EcoSim: Null models software for ecology. Version 7. In: http://garyentsminger.com/ecosim.htm. Jericho, VT 05465: Acquired Intelligence Inc. & Kesey-Bear.

Guerrieri FJ, Nehring V, Jorgensen CG, Nielsen J, Galizia CG, d'Ettorre P. 2009. Ants recognize foes and not friends. *Proc. R. Soc. Lond. B* 276:2461-2468.
Hasenkamp KR. 1974. Studies on the ecology, physiology and behavior of leaf-cutting bees (Megachile). *Forma Functiologica* 7:137-178.
Hauber ME, Sherman PW. 2001. Self-referent phenotype matching: theoretical considerations and empirical evidence. *Trends Neurosci.* 24:609-616.
Hector A, Schmid B, Beierkuhnlein C, Caldeira MC, Diemer M, Dimitrakopoulos PG, Finn JA, Freitas H, Giller PS, Good J, Harris R, Högberg P, Huss-Danell K, Joshi J, Jumpponen A, Körner C, Leadley PW, Loreau M, Minns A, Mulder CPH, O'Donovan G, Otway SJ, Pereira JS, Prinz A, Read DJ, Scherer-Lorenzen M, Schulze E-D, Siamantziouras A-SD, Spehn EM, Terry AC, Troumbis AY, Woodward FI, Yachi S, Lawton JH. 1999. Plant diversity and productivity experiments in European grasslands. *Science* 286:1123-1127.
Hefetz A. 1987. The role of Dufours gland secretions in bees. *Physiol. Entomol.* 12:243-253.
Hefetz A, Batra SWT, Blum MS. 1979. Chemistry of the mandibular gland secretion of the Indian bee *Pithitis smaragdula*. *J. Chem. Ecol.* 5:753-758.
Heinze J, Stengl B, Sledge MF. 2002. Worker rank, reproductive status and cuticular hydrocarbon signature in the ant, Pachycondyla cf. inversa. *Behav. Ecol. Sociobiol.* 52: 59-65.
Hepburn HR. 1986. *Honeybees and wax. An experimental natural history.* Berlin: Springer Verlag.
Hepburn R, Duangphakdee O, Phiancharoen M, Radloff S. 2010. Comb wax salvage by the red dwarf honeybee, *Apis florea* F. *J. Insect Behav.* 23:159-164.
Hick AJ, Luszniak MC, Pickett JA. 1999. Volatile isoprenoids that control insect behaviour and development. *Natural Product Reports* 16:39-54.
Hölldobler B. 1995. The chemistry of social regulation - multicomponent signals in ant societies. *Proc. Natl. Acad. Sci. U. S. A.* 92:19-22.
Hölldobler B, Wilson EO. 1990. *The ants*. Cambridge, MA: Harvard University Press.
Hölldobler B, Wilson EO. 2009. *The Superorganism*. New York: Norton & Company.
Howard JJ. 1985. Observations on resin collecting by six interacting species of stingless bees (Apidae, Meliponinae). *J. Kans. Entomol. Soc.* 58:337-345.
Howard RW. 1993. Cuticular hydrocarbons and chemical communication. In: Stanley-Samuelson DW, Nelson DR, ed. *Insects Lipids: Chemistry, Biochemistry and Biology*. Lincoln, Nebraska: University of Nebraska Press. p. 179-226.
Howard RW, Blomquist GJ. 2005. Ecological, behavioral, and biochemical aspects of insect hydrocarbons. *Annu. Rev. Entomol.* 50:371-393.
Howard RW, McDaniel CA, Nelson DR, Blomquist GJ, Gelbaum LT, Zalkow LH. 1982. Cuticular hydrocarbons of *Reticulitermes virginicus* (Banks)[1] and their role as potential species- and caste-recognition cues. *J. Chem. Ecol.* 8:1227-1239.
Howard RW, Perez-Lachaud G, Lachaud JP. 2001. Cuticular hydrocarbons of *Kapala sulcifacies* (Hymenoptera: Eucharitidae) and its host, the ponerine ant *Ectatomma ruidum* (Hymenoptera: Formicidae). *Ann. Entomol. Soc.* 94:707-716.
Hrncir M, Jarau S, Zucchi R, Barth FG. 2004. On the origin and properties of scent marks deposited at the food source by a stingless bee, *Melipona seminigra Apidologie* 35:3-13.
Hubbell SP, Johnson LK. 1977. Competition and nest spacing in a tropical stingless bee community. *Ecology* 58:949-963.
Hubbell SP, Johnson LK. 1978. Comparative Foraging Behavior of 6 Stingless Bee Species Exploiting a Standardized Resource. *Ecology* 59:1123-1136.
Inoue T, Nagamitsu T, Momose K, Sakagami SF, Hamid AA. 1994. Stingless bees in Sarawak. In: Inoue T, Hamid AA, ed. *Plant reproductive systems and animal seasonal*

dynamics: long-term study of dipterocarp forests in Sarawak. Otsu: Report of Monbusho international scientific research. Center for Ecological Research, Kyoto University. p. 231-237.
Inoue T, Roubik DW. 1990. Kin recognition of the stingless bee *Melipona fasciata*. In: Veeresh GK, Mallik R, Viraktamath CA, ed. *Social Insects and the Environment*. New Delhi: Oxford & IBH Publishing Co. p. 517-518.
Inoue T, Roubik DW, Suka T. 1999. Nestmate recognition in the stingless bee *Melipona panamica* (Apidae, Meliponini). *Insect. Soc.* 46:208-218.
Inoue T, Sakagami SF, Salmah S, Yamane S. 1984. The process of colony multiplication in the Sumatran stingless bee *Trigona (Tetragonula) laeviceps*. *Biotropica* 16:100-111.
Inoue T, Yumoto T, Hamid AA, Lee HS, Ogino K. 1995. Construction of a canopy observation system in a tropical rainforest of Sarawak. *Selbyana* 16:24-35.
Jarau S. 2009. *Chemical communication during food exploitation in stingless bees*.
Johnson LK. 1983. Foraging strategies and the structure of stingless bee communities in Costa Rica. In: Jaisson P, ed. *Social Insects in the Tropics 2*. Paris: Université Paris-Nord. p. 31-58.
Johnson LK, Haynes LW, Carlson MA, Fortnum HA, Gorgas DL. 1985. Alarm substances of the stingless bee, *Trigona sylvestriana*. *J. Chem. Ecol.* 11:409-416.
Johnson LK, Hubbell SP. 1974. Aggression and competition among stingless bees - field studies. *Ecology* 55:120-127.
Jordano P. 1987. Patterns of mutualistic interactions in pollination and seed dispersal: connectance, dependence asymmetries, and coevolution. *Am. Nat.* 129: 657-677
Jungnickel H, da Costa AJS, Tentschert J, Patricio E, Imperatriz-Fonseca VL, Drijfhout F, Morgan ED. 2004. Chemical basis for inter-colonial aggression in the stingless bee *Scaptotrigona bipunctata* (Hymenoptera : Apidae). *J. Insect Physiol.* 50:761-766.
Junker R, Chung AYC, Blüthgen N. 2007. Interaction between flowers, ants and pollinators: additional evidence for floral repellence against ants. *Ecol. Res.* 22:665-670.
Junker RR, Blüthgen N. 2008. Floral scents repel potentially nectar-thieving ants. *Evolut. Ecol. Res.* 10:295-308.
Junker RR, Blüthgen N. 2010. Floral scents repel facultative flower visitors, but attract obligate ones. *Annals of Botany*: doi: 10.1093/aob/mcq045.
Kaib M, Jmhasly P, Wilfert L, Durka W, Franke S, Francke W, Leuthold RH, Brandl R. 2004. Cuticular hydrocarbons and aggression in the termite *Macrotermes subhyalinus*. *J. Chem. Ecol.* 30:365-385.
Kainulainen P, Tarhanen J, Tiilikkala K, Holopainen JK. 1998. Foliar and emission composition of essential oil in two carrot varieties. *J. Agric. Food Chem.* 46:3780-3784.
Kerr WE, Jungnickel H, Morgan ED. 2004. Workers of the stingless bee *Melipona scutellaris* are more similar to males than to queens in their cuticular compounds. *Apidologie* 35:611-618.
Khoo SG, Yong HS. 1987. Nest structure and colony defence in the stingless bee *Trigona terminata* Smith. *Nature Malaysiana* 12:4-15.
Kirchner WH, Friebe R. 1999. Nestmate discrimination in the African stingless bee *Hypotrigona gribodoi* Magretti (Hymenoptera : Apidae). *Apidologie* 30:293-298.
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.
Lacy RC, Sherman PW. 1983. Kin recognition by phenotype matching. *Am. Nat.* 121:489-512.

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. 1969. Amber - a botanical inquiry. *Science* 163:1157-1169.
Langenheim JH. 2003. *Plant resins: chemistry, evolution, ecology and ethnobotany*. Portland: Timber Press.
Langenheim JH, Lincoln DE, Foster CE, Stubblebine WH. 1978. Implications of variation in resin composition among organs, tissues and populations in tropical legume *Hymenaea*. *Biochem. Syst. Ecol.* 6:299-313.
Langenheim JH, Stubblebine WH. 1983. Variation in leaf resin composition between parent tree and progeny in *Hymenea* - implications for herbivory in the humid tropics. *Biochem. Syst. Ecol.* 11:97-106.
Laurent P, Braekman J-C, Daloze D, Pasteels J. 2003. Biosynthesis of defensive compounds from beetles and ants. *Europ. J. Org. Chem.* 15:2733-2743.
Legendre A, Miao XX, Da Lage JL, Wicker-Thomas C. 2008. Evolution of a desaturase involved in female pheromonal cuticular hydrocarbon biosynthesis and courtship behavior in *Drosophila*. *Insect Biochem. Mol. Biol.* 38:244-255.
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.
Lenoir A, D'Ettorre P, Errard C, Hefetz A. 2001. Chemical ecology and social parasitism in ants. *Annu. Rev. Entomol.* 46:573-599.
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.
Leonhardt SD, Dworschak K, Eltz T, Blüthgen N. 2007. Foraging loads of stingless bees and utilisation of stored nectar for pollen harvesting. *Apidologie* 38:125-135.
Lockey KH. 1988. Lipids of the insect cuticle: origin, composition and function. *Comp. Biochem. Physiol. B-Biochem. Mol. Biol.* 89B:595-645.
Lokvam J, Braddock JF. 1999. Anti-bacterial function in the sexually dimorphic pollinator rewards of *Clusia grandiflora* (Clusiaceae). *Oecologia* 119:534-540.
Lopes AV, Machado IC. 1998. Floral biology and reproductive ecology of *Clusia nemorosa* (Clusiaceae) in northeastern Brazil. *Plant Systematics and Evolution* 213:71-90.
Loreau M, Naeem S, Inchausti P, Bengtsson J, Grime JP, Hector A, Hooper DU, Huston MA, Raffaelli D, Schmid B, Tilman D, Wardle DA. 2001. Ecology - biodiversity and ecosystem functioning: current knowledge and future challenges. *Science* 294:804-808.
Mant J, Brändli C, Vereecken NJ, Schulz CM, Francke W, Schiestl FP. 2005. Cuticular hydrocarbons as sex pheromone of the bee *Colletes cunicularius* and the key to its mimicry by the sexually deceptive orchid, *Ophrys exaltata*. *J. Chem. Ecol.* 31:1765-1787.
Marsh CW, Greer AG. 1992. Forest land-use in Sabah, Malaysia: an introduction to Danum Valley. *Philosoph. Trans. R. Soc. Lond. B* 335:331-339.
Martin S, Drijfhout F. 2009. A review of ant cuticular hydrocarbons. *J. Chem. Ecol.* 35:1151-1161.
Martin SJ, Vitikainen E, Helanterä H, Drijfhout FP. 2008. Chemical basis of nest-mate discrimination in the ant Formica exsecta. *Proc. R. Soc. Lond. B* 275:1271-1278.

Maschwitz U. 1975. Old and new chemical weapons in ants. In: Noirot C, Howse PE, Le Masne G, ed. *Defensive secretions in social insects, Symp. IUSSI*. Dijon: Université de Dijon. p. 41-45.
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.
McDaniel CA, Howard RW, Blomquist GJ, Collins AM. 1984. Hydrocarbons of the cuticle, sting apparatus, and sting shaft of *Apis mellifera* L - identification and preliminary evaluation as chemotaxonomic characters. *Sociobiology* 8:287-298.
Menzel F, Bluethgen N, Schmitt T. 2008a. Tropical parabiotic ants: highly unusual cuticular substances and low interspecific discrimination. *Frontiers in Zoology* 5:Article No.: 16.
Menzel F, Linsenmair KE, Blüthgen N. 2008b. Selective interspecific tolerance in tropical *Crematogaster* - *Camponotus* associations. *Anim. Behav.* 75:837-846.
Mesquita RDG, Franciscon CH. 1995. Flower visitors of *Clusia nemorosa* gfw-Meyer (Clusiaceae) in an Amazonian white-Sand campina. *Biotropica* 27:254-258.
Messer AC. 1985. Fresh dipterocarp resins gathered by megachilid bees inhibit growth of pollen-associated fungi. *Biotropica* 17:175-176.
Michener CD. 1974. *The Social Behavior of the Bees*. Harvard: Harvard University Press.
Michener CD. 1979. Biogeography of the bees. *Ann. Missouri Bot. Garden* 66:277-347.
Michener CD. 2000. *The Bees of the World*. Baltimore & London: John Hopkins University Press.
Milborrow BV, Kennedy JM, Dollin A. 1987. Composition of Wax Made by the Australian Stingless Bee Trigona-Australis. *Austr. J. Biol. Sci.* 40:15-25.
Momose K, Yumoto T, Nagamitsu T, Kato T, Nagamasu H, Sakai S, Harrison RD, Itioka T, Hamid AA, Inoue T. 1998. Pollination biology in a lowland dipterocarp forest in Sarawak, Malaysia. I. Characteristics of the plant pollinator community in a lowland dipterocarp forest. *Am. J. Bot.* 85:1477-1501.
Morgan ED, Jungnickel H, Keegans SJ, Do Nascimento RR, Billen J, Gobin B, Ito F. 2003. Comparative survey of abdominal gland secretions of the ant subfamily Ponerinae. *J. Chem. Ecol.* 29:95-114.
Mori A, Visicchio R, Sledge MF, Grasso DA, Le Moli F, Turillazzi S, Spencer S, Jones GR. 2000. Behavioural assays testing the appeasement allomone of *Polyergus rufescens* queens during host-colony usurpation. *Ethology, Ecology and Evolution* 12:215-322.
Moure JS. 1961. A preliminary supra-specific classification of the old world meliponine bees (Hymenoptera, Apoidea) *Studia Entomologica* 4:181-242.
Nagamitsu T, Inoue T. 1997. Aggressive foraging of social bees as a mechanism of floral resource partitioning in an Asian tropical rainforest. *Oecologia* 110:432-439.
Nielsen J, Boomsma JJ, Oldham NJ, Petersen HC, Morgan ED. 1999. Colony-level and season-specific variation in cuticular hydrocarbon profiles of individual workers in the ant *Formica truncorum*. *Insect. Soc.* 46:58-65.
Nunes TM, Nascimento FS, Turatti IC, Lopes NP, Zucchi R. 2008. Nestmate recognition in a stingless bee: does the similarity of chemical cues determine guard acceptance? *Anim. Behav.* 75:1165-1171.
Nunes TM, Turatti ICC, Lopes NP, Zucchi R. 2009a. Chemical signals in the stingless bee, *Frieseomelitta varia*, indicate caste, gender, age, and reproductive Status. *J. Chem. Ecol.* 35:1172-1180.
Nunes TM, Turatti ICC, Mateus S, Nascimento FS, Lopes NP, Zucchi R. 2009b. Cuticular hydrocarbons in the stingless bee *Schwarziana quadripunctata* (Hymenoptera, Apidae, Meliponini): differences between colonies, castes and age. *Genet. Molec. Res.* 8:589-595.

Nunez CV, de Oliveira ML, Lima RD, Diaz IEC, Sargentini E, Pereira OL, Araujo LM. 2008. Chemical analyses confirm a rare case of seed dispersal by bees. *Apidologie* 39:618-626.

Olesen JM, Bascompte J, Dupont YL, Jordano P. 2007. The modularity of pollination networks. *Proc. Natl. Acad. Sci. U. S. A.* 104:19891-19896.

Page RE, Metcalf RA, Metcalf RL, Erickson EH, Lampman RL. 1991. Extractable hydrocarbons and kin recognition in honeybee (*Apis mellifera* L). *J. Chem. Ecol.* 17:745-756.

Pankiw T. 2004. Cued in: honey bee pheromones as information flow and collective decision-making. *Apidologie* 35:217-226.

Pasteels JM, Gregoire JC, Rowellrahier M. 1983. The chemical ecology of defense in arthropods. *Annu. Rev. Entomol.* 28:263-289.

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.

Patricio E, Lopez LC, Maile R, Morgan ED. 2003. Secretions of stingless bees: the Dufour glands of some *Frieseomelitta* species (Apidae, Meliponinae). *Apidologie* 34:359-365.

Paulmier I, Bagnères AG, Afonso CMM, Dusticier G, Riviere G, Clément JL. 1999. Alkenes as a sexual pheromone in the alfalfa leaf-cutter bee *Megachile rotundata*. *J. Chem. Ecol.* 25:471-490.

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 in Plant Science* 4:184-190.

Pianaro A, Menezes C, Kerr WE, Singer RB, Patricio EFLRA, Marsaioli AJ. 2009. Stingless bees: chemical differences and potential functions in *Nannotrigona testaceicornis* and *Plebeia droryana* males and workers. *J. Chem. Ecol.* 35:1117-1128.

Prestwich GD. 1979. Interspecific Variation in the Defence Secretions of Nasutitermes Soldiers. *Biochem. Syst. Ecol.* 7:211-221.

R-Development-Core-Team, 2009. R: A language and environment for statistical computing, ISBN 3-900051-07-0, URL http://www.R-project.org. In. Vienna, Austria: R Foundation for Statistical Computing.

Raguso RA. 2008. Wake up and smell the roses: the ecology and evolution of floral scent. *Ann. Rev. Ecol. Evol. Sys.* 39: 549-569.

Rasmussen C, Cameron SA. 2007. A molecular phylogeny of the Old World stingless bees (Hymenoptera: Apidae: Meliponini) and the non-monophyly of the large genus *Trigona*. *Syst. Entomol.* 32:26-39.

Rasmussen C, Cameron SA. 2010. Global stingless bee phylogeny supports ancient divergence, vicariance, and long distance dispersal. *Biological Journal of the Linnean Society* 99:206-232.

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.
Roubik DW. 1979. Nest and colony characteristics of stingless bees from French Guiana (Hymenoptera, Apidae). *J. Kans. Entomol. Soc.* 52:443-470, 1979.
Roubik DW. 1983. Nest and colony characteristics of stingless bees from Panama (Hymenoptera, Apidae). *J. Kans. Entomol. Soc.* 56:327-355.
Roubik DW. 1989. *Ecology and Natural History of Tropical Bees*. New York: Cambridge University Press.
Roubik DW. 1996. Wild bees of Brunei Darussalam. In: Edwards DS, Booth WE, Choy SC, ed. *Tropical Rainforest Research - Current Issues: Proceedings of the Conference Held in Bandar Seri Begawan, April 1993*. Bandar Seri Begawan: Kluwer Academic Publishers. p. 566.
Said I, Costagliola G, Leoncini I, Rivault C. 2005. Cuticular hydrocarbon profiles and aggregation in four *Periplaneta* species (Insecta : Dictyoptera). *J. Insect Physiol.* 51:995-1003.
Sakagami SF, Camargo JFM. 1964. Cerumen collection accompanied by thieving and attacking in a stingless bee, *Nannotrigona (Scaptotrigona) postica* (Latreille), with a consideration on territoriality in social insects. *Revista de Biologia Tropical* 12:197-207.
Sakagami SF, Inoue T, Yamane S, Salmah S. 1989. Nests of the myrmecophilous stingless bee, *Trigona moorei* - how do bees initiate their nest within an arboreal ant nest. *Biotropica* 21:265-274.
Sakai S, Momose K, Inoue T, Hamid AA. 1997. Climate data in Lambir Hills National Park and Miri Airport, Sarawak. In: Inoue T, Hamid AA, ed. *General flowering of tropical rainforest in Sarawak*. Center for Ecological Research, Kyoto University. p. 1-10.
Salmah S, Inoue T, Sakagami SF. 1990. An analysis of apid bee richness (Apidae) in central Sumatra. In: Ohgushi R, Sakagami SF, Roubik DW, ed. *Natural History of Social Wasps and Bees in Equatorial Sumatra*. Sapporo: Hokkaido University Press. p. 139-174.
Samejima H, Marzuki M, Nagamitsu T, Nakasizuka T. 2004. The effects of human disturbance on a stingless bee community in a tropical rainforest. *Biological Conservation* 120:577-578.
Schiestl FP, Huber FK, Gomez JM. 2010. Pollinator driven selection and the evolution of floral scent bouquets. *Evolutionary Ecology* (in press).
Schmitt U, Lübke G, Francke W. 1991. Tarsal secretions mark food sources in bumblebees (Hymenoptera: Apidae). *Chemoecology* 2:35-40.
Schorkopf DLP, Hrncir M, Mateus S, Zucchi R, Schmidt VM, Barth FG. 2009. Mandibular gland secretions of meliponine worker bees: further evidence for their role in interspecific and intraspecific defence and aggression and against their role in food source signalling. *J. Exp. Biol.* 212:1153-1162.
Schwarz HF. 1948. Stingless bees of the western hemisphere. *Bulletin of the American Museum of Natural History* 90:1-546.
Simone M, Evans JD, Spivak M. 2009. Resin collection and social immunity in honey bees. *Evolution* 63:3016-3022.
Simpson J. 1960. The functions of the salivary glands of *Apis mellifera*. *J. Insect Physiol.* 4:107-121.
Soepadmo E, Saw LG, Chung RCK. 2004. *Tree flora of Sabah and Sarawak*. Government of Malaysia.
Sommeijer MJ, De Bruijn LLM, Van de Guchte C. 1985. The social food flow within the colony of a stingless bee, *Melipona favosa* (F.). *Behaviour* 92:39-58.

Sommeijer MJ, Derooy GA, Punt W, Debruijn LLM. 1983. A comparative study of foraging behavior and pollen resources of various stingless bees (Hym, Meliponinae) and honeybees (Hym, Apinae) in Trinidad, West-Indies. *Apidologie* 14:205-224.
Souza B, Roubik D, Barth O, Heard T, Enriquez E, Carvalho C, Villas-Boas J, Marchini L, Locatelli J, Persano-Oddo L, Almeida-Muradian L, Bogdanov S, Vit P. 2006. Composition of stingless bee honey: setting quality standards. *Interciencia* 31:867-875.
Sramkova A, Schulz C, Twele R, Francke W, Ayasse M. 2008. Fertility signals in the bumblebee *Bombus terrestris* (Hymenoptera : Apidae). *Naturwissenschaften* 95:515-522.
St. Leger RJ. 1995. Integument as a barrier to microbial infections. In: Binnington K, Retnakaran I, ed. *Physiology of the Insect Epidermis*. Melbourne: CSIRO Publications. p. 284-306.
Starr CK, Sakagami SF. 1987. An extraordinary concentration of stingless bee colonies in the Philippines, with notes on nest structure (Hymenoptera: Apidae: *Trigona* spp.). *Insect. Soc.* 34:96-107.
Steck K, Hansson BS, Knaden M. 2009. Smells like home: Desert ants, *Cataglyphis fortis*, use olfactory landmarks to pinpoint the nest. *Frontiers in Zoology* 6:1-8.
Strohm E, Kroiss J, Herzner G, Laurien-Kehnen C, Boland W, Schreier P, Schmitt T. 2008. A cuckoo in wolves' clothing? Chemical mimicry in a specialized cuckoo wasp of the European beewolf (Hymenoptera, Chrysididae and Crabronidae). *Frontiers in Zoology* 5:doi:10.1186/1742-9994-5-2.
Suka T, Inoue T. 1993. Nestmate recognition of the stingless bee *Trigona* (*Tetragonula*) *minangkabau* (Apidae, Meliponinae). *J. Ethol.* 11:141-147.
Suka T, Inoue T, Roubik DW. 1994. Worker oviposition and kin recognition of the stingless bee *Scaptotrigona barrocoloradensis*. In: Lenoir A, Arnold G, Lepage M, ed. *Les Insectes Sociaux*. Villetaneuse: Universite Paris Nord. p. 338.
Temeles EJ. 1994. The role of neighbours in territorial systems: when are they 'dear enemies'? *Anim. Behav.* 47:339-350.
Terzo M, Urbanova K, Valterova I, Rasmont P. 2005. Intra and interspecific variability of the cephalic labial glands' secretions in male bumblebees: the case of *Bombus (Thoracobombus) ruderarius* and *B. (Thoracobombus) sylvarum* [Hymenoptera, Apidae]. *Apidologie* 36:85-96.
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.
Thomas ML, Simmons LW. 2008. Cuticular hydrocarbons are heritable in the cricket *Teleogryllus oceanicus*. *J. Evol. Biol.* 21:801-806.
Turner IM. 2001. *The ecology of trees in the tropical rain forest*.
van Veen JW, Arce HGA. 1999. Nest and colony characteristics of log-hived *Melipona beechii* (Apidae: Meliponinae). *J. Apic. Res.* 38:43-48.
van Zweden JS, Dreier S, d'Ettorre P. 2009. Disentangling environmental and heritable nestmate recognition cues in a carpenter ant. *J. Insect Physiol.* 55:158-163.
Vander Meer RK, Morel L. 1998. Nestmate recognition in ants. In: Vander Meer RK, Winston M, Espelie KE, ed. *Pheromone communication in social insects*. Boulder: Westview. p. 79-103.
Velikova M, Bankova V, Marcucci MC, Tsvetkova I, Kujumgiev A. 2000a. Chemical composition and biological activity of propolis from Brazilian Meliponinae. *Z. Naturforsch. C* 55:785-789.
Velikova M, Bankova V, Tsvetkova I, Kujumgiev A, Marcucci MC. 2000b. Antibacterial ent-kaurene from Brazilian propolis of native stingless bees. *Fitoterapia* 71:693-696.

Vergoz V, Schreurs HA, Mercer AR. 2007. Queen pheromone blocks aversive learning in young worker bees. *Science* 317:484-386.
Villa JD, Weiss MR. 1990. Observations on the use of visual and olfactory cues by *Trigona* spp foragers. *Apidologie* 21:541-545.
Visicchio R, Sledge MF, Mori A, Grasso DA, Le Moli F, Turillazzi S, Moneti G, Spencer S, Jones GR. 2000. Dufour's gland contents of queens of the slave-making ant *Polyergus rufescens* and its host species *Formica cunicularia*. *Ethology, Ecology and Evolution* 12:67-73.
Vogel S. 1966. Parfümsammelnde Bienen als Bestäuber von Orchidaceen und *Gloxinia*. *Österreichische Botanische Zeitschrift* 113:302-361.
Wallace HM, Howell MG, Lee DJ. 2008. Standard yet unusual mechanisms of long-distance dispersal: seed dispersal of *Corymbia torelliana* by bees. *Divers. Distrib.* 14:87-94.
Wallace HM, Lee DJ. 2009. Resin-foraging by colonies of *Trigona sapiens* and *T. hockingsi* (Hymenoptera: Apidae, Meliponini) and consequent seed dispersal of *Corymbia torelliana* (Myrtaceae). *Apidologie* Preprint.
Wallace HM, Trueman SJ. 1995. Dispersal of *Eucalyptus torelliana* seeds by the resin-collecting stingless bee, *Trigona carbonaria*. *Oecologia* 104:12-16.
Wheeler JW, Blum MS, Daly HV, Kislow CJ, Brand JM. 1977. Chemistry of mandibular gland secretions of small Carpenter bees (*Ceratina* spp) (Hymenoptera-Anthophoridae). *Ann. Entomol. Soc.* 70:635-636.
Wille A. 1983. Biology of the stingless bee. *Annu. Rev. Entomol.* 28:41-46.
Wille A, Michener CD. 1973. The nest architecture of stingless bees with special reference to those of Costa Rica (Hymenoptera, Apidae). *Revista de Biologia Tropical* 21:1-278.
Wilms W, Imperatriz-Fonesca VL, Engels W. 1996. Resource partitioning between highly eusocial bees and possible impact of the introduced Africanized honeybee on native stingless bees in the Brazilian Atlantic rainforest. *Studies of Neotropical Fauna and Environment* 31:137-151.
Wilson EO. 1971. *The Insect Societies*. Belknap Press of the Harvard University Press.
Winfree R, Aguilar R, Vazquez DP, LeBuhn G, Aizen MA. 2009. A meta-analysis of bees' responses to anthropogenic disturbance. *Ecology* 90:2068-2076.
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.
Wood MJ, Ratnieks FLW. 2004. Olfactory cues and *Vespula* wasp recognition by honey bee guards. *Apidologie* 35:461-468.
Wright GA, Lutmerding A, Dudareva N, Smith BH. 2005a. Intensity and the ratios of compounds in the scent of snapdragon flowers affect scent discrimination by honeybees (*Apis mellifera*). *Journal of Comparative Physiology a-Neuroethology Sensory Neural and Behavioral Physiology* 191:105-114.
Wright GA, Schiestl FP. 2009. The evolution of floral scent: the influence of olfactory learning by insect pollinators on the honest signalling of floral rewards. *Functional Ecology* 23:841-851.
Wright GA, Thomson MGA, Smith BH. 2005b. Odour concentration affects odour identity in honeybees. *Proc. R. Soc. Lond. B* 272:2417-2422.
Zimmermann Y, Ramirez SR, Eltz T. 2009. Chemical niche differentiation among sympatric species of orchid bees. *Ecology* 90:2994-3008.

XIV. Supplementary material

Table S1

Foraging and chemical networks analyzed for bee and tree species in Borneo.

Number	Network	Year	Location	n1	n2	H_2'
Foraging networks (n1 - n2)						
1	tree species - bee species	2008	RDC	15	13	0.20
2	bee species - resin colour	2007	RDC, KSR, DVC	2*	25	0.12
3	bee species - resin colour	2008	RDC, KSR, DVC	3**	21	0.27
4	*Tetragonilla collina* colonies - resin colour	2007	RDC, KSR, DVC	4	23	0.09
5	*Tetragonilla collina* colonies - resin colour	2008	RDC, KSR, DVC	6	17	0.23
6	*Tetragonula melanocephala* colonies - resin colour	2007	RDC, KSR, DVC	4	17	0.18
7	*Tetragonula melanocephala* colonies - resin colour	2008	RDC, KSR, DVC	3	7	0.12
8	*Tetragonula geissleri* colonies - resin colour	2008	RDC, KSR, DVC	2	9	0.20
Chemical networks (n1 - n2)						
9	tree species - resin compounds (terpenes)	2007	RDC, DVC	20	265	0.58
10	nest material - all compounds	2007	KSR, DVC	6	247	0.42
11	nest material - wax compounds	2007	KSR, DVC	6	91	0.45
12	nest material - terpenoid compounds	2007	KSR, DVC	6	156	0.38
13	bee species - all compounds	2007	KSR, DVC	6	194	0.50
14	bee species - non-terpenoid compounds	2007	KSR, DVC	6	80	0.66
15	bee species - terpenoid compounds	2007	KSR, DVC	6	114	0.26
16	bee species - only sesquiterpenes	2007	KSR, DVC	6	67	0.17
17	bee species - only triterpenes	2007	KSR, DVC	6	40	0.16
18	*Tetragonilla collina* - all compounds	2007	KSR, DVC	9	124	0.08
19	*Tetragonilla collina* - non-terpenoid compounds	2007	KSR, DVC	9	49	0.05
20	*Tetragonilla collina* - terpenoid compounds	2007	KSR, DVC	9	75	0.13
21	*Tetragonula fuscobalteata* - all compounds	2007	KSR, DVC	8	127	0.09
22	*Tetragonula fuscobalteata* - non-terpenoid compounds	2007	KSR, DVC	8	57	0.11
23	*Tetragonula fuscobalteata* - terpenoid compounds	2007	KSR, DVC	8	70	0.08
24	*Tetragonula geissleri* - all compounds	2007	KSR, DVC	2	88	0.03
25	*Tetragonula geissleri* - non-terpenoid compounds	2007	KSR, DVC	2	41	0.02
26	*Tetragonula geissleri* - terpenoid compounds	2007	KSR, DVC	2	47	0.03
27	*Tetragonula melanocephala* - all compounds	2007	KSR, DVC	5	102	0.09
28	*Tetragonula melanocephala* - non-terpenoid compounds	2007	KSR, DVC	5	46	0.14
29	*Tetragonula melanocephala* - terpenoid compounds	2007	KSR, DVC	5	56	0.06
30	*Lepidotrigona terminata* - all compounds	2007	KSR, DVC	4	62	0.14
31	*Lepidotrigona terminata* - non-terpenoid compounds	2007	KSR, DVC	4	38	0.11
32	*Lepidotrigona terminata* - terpenoid compounds	2007	KSR, DVC	4	24	0.19

* *T. collina* & *T. melanocephala*
** *T. collina, T. melanocephala* & *T. geissleri*

Table S2

Percentages [± SD] of compounds from hexane extracts of surface profiles from eight Australian stingless bee species (comprising two genera: *Tetragonula* and *Austroplebeia*) listed according to their diagnostic ions (MS), Kovats retention indices (KI) and retention times (RT). Bold compounds or compounds marked by a plus indicate compounds that were also present in resin of seed capsules from *Corymbia torelliana trees*.

Nr.	MS	Compound	KI	RT	*A. australis*	*A. simeii*	*A. spec*	*T. carbonaria*	*T. clypearis*	*T. davenportii*	*T. hockingsii*	*T. sapiens*
1	128	Nonanone	-	4.32	4.52 % ± 5.66	1.25 % ± 1.56	10.59 % ± 8.43	0.55 % ± 1.09	8.64 % ± 7.48	0.08	0.12 % ± 0.08	0.28 % ± 0.27
2	136	**Tricyclene**	921	4.98	0.07 % ± 0.19	0.32 % ± 0.28	-	0.44 % ± 0.55	-	0.31	0.26 % ± 0.04	0.11 % ± 0.08
3	136	***α*-Pinene**	932	5.15	0.83 % ± 1.69	2.05 % ± 3.55	-	3.37 % ± 5.74	-	0.38	0.8 % ± 0.66	0.29 % ± 0.28
4	136	Sabinene	969	5.82	-	-	-	0.15 % ± 0.53	-	-	-	-
5	136	+	-	5.93	0.11 % ± 0.32	0.05 % ± 0.08	-	1.78 % ± 1.74	6.05 % ± 4.17	0.12	1.63 % ± 2.25	0.44 % ± 0.47
6	136	***β*-Pinene**	974	6.14	-	-	-	0.29 % ± 0.65	-	0.13	0.08 % ± 0.11	0.34 % ± 0.53
7	142	Decane	1000	6.34	2.72 % ± 2.3	1.58 % ± 1.35	9.75 % ± 5.48	0.53 % ± 0.76	6.04 % ± 2.09	0.08	0.05 % ± 0.08	0.39 % ± 0.36
8	-	-	-	6.44	-	-	-	0.25 % ± 0.33	-	-	-	0.08 % ± 0.12
9	136	para-Mentha-1(7),8-diene	1003	6.62	-	-	-	0.24 % ± 0.36	0.28 % ± 0.56	-	0.09 % ± 0.13	0.18 % ± 0.17
10	136	**Limonene**	1024	7.10	-	-	-	0.04 % ± 0.15	-	-	-	-
11	-	methyl-alkane	-	7.67	3.12 % ± 5.93	-	2.98 % ± 2.21	0.36 % ± 0.54	0.5 % ± 0.44	-	-	0.02 % ± 0.04
12	-	methyl-alkane	-	7.82	1.69 % ± 3.31	-	-	0.19 % ± 0.32	-	-	-	-
13	-	-	-	8.48	-	0.46 % ± 0.8	-	0.41 % ± 0.68	0.82 % ± 0.98	0.09	0.12 % ± 0.05	0.78 % ± 0.48
14	136	Terpinolene	1086	8.51	-	-	-	0.05 % ± 0.18	-	-	-	-
15	-	+	-	8.57	-	0.13 % ± 0.23	-	0.41 % ± 0.56	-	0.12	0.09 % ± 0.13	-
16	-	-	-	8.63	0.24 % ± 0.52	-	-	0.05 % ± 0.1	-	-	-	-
17	156	Undecane	1100	8.84	2.3 % ± 2.06	0.93 % ± 0.59	5.83 % ± 3.23	0.41 % ± 0.5	4.58 % ± 3.52	0.07	0.13 % ± 0.11	0.34 % ± 0.09
18	-	aliphatic compound	-	8.83	1.84 % ± 3.25	0.09 % ± 0.16	-	0.18 % ± 0.34	-	-	-	-
19	-	-	-	8.98	1.19 % ± 1.87	0.27 % ± 0.47	-	0.22 % ± 0.28	-	-	-	-
20	170	Dodecane	1200	11.57	2.15 % ± 1.77	0.58 % ± 0.4	4.52 % ± 2.24	0.27 % ± 0.32	3.67 % ± 2.51	0.3	0.15 % ± 0.05	0.3 % ± 0.15
21	-	+	-	13.21	-	0.07 % ± 0.11	-	0.68 % ± 0.64	0.13 % ± 0.25	0.24	0.41 % ± 0.31	0.7 % ± 0.22
22	-	methyl-alkane	-	13.55	2.24 % ± 2.82	0.28 % ± 0.17	1.96 % ± 1.18	0.42 % ± 0.34	1.1 % ± 0.63	0.04	0.07 % ± 0	0.17 % ± 0.04
23	184	Tridecane	1300	14.25	2.11 % ± 1.51	0.79 % ± 0.57	5.04 % ± 3.21	0.38 % ± 0.41	3.59 % ± 1.77	0.13	0.12 % ± 0.01	0.36 % ± 0.21
24	-	methyl-alkane	-	14.79	1.24 % ± 1.06	0.26 % ± 0.17	1.67 % ± 1.24	0.2 % ± 0.14	1.08 % ± 0.81	0.04	0.04 % ± 0.01	0.1 % ± 0.03
25	204	***α*-Copaene**	1374	16.30	-	0.16 % ± 0.14	-	0.33 % ± 0.34	-	0.03	0.17 % ± 0.03	0.17 % ± 0.14
26	196	Tetradecene	1388	16.61	0.25 % ± 0.49	-	-	0.06 % ± 0.08	-	-	-	-
27	198	Tetradecane	1400	16.82	0.7 % ± 0.41	0.32 % ± 0.23	1.6 % ± 0.82	0.12 % ± 0.12	1.26 % ± 0.57	0.05	0.06 % ± 0.02	0.15 % ± 0.04
28	204	**E-Caryophyllene**	1417	17.44	0.02 % ± 0.06	0.3 % ± 0.31	-	1.13 % ± 0.81	1.62 % ± 0.51	0.21	0.52 % ± 0.49	0.44 % ± 0.36
29	204	***β*-Gurjunene**	1431	17.72	-	-	-	-	-	-	0.06 % ± 0.06	0.17 % ± 0.41
30	204	**cis-Muurola-3,5-diene**	1448	18.17	-	-	-	0.04 % ± 0.04	0.92 % ± 0.89	0.23	0.09 % ± 0.03	0.04 % ± 0.02

Table S2. continued.

Nr.	MS	Compound	KI	RT	*A. australis*	*A. simeii*	*A. spec*	*T. carbonaria*	*T. clypearis*	*T. davenportii*	*T. hockingsii*	*T. sapiens*
31	-	methyl-alkane	-	18.99	0.92 % ± 0.76	0.29 % ± 0.2	1.65 % ± 0.85	0.15 % ± 0.09	1 % ± 0.48	0.05	0.06 % ± 0.02	0.14 % ± 0.04
32	212	Pentadecane	1500	19.31	0.4 % ± 0.37	0.15 % ± 0.17	1.03 % ± 0.54	0.06 % ± 0.08	0.86 % ± 0.5	0.04	0.02 % ± 0.03	0.1 % ± 0.03
33	202	**trans-Calamene**	1521	19.93	-	-	-	0.06 % ± 0.06	0.47 % ± 0.38	0.37	0.06 % ± 0.05	0.06 % ± 0.02
34	-	methyl-alkane	-	20.10	0.82 % ± 0.53	0.23 % ± 0.11	1.27 % ± 0.64	0.1 % ± 0.06	0.78 % ± 0.39	0.04	0.05 % ± 0.01	0.11 % ± 0.03
35	222	Elemol	1548	20.56	-	-	-	0.08 % ± 0.13	-	0.55	0.02 % ± 0.01	0.24 % ± 0.21
36	250	**$C_{15}H_{22}O_3$**	**-**	21.80	0.24 % ± 0.59	5.18 % ± 5.1	-	18.15 % ± 14.86	22.57 % ± 11.71	11.07	11.77 % ± 8.49	13.91 % ± 4.98
37	248	**Hillone**	1607	21.83	0.21 % ± 0.33	1.33 % ± 1.72	-	1.89 % ± 1.16	6.45 % ± 1.01	0.42	1.29 % ± 1.18	1.72 % ± 0.7
38	-	aliphatic compound	-	23.95	0.61 % ± 0.35	0.24 % ± 0.13	1.2 % ± 0.65	0.11 % ± 0.08	0.96 % ± 0.7	0.05	0.08 % ± 0.02	0.16 % ± 0.02
39	-	methyl-alkane	-	24.91	0.58 % ± 0.32	0.25 % ± 0.1	0.9 % ± 0.34	0.07 % ± 0.07	0.78 % ± 0.56	0.05	0.06 % ± 0.03	0.13 % ± 0.03
40	-	-	-	25.37	0.01 % ± 0.02	0.08 % ± 0.07	-	0.01 % ± 0.02	0.3 % ± 0.38	0.06	-	0.03 % ± 0.05
41	254	Octadecane	1800	26.11	0.24 % ± 0.22	0.11 % ± 0.09	0.51 % ± 0.18	0.04 % ± 0.04	0.46 % ± 0.24	-	0.03 % ± 0.05	0.01 % ± 0.02
42	-	+	-	26.23	0.03 % ± 0.09	0.53 % ± 0.54	-	0.45 % ± 0.6	0.45 % ± 0.32	0.02	0.43 % ± 0.6	0.34 % ± 0.08
43	-	-	-	26.46	-	0.32 % ± 0.36	-	0.49 % ± 0.69	0.22 % ± 0.15	-	0.22 % ± 0.31	0.15 % ± 0.04
44	242	Hexadecanol	1874	27.80	-	-	-	0.02 % ± 0.07	-	0.57	0.22 % ± 0.3	-
45	268	Nonadecane	1900	28.16	0.25 % ± 0.2	0.13 % ± 0.05	0.64 % ± 0.27	0.05 % ± 0.04	0.32 % ± 0.14	0.03	0.06 % ± 0.03	0.15 % ± 0.08
46	-	methyl alkane	-	28.32	0.47 % ± 0.34	0.24 % ± 0.08	1.04 % ± 0.42	0.08 % ± 0.07	0.73 % ± 0.42	0.05	0.09 % ± 0.06	0.21 % ± 0.03
47	-	-	-	28.41	2 % ± 4.34	-	-	-	-	-	-	-
48	270	Hexadecanoic acid, methyl ester	1921	28.65	-	-	-	0.18 % ± 0.18	-	0.02	-	-
49	272	diterpene	-	28.81	-	-	-	0.2 % ± 0.21	-	-	-	-
50	-	methyl-alkane	-	29.16	0.28 % ± 0.23	0.15 % ± 0.06	0.73 % ± 0.26	0.05 % ± 0.06	0.41 % ± 0.21	0.06	0.05 % ± 0.02	0.12 % ± 0.1
51	272	diterpene	-	29.34	0.29 % ± 0.6	-	-	0.02 % ± 0.07	-	-	-	-
52	256	Hexadecanoic acid	1959	29.39	-	-	-	0.05 % ± 0.14	-	0.05	0.44 % ± 0.62	-
53	-	+	-	29.65	-	-	-	0.98 % ± 0.71	0.09 % ± 0.11	0.11	0.95 % ± 0.2	1.98 % ± 1.42
54	-	+	-	29.97	-	0.8 % ± 0.75	-	2.74 % ± 2.2	1.4 % ± 0.78	0.51	3.31 % ± 2.87	3.27 % ± 1.39
55	284	Hexadecanoic acid, ethyl ester	1992	30.01	0.15 % ± 0.27	-	-	0.38 % ± 0.74	0.26 % ± 0.34	0.78	0.14 % ± 0.2	0.04 % ± 0.11
56	282	Eicosane	2000	30.12	0.36 % ± 0.24	0.27 % ± 0.03	0.58 % ± 0.07	0.13 % ± 0.15	0.6 % ± 0.18	0.1	0.15 % ± 0.03	0.23 % ± 0.05
57	284	Hexadecyl acetate	2003	30.25	-	-	-	0.57 % ± 0.98	-	-	0.13 % ± 0.18	-
58	-	alcohol	-	30.30	-	-	-	0.3 % ± 0.38	-	0.25	0.07 % ± 0.1	0.01 % ± 0.03
59	-	-	-	31.02	0.24 % ± 0.67	-	-	-	-	-	-	-
60	272	diterpene	-	31.20	-	-	-	0.33 % ± 0.37	-	0.22	-	-

Table S2. continued.

Nr.	MS	Compound	KI	RT	*A. australis*	*A. simeii*	*A. spec*	*T. carbonaria*	*T. clypearis*	*T. davenportii*	*T. hockingsii*	*T. sapiens*
61	296	Heneicosane	2100	32.05	0.3 % ± 0.26	0.12 % ± 0.03	0.26 % ± 0.23	0.06 % ± 0.06	0.31 % ± 0.13	0.08	0.1 % ± 0.05	0.24 % ± 0.12
62	-	aliphatic compound	-	32.32	0.24 % ± 0.13	0.15 % ± 0.05	0.45 % ± 0.09	0.06 % ± 0.05	0.27 % ± 0.11	0.06	0.07 % ± 0.02	0.14 % ± 0.03
63	288	diterpene	-	32.66	0.05 % ± 0.1	-	-	1.13 % ± 1.16	-	0.08	0.03 % ± 0.05	-
64	284	Octadecanoic acid	-	33.13	-	0.1 % ± 0.09	0.3 % ± 0.27	0.04 % ± 0.11	0.1 % ± 0.11	0.08	-	0.03 % ± 0.06
65	-	aliphatic compound	-	33.14	0.26 % ± 0.2	0.04 % ± 0.07	0.14 % ± 0.24	0.04 % ± 0.13	0.18 % ± 0.23	0.06	0.1 % ± 0.02	0.11 % ± 0.07
66	286	diterpene	-	33.24	-	-	-	0.2 % ± 0.34	-	0.18	0.18 % ± 0.25	0.15 % ± 0.15
67	290	diterpene	-	33.50	1.24 % ± 3.51	-	-	-	-	-	-	-
68	286	diterpene	-	33.62	-	-	-	0.81 % ± 0.69	-	0.51	0.38 % ± 0.53	-
69	310	Docosane	2200	33.84	0.3 % ± 0.27	0.12 % ± 0.05	0.26 % ± 0.23	0.05 % ± 0.06	0.2 % ± 0.1	0.06	0.1 % ± 0.05	0.14 % ± 0.05
70	312	Octadecanol acetate	2209	33.92	-	-	-	-	0.29 % ± 0.54	-	-	0.03 % ± 0.06
71	286	diterpene	-	34.11	-	-	-	1.52 % ± 1.4	-	0.91	0.51 % ± 0.62	0.05 % ± 0.07
72	286	diterpene	-	34.29	-	-	-	0.24 % ± 0.35	-	0.27	0.24 % ± 0.34	0.19 % ± 0.17
73	-	+	-	34.68	-	0.05 % ± 0.08	-	0.16 % ± 0.12	0.09 % ± 0.06	0.08	0.25 % ± 0.1	0.37 % ± 0.14
74	306	diterpene	-	34.92	-	-	-	0.91 % ± 1.58	-	0.44	0.76 % ± 1.07	-
75	322	Tricosene	-	35.11	0.06 % ± 0.08	0.11 % ± 0.11	-	0.1 % ± 0.15	0.41 % ± 0.6	0.13	0.13 % ± 0.18	0.06 % ± 0.14
76	322	Tricosene	-	35.09	-	-	-	0.01 % ± 0.03	0.24 % ± 0.34	-	-	0.02 % ± 0.06
77	286	diterpene	-	35.17	-	-	-	0.1 % ± 0.24	-	0.41	0.28 % ± 0.39	-
78	322	Tricosene	-	35.17	-	-	-	-	1.98 % ± 2.67	-	0.11 % ± 0.16	0.23 % ± 0.56
79	-	diterpene	-	35.22	-	-	-	0.29 % ± 0.47	-	0.25	-	-
80	-	alcohol	-	35.36	-	-	-	0.01 % ± 0.03	-	-	0.09 % ± 0.13	0.45 % ± 0.29
81	324	Tricosane	2300	35.58	7.68 % ± 10.32	0.67 % ± 0.12	0.16 % ± 0.27	1.99 % ± 1.56	0.28 % ± 0.15	0.94	2.99 % ± 3.07	0.6 % ± 0.28
82	286	diterpene	-	35.69	-	-	-	5.72 % ± 6.01	-	4.71	0.74 % ± 1.04	-
83	-	-	-	35.77	-	-	-	-	-	0.79	0.45 % ± 0.63	-
84	-	-	-	35.76	-	-	-	0.13 % ± 0.51	-	-	0.13 % ± 0.19	0.06 % ± 0.09
85	-	-	-	35.92	-	-	-	0.11 % ± 0.3	-	2.02	0.38 % ± 0.54	-
86	386	**terpene**	-	35.94	-	0.09 % ± 0.08	-	0.35 % ± 0.31	0.11 % ± 0.07	-	0.25 % ± 0.35	0.62 % ± 0.25
87	286	diterpene	-	35.97	-	-	-	4.42 % ± 4.92	-	3.36	1.29 % ± 1.83	-
88	-	-	-	36.05	-	-	-	0.2 % ± 0.78	-	-	-	-
89	302	diterpene	-	36.10	-	-	-	0.02 % ± 0.09	-	1.16	0.84 % ± 1.19	-
90	302	diterpene	-	36.12	-	-	-	0.18 % ± 0.71	-	-	-	-

Table S2. continued.

Nr.	MS	Compound	KI	RT	*A. australis*	*A. simeii*	*A. spec*	*T. carbonaria*	*T. clypearis*	*T. davenportii*	*T. hockingsii*	*T. sapiens*
91	386	**terpene**	-	36.16	0.03 % ± 0.09	0.16 % ± 0.19	-	0.82 % ± 0.74	0.15 % ± 0.1	0.55	1.05 % ± 0.32	1.03 % ± 0.49
92	-	-	-	36.25	-	-	-	0.1 % ± 0.4	-	0.76	0.35 % ± 0.49	-
93	386	terpene	-	36.66	-	-	-	-	-	-	0.33 % ± 0.46	-
94	386	**terpene**	-	36.42	0.02 % ± 0.06	0.22 % ± 0.25	-	0.82 % ± 0.5	0.19 % ± 0.13	0.82	1.32 % ± 0.08	1.19 % ± 0.48
95	386	**terpene**	-	36.47	-	0.16 % ± 0.28	-	0.82 % ± 0.43	0.22 % ± 0.15	0.98	1.44 % ± 0.04	1.16 % ± 0.51
96	302	diterpene	-	36.47	-	-	-	0.09 % ± 0.36	-	-	-	-
97	302	diterpene	-	36.64	-	-	-	0.83 % ± 2.58	-	8.82	3.71 % ± 5.25	-
98	386	**terpene**	-	36.63	0.01 % ± 0.04	0.1 % ± 0.11	-	0.62 % ± 0.38	0.11 % ± 0.07	-	0.61 % ± 0.86	0.7 % ± 0.33
99	386	terpene	-	36.91	0.02 % ± 0.05	0.02 % ± 0.04	-	0.37 % ± 0.21	0.12 % ± 0.09	-	0.25 % ± 0.36	0.33 % ± 0.25
100	-	diterpene	-	36.94	-	-	-	0.05 % ± 0.18	-	0.23	-	-
101	-	-	-	37.13	-	1.06 % ± 1.33	-	2.42 % ± 2.15	1.09 % ± 0.82	2.71	3.17 % ± 4.48	2 % ± 1.05
102	386	**terpene**	-	37.15	0.01 % ± 0.03	-	-	-	0.35 % ± 0.7	-	-	-
103	-	-	-	37.18	-	-	-	0.42 % ± 1.16	-	0.35	0.43 % ± 0.61	-
104	386	**terpene**	-	37.25	0.17 % ± 0.47	1.92 % ± 1.94	-	3.82 % ± 2.06	1.28 % ± 0.86	2.29	6.21 % ± 0.82	8.07 % ± 2.29
105	302	diterpene	-	37.30	-	-	-	0.35 % ± 1.37	-	-	-	-
106	302	diterpene	-	37.28	-	-	-	0.19 % ± 0.68	-	0.52	0.3 % ± 0.42	-
107	-	-	-	37.51	0.3 % ± 0.84	-	-	-	-	-	-	-
108	386	**terpene**	-	37.54	0.1 % ± 0.23	1.79 % ± 2.1	-	4.29 % ± 2.39	1.64 % ± 0.45	2.3	5.19 % ± 1.14	6.24 % ± 1.87
109	-	-	-	37.77	-	-	-	0.2 % ± 0.78	-	0.48	-	-
110	-	diterpene	-	38.00	-	-	-	0.22 % ± 0.69	-	1.69	0.82 % ± 1.16	-
111	306	aliphatic compound	-	38.28	-	0.04 % ± 0.07	-	0.24 % ± 0.73	0.07 % ± 0.14	1.16	0.79 % ± 1.12	-
112	350	Pentacosene	-	38.41	0.17 % ± 0.17	27.33 % ± 9.43	-	0.36 % ± 0.23	0.07 % ± 0.08	1.15	1.88 % ± 1.65	0.05 % ± 0.12
113	-	-	-	38.51	-	-	-	0.36 % ± 1.4	-	0.82	-	-
114	350	Pentacosene	-	38.52	-	1.11 % ± 0.51	-	-	0.06 % ± 0.12	-	-	0.14 % ± 0.04
115	-	+	-	38.76	2.39 % ± 6.75	0.08 % ± 0.13	-	0.13 % ± 0.17	-	0.35	0.48 % ± 0.01	0.54 % ± 0.15
116	352	Pentacosane	2500	38.84	12.96 % ± 9.01	25.56 % ± 6.4	0.73 % ± 0.17	9.25 % ± 6.5	0.03 % ± 0.04	4.6	6.12 % ± 2	0.26 % ± 0.08
117	-	+	-	39.28	-	0.61 % ± 1.06	-	0.66 % ± 1.94	-	2.63	4.54 % ± 1.63	2.9 % ± 2.13
118	-	-	-	39.22	-	-	-	0.06 % ± 0.18	-	-	-	-
119	-	methylalkan	-	39.33	0.99 % ± 1.42	-	-	-	-	-	-	-
120	-	-	-	39.37	0.36 % ± 0.67	-	-	-	-	-	-	-

Table S2. continued.

Nr.	MS	Compound	KI	RT	*A. australis*	*A. simeii*	*A. spec*	*T. carbonaria*	*T. clypearis*	*T. davenportii*	*T. hockingsii*	*T. sapiens*
121	-	+	-	39.42	-	0.37 % ± 0.64	-	0.37 % ± 0.9	-	1.77	2.82 % ± 0.11	2.37 % ± 0.86
122	-	-	-	39.45	-	-	-	0.28 % ± 1.08	-	-	-	-
123	-	-	-	39.48	-	-	-	0.09 % ± 0.34	-	1.96	0.49 % ± 0.69	-
124	-	+	-	39.71	-	0.09 % ± 0.15	-	0.13 % ± 0.19	-	0.7	1.11 % ± 0.15	0.79 % ± 0.2
125	-	-	-	39.74	-	-	-	0.12 % ± 0.41	-	0.59	0.38 % ± 0.54	-
126	-	-	-	39.87	-	-	-	0.02 % ± 0.06	-	0.86	-	-
127	-	terpene	-	40.17	-	-	-	0.08 % ± 0.3	-	0.45	0.14 % ± 0.2	-
128	366	Hexacosane	2600	40.35	0.3 % ± 0.14	0.39 % ± 0.25	0.07 % ± 0.12	0.2 % ± 0.13	-	0.2	0.14 % ± 0.04	0.06 % ± 0.03
129	300	diterpene	-	40.64	-	-	-	0.18 % ± 0.29	-	0.78	0.23 % ± 0.32	-
130	-	diterpene	-	40.84	-	0.03 % ± 0.06	-	0.04 % ± 0.14	-	0.1	-	-
131	334	aliphatic compound	-	41.35	0.02 % ± 0.05	-	-	-	-	-	-	1.19 % ± 0.42
132	378	Heptacosene	-	41.47	1.8 % ± 2.46	4.18 % ± 3.07	7.85 % ± 4.65	0.29 % ± 0.21	-	1.16	0.58 % ± 0.69	-
133	378	Heptacosene	-	41.52	0.21 % ± 0.4	0.31 % ± 0.28	1.62 % ± 0.84	0.02 % ± 0.04	-	0.06	-	0.05 % ± 0.12
134	-	**terpene**	-	41.76	-	0.11 % ± 0.19	-	0.12 % ± 0.11	-	0.1	0.25 % ± 0.04	0.54 % ± 0.14
135	380	Heptacosane	2700	41.84	9.1 % ± 7.32	4.57 % ± 2.33	11.14 % ± 2.17	6.91 % ± 3.32	2.04 % ± 1.08	4.36	1.93 % ± 0.68	2.45 % ± 0.46
136	-	methyl-alkane	-	42.27	0.76 % ± 1.02	-	-	-	-	-	-	-
137	392	Octacosene	-	42.84	0.09 % ± 0.14	0.12 % ± 0.11	0.25 % ± 0.44	-	-	-	-	-
138	-	+	-	43.60	-	0.06 % ± 0.11	-	0.05 % ± 0.14	-	0.13	0.34 % ± 0.03	0.41 % ± 0.21
139	404	Nonacosadiene	-	43.87	0.64 % ± 0.83	-	-	-	0.35 % ± 0.7	-	-	-
140	404	Nonacosadiene	-	43.86	0.25 % ± 0.42	-	-	-	-	-	-	-
141	406	Nonacosene	-	44.13	0.93 % ± 1.05	-	-	-	0.08 % ± 0.16	-	-	0.32 % ± 0.43
142	406	Nonacosene	-	44.31	6.46 % ± 6.16	1.08 % ± 0.95	17.46 % ± 14.79	0.11 % ± 0.15	-	1.8	0.82 % ± 1.11	0.28 % ± 0.32
143	-	-	-	44.50	-	-	-	0.37 % ± 1.45	-	-	1.23 % ± 1.75	-
144	-	aliphatic compound	-	44.50	1 % ± 2.84	-	1.87 % ± 3.23	-	-	-	-	-
145	406	Nonacosene	-	44.56	0.05 % ± 0.15	-	0.22 % ± 0.39	-	-	-	-	0.95 % ± 0.65
146	408	Nonacosane	2900	44.64	3.61 % ± 4.35	0.73 % ± 0.54	1.02 % ± 1.31	2.19 % ± 1.99	6.82 % ± 1.69	3.73	3.72 % ± 2.72	9.62 % ± 4.03
147	-	-	-	45.00	-	-	-	0.1 % ± 0.4	-	-	0.26 % ± 0.37	-
148	-	-	-	45.02	0.09 % ± 0.25	-	-	-	-	0.05	-	-
149	432	Untriacontadiene	-	46.57	0.85 % ± 1.35	-	-	-	-	-	-	-
150	432	Untriacontadiene	-	46.64	0.31 % ± 0.29	-	-	-	-	-	-	0.01 % ± 0.01

Table S2. continued.

Nr.	MS	Compound	KI	RT	*A. australis*	*A. simeii*	*A. spec*	*T. carbonaria*	*T. clypearis*	*T. davenportii*	*T. hockingsii*	*T. sapiens*
151	432	Untriacontadiene	-	46.74	0.24 % ± 0.45	-	-	-	-	-	-	0.2 % ± 0.32
152	434	Untriacontene	-	46.78	1.14 % ± 1.2	-	-	-	-	-	-	0.44 % ± 0.63
153	434	Untriacontene	-	46.90	0.45 % ± 0.62	1.41 % ± 2.44	-	-	0.07 % ± 0.14	-	-	0.44 % ± 0.87
154	434	Untriacontene	-	46.98	2.26 % ± 2.68	1.1 % ± 1.47	1.11 % ± 1.33	0.01 % ± 0.03	-	0.25	0.26 % ± 0.31	0.21 % ± 0.19
155	436	Untriacontane	3100	47.26	3.15 % ± 5.38	1.16 % ± 0.98	0.17 % ± 0.29	0.16 % ± 0.16	0.75 % ± 0.32	1.32	0.85 % ± 0.55	1.91 % ± 1.82
156	-	-	-	47.96	0.13 % ± 0.36	-	-	-	-	-	-	-
157	460	Tritriacontadiene	-	49.16	0.39 % ± 0.94	-	-	-	-	-	-	-
158	460	Tritriacontadiene	-	49.28	0.21 % ± 0.58	-	-	-	-	-	-	-
159	460	Tritriacontadiene	-	49.40	0.22 % ± 0.31	-	-	-	-	-	-	-
160	462	Tritriacontene	-	49.44	0.28 % ± 0.42	-	-	-	-	-	-	-
161	426	triterpene	-	49.49	-	-	-	-	-	-	0.03 % ± 0.04	0.72 % ± 0.61
162	462	Tritriacontene	-	49.64	0.62 % ± 1.04	1.48 % ± 1.3	0.29 % ± 0.51	-	-	-	-	0.07 % ± 0.18
163	462	Tritriacontene	-	49.71	0.08 % ± 0.23	0.14 % ± 0.16	-	-	-	-	-	0.03 % ± 0.08
164	424	triterpene	-	49.88	-	-	-	0.03 % ± 0.07	-	1.57	0.13 % ± 0.18	0.23 % ± 0.4
165	464	Tritriacontane	3300	49.95	1.12 % ± 2.18	0.54 % ± 0.51	0.18 % ± 0.31	-	-	-	0.03 % ± 0.05	0.08 % ± 0.2
166	424	triterpene	-	50.10	-	-	-	0.02 % ± 0.03	0.04 % ± 0.07	-	0.02 % ± 0.03	0.51 % ± 0.44
167	424	triterpene	-	50.20	-	-	-	0.01 % ± 0.05	-	1.1	0.1 % ± 0.15	-
168	426	triterpene	-	50.42	-	-	-	-	-	0.34	0.1 % ± 0.15	0.18 % ± 0.24
169	-	triterpene	-	50.41	-	-	-	-	-	-	-	0.29 % ± 0.39
170	426	triterpene	-	50.47	-	-	-	-	-	-	-	0.1 % ± 0.25
171	426	triterpene	-	50.70	-	-	-	0.03 % ± 0.1	-	0.11	0.52 % ± 0.14	2.25 % ± 2.13
172	438	triterpene	-	50.78	-	-	-	-	-	0.55	-	-
173	426	triterpene	-	50.83	-	-	-	0.02 % ± 0.05	-	-	-	1.81 % ± 0.81
174	424	triterpene	-	50.86	-	-	-	-	-	0.53	0.28 % ± 0.39	-
175	426	triterpene	-	50.92	-	-	-	-	-	-	-	0.73 % ± 1.78
176	424	triterpene	-	51.45	-	-	-	-	-	-	0.15 % ± 0.21	0.57 % ± 0.93
177	424	triterpene	-	50.97	-	-	-	0.06 % ± 0.14	-	-	-	2.96 % ± 2.33
178	424	triterpene	-	50.86	-	-	0.18 % ± 0.32	0.06 % ± 0.12	0.15 % ± 0.17	1.7	-	-
179	480	Hexadecanoic acid, hexadecyl ester	-	50.99	-	0.1 % ± 0.1	-	-	-	0.6	2.13 % ± 3.01	1.03 % ± 2.52

Table S2. continued.

Nr.	MS	Compound	KI	RT	*A. australis*	*A. simeii*	*A. spec*	*T. carbonaria*	*T. clypearis*	*T. davenportii*	*T. hockingsii*	*T. sapiens*
180	426	triterpene	-	51.36	-	-	-	0.02 % ± 0.1	-	0.07	0.53 % ± 0.24	1.33 % ± 0.4
181	426	triterpene	-	51.49	-	-	-	0.07 % ± 0.23	-	0.51	1.74 % ± 0.76	4.16 % ± 1.81
182	468	triterpene	-	52.09	-	-	-	0.02 % ± 0.06	0.37 % ± 0.32	-	0.08 % ± 0.11	0.38 % ± 0.73
183	468	triterpene	-	52.75	-	-	-	-	-	-	0.36 % ± 0.51	-
184	468	triterpene	-	52.92	-	-	-	-	-	-	0.61 % ± 0.87	-
185	468	triterpene	-	52.36	-	-	-	-	-	0.17	-	0.32 % ± 0.68
186	-	triterpene	-	52.45	-	-	-	-	-	-	0.3 % ± 0.43	0.55 % ± 0.63
187	468	triterpene	-	53.08	-	-	-	0.12 % ± 0.17	0.1 % ± 0.2	0.06	-	0.65 % ± 0.68
188	468	triterpene	-	53.11	-	-	-	-	-	1	1.56 % ± 1.65	0.37 % ± 0.47
189	-	-	-	53.49	-	-	0.47 % ± 0.82	0.07 % ± 0.25	-	-	0.15 % ± 0.21	-
190	-	-	-	54.14	0.12 % ± 0.33	-	-	-	-	-	-	0.14 % ± 0.34
191	-	triterpene	-	54.49	-	-	-	-	-	-	-	0.31 % ± 0.5
192	-	ester	-	54.76	-	0.23 % ± 0.31	-	-	-	1.16	1.5 % ± 2.12	-
193	-	triterpene	-	54.84	-	-	-	-	-	-	-	0.41 % ± 0.71
194	-	-	-	55.02	-	-	0.3 % ± 0.52	0.01 % ± 0.02	-	0.18	-	0.24 % ± 0.42
195	-	-	-	55.56	-	-	-	-	-	2.03	-	-
196	-	triterpene	-	56.44	-	-	-	-	-	-	-	0.14 % ± 0.35
197	468	triterpene	-	56.83	-	-	-	-	-	-	-	0.15 % ± 0.37
					N = 8	N = 3	N = 3	N = 15	N = 4	N = 1	N = 2	N = 6

List of Publications

(6) Leonhardt SD, Jung LM, Schmitt T & Blüthgen N (in press) Terpenoids tame aggressor: role of chemicals in stingless bee communal nesting. ***Behavioral Ecology and Sociobiology.***

(5) Leonhardt SD, Blüthgen N & Schmitt T (2009) Smelling like resin: terpenoids account for species-specific cuticular profiles in Southeast-Asian stingless bees. ***Insectes Sociaux*** 56 (2): 157-170.

(4) Leonhardt SD & Blüthgen N (2009) A sticky affair: resin collection by Bornean stingless bees. ***Biotropica*** 41 (6): 730-736.

(3) Leonhardt SD, Tung J, Leal M & Drea CM (2008) Seeing red: behavioral evidence of trichromatic color vision in strepsirrhine primates. ***Behavioral Ecology*** 20 (1): 1-12.

(2) Leonhardt SD, Brandstaetter AS & Kleineidam CJ (2007). Reformation process of the neuronal template for nestmate recognition cues in the carpenter ant (*Camponotus floridanus*). ***Journal of Comparative Physiology A – Neuroethology, Sensory, Neural and Behavioral Physiology*** 193 (9): 993-1000.

(1) Leonhardt SD, Dworschak K, Eltz T & Blüthgen N (2007) Foraging loads of stingless bees and utilisation of stored nectar for pollen harvesting. ***Apidologie*** 38 (2): 125-135.

Congress Contributions

(5) Leonhardt SD, Schmitt T, Blüthgen N (2009): Does a group of chemical compounds trigger interspecific associations in social bees? Talk at the *1st Central European Meeting of the International Union for the Study of Social Insects (IUSSI)*, München, Germany.

(4) Leonhardt SD, Blüthgen N, Schmitt T (2009): An unexpected "appeasement signal": the role of sesquiterpenes in the chemical profile of tropical stingless bees. Talk at the *25th annual meeting of the International Society of Chemical Ecology (ISCE)*, Neuchâtel, Switzerland.

(3) Leonhardt SD, Schmitt T, Blüthgen N (2009): Resin collection in a tropical stingless bee community. Talk at the *Joint meeting of Association for Tropical Biology and Conservation (ATBC) & Society for Tropical Ecology (GTÖ)*, Marburg, Germany.

(2) Leonhardt SD, Blüthgen N, Schmitt T (2009): Cuticular terpenoids in Old World stingless bees – role of trees? Talk at the *15th PhD Meeting of Evolutionary Biology of the Deutsche Zoologische Gesellschaft (DZG)*, München, Germany.

(1) Leonhardt SD, Blüthgen N, Schmitt T (2008): A sticky affair: collection and use of resin by Bornean stingless bees (Meliponini, Apidae). Talk at the *Multitrophic Interactions Workshop*, Göttingen, Germany.