

## Research



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# Genes versus environment: geography and phylogenetic relationships shape the chemical profiles of stingless bees on a global scale

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Chemical compounds are highly important in the ecology of animals. In social insects, compounds on the body surface represent a particularly interesting trait, because they comprise different compound classes that are involved in different functions, such as communication, recognition and protection, all of which can be differentially affected by evolutionary processes. Here, we investigate the widely unknown and possibly antagonistic influence of phylogenetic and environmental factors on the composition of the cuticular chemistry of tropical stingless bees. We chose stingless bees because some species are unique in expressing not only self-produced compounds, but also compounds that are taken up from the environment. By relating the cuticular chemistry of 40 bee species from all over the world to their molecular phylogeny and geographical occurrence, we found that distribution patterns of different groups of compounds were differentially affected by genetic relatedness and biogeography. The ability to acquire environmental compounds was, for example, highly correlated with the bees' phylogeny and predominated in evolutionarily derived species. Owing to the presence of environmentally derived compounds, those species further expressed a higher chemical and thus functional diversity. In Old World species, chemical similarity of both environmentally derived and self-produced compounds was particularly high among sympatric species, even when they were less related to each other than to allopatric species, revealing a strong environmental effect even on largely genetically determined compounds. Thus, our findings do not only reveal an unexpectedly strong influence of the environment on the cuticular chemistry of stingless bees, but also demonstrate that even within one morphological trait (an insect's cuticular profile), different components (compound classes) can be differentially affected by different drivers (relatedness and biogeography), depending on the functional context.

## 1. Introduction

Different species of plants and animals can show striking variation in their phenotypes, even when they are closely related to each other. How (interacting) evolutionary processes drive these differences between species' phenotypes is, however, still unclear for most organisms [1,2]. This is particularly true for chemical traits in insects [3]. An interesting chemical trait to investigate is the cuticular profile of social insects, because it represents a complex blend of several compound classes that can have largely different functions and whose composition is likely to be shaped by different evolutionary processes. The cuticular chemistry is known to be affected by genetic relatedness [3–5], season [6], geographical location [4,7–9] and diet [10–12], but few studies have investigated whether environmental and/or genetic factors differentially affect different classes of cuticular compounds. Cuticular profiles predominantly

**Table 1.** Functions of self-produced (SP) and environmentally derived (ED) compound classes typically present in the cuticular chemical profiles of stingless bees (functions in brackets have not been proved directly, but are derived from behavioural assays).

compound class	origin	function	reference
monoterpenes	ED/SP	alarm pheromones, scent marks, (protection against predators)	[31,32]
sesquiterpenes	ED	appeasement of heterospecifics, scent marks, (protection against predators)	[31,33,34]
diterpenes	ED	unknown	—
triterpenes	ED	(protection against predators)	[31]
other terpenoids	ED/(SP)	unknown	—
<i>n</i> -alkanes	SP	protection against desiccation, damage and predators, nest-mate recognition, scent marks	[19,35,36]
alkenes and alkadienes	SP	protection against desiccation, damage and predators, nest-mate recognition, scent marks	[19,35–38]
methyl-alkanes	SP	unknown	—
esters, alcohols, aldehydes and fatty acids	SP	alarm pheromones, nest-mate recognition (fatty acids), scent marks	[35,36,39]

comprise non-polar hydrocarbons (*n*-alkanes, alkenes and methyl-branched alkanes), but can be enriched by polar compounds (such as alcohols, esters, ketones, aldehydes or oxidized terpenes; [13–19]). These compounds have a variety of functions. They protect insects against desiccation, cuticle abrasion, infection and predators, but can also play a dominant role in the communication within and among insect species [19–21]. They can differ qualitatively and/or quantitatively among species [5,19,22–25], but the evolutionary mechanisms and factors responsible for the diversification of cuticular profiles are still poorly understood. Moreover, because different compound groups fulfil different functions in different species or even within the same species, they may be differentially affected by abiotic and biotic environmental factors [26,27] as well as by phylogenetic constraints, which may lead to antagonistic evolutionary processes [3,5]. However, the composition of compound classes can also vary independently of their functional context, for instance with season [6] and/or geographical location [4,7–9], which suggests an additional influence of environmental factors.

An essential tool for better understanding the different factors driving the diversity and pattern formation of chemical profiles is studies that relate the cuticular chemistry of insects to their phylogenetic relatedness [3]. Such studies are still scarce [3]; and, except for the ground-breaking work of Jim Cane [28], they are basically absent in an important group of social insects: bees. The lack of chemical phylogenies for bees is surprising giving that—compared with other Hymenoptera—some groups of bees show particularly diverse cuticular profiles, which can include both genetically determined and environmentally derived compounds [29].

We have composed a comprehensive database for the cuticular chemical profiles of 40 stingless bee species (Hymenoptera: Apidae: Meliponini) of Southeast Asia, Australia and Central America. Here, we match the data on cuticular profiles of stingless bees with a recently published extensive phylogeny [30] to better understand the influence of phylogenetic and environmental factors on the diversification of different groups of compounds typically found in cuticular profiles of stingless bees (table 1). Stingless bees represent a highly diversified group of eusocial bees that are typically

found in the tropical and subtropical regions of the world. The phylogeny of the group suggests three separate 80–65 Ma lineages: a New World clade, an Afrotropical clade and a Southeast Asian clade [30]. To date, stingless bees appear to be unique among social insects in that some species' cuticular profiles include environmentally derived compounds acquired from plant resins in their cuticular profiles [9,22,29,40]. Resin-derived compounds have so far only been described for species of Southeast Asia and Australia, whereas none of the studies on chemical profiles of New World stingless bees reported any compounds that could be traced back to plant resins [37,41–48]. Resin-derived compounds protect their bearers against predators [31] as well as (most probably) microbes (table 1). They can further reduce aggression in conspecifics ([33]; table 1). By enriching their chemical profiles with these compounds, the bees tremendously increase their cuticular profile diversity and hence the functional diversity of their chemical repertoire, thereby exceeding most (if not all) other eusocial insects [29]. Consequently, the cuticular chemistry of stingless bees represents a complex and highly diverse blend of self-produced and environmentally derived compounds ([22,38,42,43,45,47]; table 1) and hence a complex trait that comprises the complete spectrum from genetically determined to environmentally derived components. It is highly likely that the different components (i.e. compound classes) are differentially affected by phylogenetic and environmental factors. Stingless bees therefore represent a unique group of insects for disentangling the differential influence of evolutionary relatedness and geography on a complex trait.

By comparing the bees' cuticular chemistry with their phylogeny, we aim to elucidate whether resin-derived compounds are indeed a unique feature of Australasian stingless bees or whether bees in the New World also secondarily evolved the ability to include compounds from plant resins in their cuticular profiles, and, if so, how frequently this ability has evolved, and whether it shows a phylogenetic or geographical signal. Because resin-derived compounds are directly transferred from plant resins to the bees' body surfaces without being structurally modified, and because different species tend to collect resin from largely the same plant species [29], we expect that the composition of this

compound group is strongly influenced by the local plant flora and hence by a species' geographical origin.

With regard to self-produced compound groups (i.e. compounds produced in glands and compounds acquired from self-produced comb wax, table 1), we expect to find a strong phylogenetic signal with high chemical similarity among closely related species.

## 2. Material and methods

### (a) Sampling of bees

Bees for chemical analyses were collected between 2006 and 2009. We caught between two and seven departing foragers (depending on the bees' body size) from each colony by attaching a clean, clear plastic bag to the nest entrance. Multiple colonies per species were sampled in Southeast Asia, Australia and Central America. Species were identified with the help of taxonomic keys and/or local experts. The bees were killed in a freezer and their legs inspected for contamination with pollen or resin. If such remnants of plant products were still attached to the corbiculae (enlarged part of the hind legs), the legs were removed before transferring the bees into 2 ml vials containing pure hexane (Sigma–Aldrich, Munich, Germany). To avoid extraction of gland contents, the bees were kept in hexane for 3 min only.

Specimens used for the phylogenetic analyses were selected from Rasmussen & Cameron [30]. As there was no direct match between the specimens sampled for chemical analyses here and those included for the phylogenetic analyses, we selected a subset of taxa for the phylogeny that either represent the same species as studied here, or the closest relative as based on morphology and published literature.

### (b) Chemical data

The chemical profiles were analysed by a Hewlett Packard HP 6890 Series gas chromatograph coupled to a Hewlett Packard HP 5973 Mass Selective Detector (Agilent Technologies, Böblingen, Germany). We used a DB-5 fused silica capillary column (30 m × 0.25 mm ID; d.f. = 0.25 µm; J & W, Folsom, CA, USA) and helium as carrier gas (constant flow of 1 ml min<sup>-1</sup>). Injection was carried out at 250 °C in the splitless mode for 1 min. Temperature was raised from 60 °C to 300 °C with a 5 °C min<sup>-1</sup> heating rate and held at 300 °C for 10 min. Electron impact mass spectra were recorded at 70 eV. Compounds were characterized by comparing their mass spectra and retention indices with mass spectra and/or retention indices of three commercially available libraries (Wiley 275, NIST 98 and Adams EO library 2205), and, where standards were available, by comparing them with synthetic standards. Mass spectra of terpenoids were further compared with mass spectra of compounds from tree resins that are known to contain terpenoids [49]. Peaks with the same mass spectra and retention indices were regarded as the same compounds across species. We classified compounds into (i) resin-derived compounds (mainly mono-, sesqui- and putatively identified di- and triterpenes, as well as other terpenoids), (ii) potentially self-produced compounds (*n*-alkanes, alkenes, alkadienes, methyl-branched alkanes, aldehydes, alcohols, esters and lactones), and (iii) unidentified compounds.

### (c) Phylogenetic data

The original Bayesian tree based on five gene fragments (16S rRNA, ArgK, EF-1a and opsin) from Rasmussen & Cameron [30] was used for estimating the relationships amongst taxa in this study. The original tree was inferred in MRBAYES v. 3.1.2. [50] with an analysis including 12 million generations, four chains, mixed-models, flat priors and saving trees every 1000 generations. Further details are available in Rasmussen & Cameron [30]. Pruning of the dataset

and the tree to represent the 40 species analysed chemically was done in MESQUITE v. 2.75 [51]. Twelve species for which we had the chemical profiles were not part of the original phylogeny, so for those species, we included a closely related species.

### (d) Statistical analysis

To account for the chemical variation ultimately caused by differences in the geographical location or colony membership of the individuals sampled, we pooled all colonies from a given species. Overall, we included 773 compounds in our chemometrical analyses. For visualization of chemical data, we performed agglomerative cluster analyses based on the unweighted pair group method using arithmetic means of Bray–Curtis dissimilarities. The Bray–Curtis dissimilarity matrix was based on all chemical compounds that accounted for at least 0.5 per cent in the chemical profile of at least one bee species. We chose the Bray–Curtis dissimilarity because it weighs presence more than absence, which is more suitable for zero-inflated datasets, such as represented by chemical datasets, than the Euclidean distance [52]. Additional analyses were performed with distances based solely on the presence/absence (0,1 matrix, using the Sørensen coefficient) of compounds.

To understand whether cuticular profiles were more strongly affected by genetic and/or geographical factors, we tested for correlations between (i) the chemical and phylogenetic data, as well as (ii) the chemical and geographical data, using Mantel tests based on Bray–Curtis dissimilarities of the chemical data, on uncorrected *p*-distances of the phylogenetic data and on the actual distances (km) between our sampling coordinates (999 permutations, library vegan in R, R Foundation for Statistical Computing, v. 2011, Vienna, Austria, ISBN 3-900051-07-0, <http://www.R-project.org>). To test whether different compound classes showed different phylogenetic and/or geographical patterns, we performed separate Mantel tests for (i) all compounds, (ii) only resin-derived compounds, (iii) only non-resin-derived/self-produced compounds, (iv) only *n*-alkanes, (v) only alkenes and alkadienes, and (vi) only esters, alcohols, aldehydes and fatty acids. Environmentally derived compounds easily contaminate profiles of insects (e.g. volatile terpenes of the floral bouquet are present as traces in chemical profiles of honeybees foraging on flowers; T. Schmitt, personal observation). We thus considered only those species capable of transferring resin-derived compounds to their body surfaces for which the compounds accounted for at least one fifth (20%) of the profiles' overall peak area. Note that the cleptoparasitic *Lestrimelitta limao* was not included in this group, although monoterpenes (citral) accounted for 65 per cent of the peak area of all compounds in its profile. However, citral is known to be produced by the bees themselves and to be released from their mandibular glands [32]. Because resin-derived compounds accounted for such significant proportions in only 22 out of the 40 species studied (see figure 1 and electronic supplementary material, table S1), they distort the ratio and hence proportions of resin-derived to self-produced compounds. To restrict analyses (iii) and (vi) to self-produced compounds, we standardized their proportions (with the sum of all self-produced compounds equalling 1). We performed additional Mantel tests separately for all Old World (Australia and Southeast Asia) and all New World bees (Central America) to see whether the two major stingless bee lineages responded differentially to genetic and geographical/environmental constraints.

We inferred whether the ability to acquire resin-derived compounds was associated with the phylogeny by the parsimony-based permutation tail probability test [53] in MACCLADE v. 4.08 [54], using 1000 permuted replicates of the host character and comparing the number of steps of the original tree with that of the null distribution.

Finally, we tested whether the chemical diversity of profiles (calculated as the Shannon diversity of all compounds of a species)



**Figure 1.** Phylogeny of 37 stingless bee species (adapted from Rasmussen & Cameron [30]). The upper branch comprises all the species from the New World (NW: here restricted to Central America), whereas the lower branch comprises all Old World species (OW: here restricted to Borneo and Australia). Bees marked in bold represent species with significant amounts of resin-derived compounds (greater than 20%) in their cuticular profiles (see the electronic supplementary material, table S1). Asterisks indicate species for which closely related species were used for chemical and phylogenetic analyses. *Tetragonula davenporti*, *Austroplebeia australis* and *Austroplebeia cincta* are not displayed.

correlated with the species' phylogenetic age. Age was estimated from the complete Bayesian phylogeny using penalized likelihood implemented in r8s 1.71 [55] with the *Liotrigonopsis* age fixed at 44.1 Ma, as in Rasmussen & Cameron [30]. We controlled for multiple testing using a false discovery rate (FDR) correction of  $p$ -values. All analyses, except when noted, were performed in R [56].

### 3. Results

Chemical profiles of 40 stingless bees from Southeast Asia, Australia and Central America comprised both self-produced compounds (i.e.  $n$ -alkanes, alkenes, methyl-branched alkanes,

esters and alcohols) and compounds derived from plant resins (i.e. mono-, sesqui-, di-, triterpenes as well as other terpenoids and some unidentified compounds; electronic supplementary material, table S1).

#### (a) The ability to acquire resin-derived compounds and phylogeny

As expected, resin-derived compounds were common in species of Southeast Asia and Australia, where they accounted for at least 20 per cent of the total peak area in 10 out of 15 (67%) species (when *Tetragonula davenporti*, *Austroplebeia australis*,

**Table 2.** Mantel test results (Mantel coefficient of correlation ( $r_M$ ) and  $p$ -values) for chemical distance matrices based on proportions (prop.) and presence/absence (P/A) of compound groups correlated against phylogenetic and geographical distances in stingless bee species from Southeast Asia, Australia and Central America. Italic  $p$ -values are significant after FDR correction.

		phylogenetic correlations				geographical correlations			
		prop.		P/A		prop.		P/A	
		$r_M$	$p$	$r_M$	$p$	$r_M$	$p$	$r_M$	$p$
all compounds		0.35	0.001	0.36	0.001	0.35	0.001	0.42	0.001
resin-derived compounds	all	0.49	0.001	0.45	0.001	0.54	0.001	0.48	0.001
self-produced compounds	all	0.20	0.006	0.30	0.001	0.18	0.008	0.30	0.001
	alkanes	0.01	0.43	0.10	0.101	0.03	0.606	0.08	0.169
	alkenes and alkadienes	0.20	0.004	0.16	0.010	0.16	0.014	0.14	0.030
	esters, alcohols, aldehydes and acids	0.26	0.001	0.43	0.001	0.25	0.001	0.37	0.001

*Austroplebeia cincta* and *Austroplebeia symei* were taken into account; see figure 1 and electronic supplementary material, table S1). They were absent in the minute taxa, that is, the taxa embedded within the separate Afrotropical clade of stingless bees [30]. Resin-derived compounds were also found in 12 out of 25 (48%) species of Central America (see figure 1 and electronic supplementary material, table S1). Again, they were lacking or present in only minor amounts in the first derived, ancestral groups, including *Trigonisca s.l.* (i.e. *Dolichotrigona*) and *Melipona* (see figure 1 and electronic supplementary material, table S1).

The ability to acquire resin-derived compounds was highly associated with the global phylogeny ( $p$ -value < 0.001) and showed significantly fewer steps than if resin-derived compounds had been found randomly distributed among the taxa (figure 1).

### (b) Correlations between chemistry and phylogeny

When all compound groups and bee species were included in the analysis, phylogenetically more closely related species were chemically similar independent of whether proportions or the presence/absence of compounds were considered (see table 2 and electronic supplementary material, figure S1a). The correlation between chemical similarity and phylogenetic relatedness was even higher when only resin-derived compounds were taken into account (see table 2 and electronic supplementary material, figure S2). By contrast, when only  $n$ -alkanes or alkenes and alkadienes were included in the analysis, closely related species showed no (alkanes) or little (alkenes and alkadienes) similarity (table 2), whereas they were highly similar with regard to oxidized compounds (i.e. alcohols, aldehydes, acids and esters; table 2).

When species of the New World (Central America) and the Old World (Southeast Asia and Australia) were considered separately, the cuticular chemistry of species of both lineages generally showed no correlation with their phylogeny, except for alkenes and alkadienes as well as oxidized compounds in New World bees (table 3).

### (c) Correlations between chemistry and geography

When all compound groups and bee species were included in the analysis, similarity in cuticular profiles was generally highest among sympatric species (table 2). It was particularly high when only environmentally derived compounds were considered (table 2). However, alkanes as well as alkenes and alkadienes showed no or little correlation with the geographical distribution of species. Interestingly, when we performed separate analyses for New and Old World bees, we found no geographical signal in New World species (except for a weak signal in the proportions of oxidized compounds), but a strong geographical signal for all compound groups in Old World bees (table 3).

### (d) Chemical diversity and species age

The chemical diversity of the bees' profiles decreased with phylogenetic age (Pearson correlation:  $r = 0.32$ ,  $p = 0.04$ ), indicating that phylogenetically more derived species have a more complex composition of compounds on their body surface (figure 2).

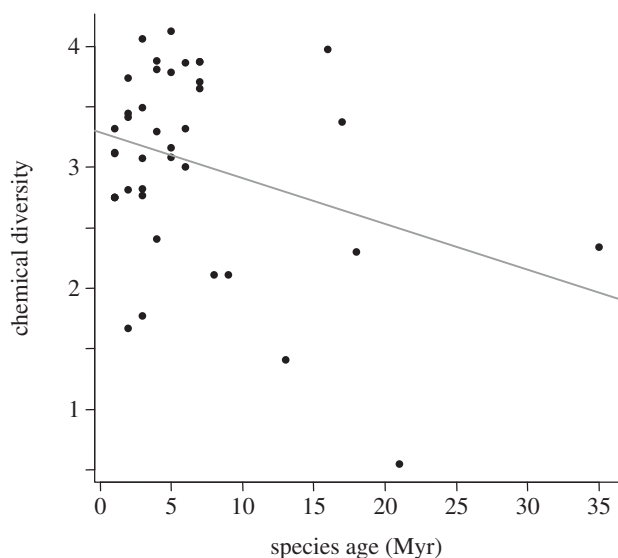
## 4. Discussion

The cuticular chemistry of social insects is known to be influenced by genetic and environmental factors, but we are, to our best knowledge, the first to investigate whether environmental and/or genetic factors differentially affect different components of an insect's cuticular profile (i.e. different groups/classes of cuticular compounds). We chose stingless bees as our model organism because they represent a particularly interesting insect taxon to address the relationship between phylogeny, geographical origin and variation in cuticular chemistry owing to their global distribution, highly social system and complex chemical ecology.

By comparing the chemical profiles of 40 species with their molecular phylogeny and geographical range, we show that the ability to include resin-derived compounds in

**Table 3.** Mantel test results (Mantel coefficient of correlation ( $r_M$ ) and  $p$ -values) for chemical distance matrices based on proportions (prop.) and presence/absence (P/A) of compound groups correlated against phylogenetic and geographical distances separately for New and Old World bees. *Italic  $p$ -values are significant after FDR correction.*

	New World bees						Old World bees												
	phylogenetic correlations			geographical correlations			phylogenetic correlations			geographical correlations									
	prop.	P/A	$r_M$	prop.	P/A	$r_M$	prop.	P/A	$r_M$	prop.	P/A	$r_M$	prop.	P/A	$r_M$				
all compounds	0.14	0.100	0.18	0.097	0.118	0.14	0.086	0.15	0.103	0.03	0.521	0.23	0.125	0.07	0.251	0.29	0.026	0.80	0.001
resin-derived compounds	0.10	0.154	0.13	0.118	0.14	0.728	0.22	0.839	0.03	0.549	0.06	0.256	0.01	0.374	0.620	0.81	0.008	0.95	0.002
self-produced compounds	0.11	0.175	0.18	0.086	0.15	0.103	0.03	0.521	0.23	0.125	0.07	0.251	0.29	0.026	0.80	0.001			
alkanes	0.05	0.292	0.10	0.171	0.03	0.401	0.07	0.622	0.10	0.600	0.02	0.379	0.18	0.099	0.91	0.003			
alkenes and alkanenes	0.21	0.047	0.17	0.096	0.03	0.364	0.01	0.460	0.13	0.222	0.20	0.142	0.01	0.450	0.55	0.001			
esters, alcohols, aldehydes and acids	0.29	0.003	0.57	0.001	0.16	0.027	0.10	0.243	0.03	0.378	0.19	0.099	0.35	0.073	0.51	0.001			



**Figure 2.** Correlation between the chemical diversity (calculated as Shannon diversity) of the surface profiles of 40 stingless bee species and their phylogenetic age (adapted from Rasmussen & Cameron [30]).

the cuticular profile is highly correlated with the bees' phylogeny and particularly common in evolutionary derived species, but lacking in all less derived groups. Moreover, this trait has evolved at least twice, in both the Old World and the New World lineage, and was lost only once in our phylogeny (i.e. in *Trigona corvina*), indicating that this ability has become a beneficial and hence stable trait in the more recent history of stingless bees. The global distribution of this trait was unexpected given that none of the previous studies on cuticular profiles of New World stingless bees reported on resin-derived compounds [37,41–48]. However, most of these studies investigated species in genera from less derived lineages that, according to our results, lack resin-derived compounds (except for Nunes *et al.* [37,45,46], who studied the cuticular profile of *Frieseomelitta varia*).

### (a) Resin and resin-derived compounds as a beneficial trait in stingless bees

Stingless bees have—as indicated by their common name—a secondarily strongly reduced, non-functional sting. Instead, they show a highly sophisticated nesting behaviour, with nests built in various kinds of holes and crevices or even at exposed locations [57]. Hence, nesting location and structure represent an important factor in the ecology of stingless bees and are likely to have influenced their chemical profiles. Like honeybees, stingless bees acquire part of their cuticular profile from the surrounding nest material only after emerging from their brood cells [37]. It is possible that they acquire resin-derived compounds in a similar way, because stingless bees are known to mix plant resins collected with wax while building their nests [57]. Resin consequently represents a resource that is essential for the nesting ecology of stingless bees and thus for their colony growth and development [58,59]. Moreover, the inclusion of foreign material in the nest (material) was found to be a key innovation in the evolution and diversification of bees in general [60] and has probably facilitated the evolution of sociality in stingless

bees as well as their successful diversification in tropical ecosystems worldwide [59].

Hence, the bees' nest environment presumably represents an atmosphere rich in compounds derived from plant resins. The likely subsequent transfer of these compounds to their body surfaces, which results in an increased chemical diversity, may thus represent an additional advantage of those species that are capable of doing so, which may explain why it was lost only once (in *T. corvina*). *Trigona corvina* is unique among the species sampled in our study in that it has entirely exposed nests that are located on tree branches and consist mainly of pollen exines from bee excrements and not of a mixture of wax and resin [61,62]. Their unique nesting behaviour and highly aggressive nest defence may explain why they do not rely on resin and hence resin-derived compounds.

The presence of resin-derived compounds in the chemical profiles of particularly evolutionary derived species may also explain why they show a higher chemical diversity than less derived species, because the profiles of the derived bees comprise both self-produced and (additionally) environmentally derived compounds. With the number of compounds increased, their functional diversity is likely to increase as well [29]. Compounds on the surface of insects fulfil various purposes such as protecting their bearers against cuticle abrasion, desiccation and microbial or predator attack, as well as providing cues for communication and recognition [19]. Consequently, an increased number of compounds and a higher diversity of compound classes in the bees' chemical profiles may render these functions more efficient and/or provide the base for new functions, e.g. appeasement of other species [33], thereby potentially increasing the fitness of those taxa.

### (b) Chemical similarity, biogeography and phylogenetic relatedness

Our analyses further revealed that the distribution patterns of different groups of chemical compounds could be differentially affected by genetic relatedness and geography, likely due to different functions of these compound groups.

#### (i) Resin-derived compounds

When we confined our analysis to species capable of acquiring resin-derived compounds (thus excluding all species without those compounds in their chemical profiles), the chemical similarity of resin-derived cuticular compounds was highest for species from the same geographical origin (i.e. continent; see the electronic supplementary material, figure S2). This pattern indicates a strong influence of the surrounding resin plant flora typical for each continent (e.g. eucalypts in Australia and dipterocarp trees in Southeast Asia). As bees do not alter, but only filter resin compounds [29], species of a specific geographical region generally share the same resin-derived compounds. However, the profile chemistry of bees of the same geographical region can still differ qualitatively, e.g. sesquiterpenes (typical for dipterocarps of Southeast Asia) can be present in one bee species of Borneo, but lacking in another [22].

By including resin compounds in their cuticular profiles, the bees can make use of their inherent antimicrobial and repellent properties and hence do not need to modify them, which probably explains the strong influence of the

environment and hence biogeography on this group of cuticular compounds.

## (ii) Genetically determined compounds

Among genetically determined compounds, different classes were differentially influenced by relatedness and geography. For instance, oxidized compounds were more similar among closely related than among distantly related species, whereas non-polar hydrocarbons were less correlated with the bees' phylogeny (table 2). We thus propose that the degree of correlation between a compound class and genetic relatedness or geography depends on its functional context.

Interestingly, not only environmentally derived compounds but also self-produced and highly genetically determined compounds (i.e. non-polar aliphatic hydrocarbons) showed a biogeographic pattern (table 2), with relatively distantly related species that coexist in the same region (e.g. *Austroplebeia* species and *Tetragonula* species in Australia) showing more similar hydrocarbon profiles than more closely related species inhabiting different regions (e.g. *Tetragonula* species of Australia and *Tetragonula* species of Borneo; electronic supplementary material, figure S1*a*). This finding fully contradicts our original hypothesis that genetically determined compounds correlate with phylogeny, but not with geography.

Consequently, the environment can have an unexpectedly strong influence on the composition of genetically determined compounds in stingless bees. However, this strong geographical signal of self-produced compounds was primarily found in Australasia, whereas our analysis revealed no geographical signal for New World bees (table 3).

Here, we found instead that closely related species of Central America shared similar alkene, alkadiene and particularly oxidized compound profiles, revealing a strong phylogenetic signal for those compound groups (table 3). This correlation between chemical similarity and relatedness found for alkenes agrees with findings in ants, where variation in alkenes was also smaller among workers of closely related than of distantly related species [4,5]. Van Wilgenburg *et al.* [5] suggested that this pattern indicates a gradual mode of evolution for alkenes in ants and no essential role of them in the discrimination of closely related species [4,5], but see Martin & Drijfhout [23] who found no correlation between cuticular hydrocarbons and phylogeny in ants. In bumblebees, chemical distances of alkenes also closely match phylogenetic distances (T. Schmitt, C. Jarvers, S. Leonhardt, personal observation), whereas the composition of *n*-alkanes is relatively stable across species [24], further pointing to potentially different functions of these two compound groups. The entire bouquet of Dufour's gland secretions (used for nest cell linings) in turn nicely matches the phylogenies in Colletidae, Halictidae, Oxaeidae and Andrenidae [28,63], indicating a uniform function of all components within Dufour's glands.

Note that compared with our global analyses with all bee species from all continents included, the phylogenetic and geographical correlations with the bees' cuticular chemistry were overall weaker and partly disappeared when we performed separate analyses for the two stingless bee lineages (tables 2 and 3). This change in significances indicates that the most prominent differences in cuticular chemistry—with regard to both genetically determined and environmentally derived compounds—were found between the major

stingless bee lineages, which have diverged both genetically and geographically [30]. Our analysis of the New World lineage was largely confined to bees sampled in Central America, and hence to a relatively narrow geographical range, which may explain why we found only phylogenetic patterns in the cuticular chemistry of the New World bee species. Our sampling range in the Old World was comparatively larger and hence able to detect a strong geographical signal for both environmentally derived and self-produced genetically determined compounds.

Our findings suggest that the environment has a generally stronger influence on cuticular profiles in stingless bees than previously expected for insects in general [5]. This is in accordance with the views of many evolutionary biologists, who have regularly emphasized the important effect of geographical/environmental factors and their interaction with genetic factors on phenotypic variation between species ([64], reviewed by Mitchel-Olds *et al.* [1]). Our results further agree with patterns found for various traits in other animal groups (e.g. body size in terrestrial vertebrates or thermal niche breadth in mammals) that also revealed a comparatively stronger influence of spatial/environmental than phylogenetic factors [65,66].

## 5. Conclusion

We here provide the first comparison between the phylogeny, geographical origin and the cuticular chemistry of an ecologically important and widely distributed group of social bees. Our findings indicate that the ability to derive compounds from plant resins and include them in the cuticular chemical profile is a relatively stable and hence most probably beneficial trait of evolutionary more derived species, which is strongly correlated with the bees' phylogeny. It was adopted several times by different stingless bee lineages and is generally lacking in less derived species. Moreover, different classes of cuticular compounds were differentially affected by genetic relatedness and geography and hence correlated more or less with the bees' phylogeny, resulting in different distribution patterns that can probably be explained by the different functions of those compound groups. Insect cuticular profiles consequently represent complex traits that are simultaneously affected by both genes and environment. For stingless bees, we found an unexpectedly strong geographical and hence environmental effect on the chemical composition of both environmental and genetic components of their cuticular profiles.

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