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RESEARCH ARTICLE

Nest-specific composition of the trail pheromone of the stingless bee *Trigona corvina* within populations

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Abstract Social insects have evolved highly developed communication systems, enabling them to coordinate complex interactions in their colonies. Pheromones play a major role in the coordination of many tasks. In *Trigona corvina*, a stingless bee that occurs in Central America, foragers use pheromones produced in their labial glands to scent mark solid substrates between a food source and their nest. Newly recruited bees subsequently follow these scent marks until they reach the food source. A recent study has revealed nest-specific differences in the composition of these trail pheromones in colonies of *T. corvina*, suggesting that pheromone specificity may serve to avoid competition between foragers from different nests. However, the nests used in this study came from different populations and their foragers certainly never met in the field (Jarau et al., 2010). The aim of the present study was to investigate whether differences in the trail pheromones of foragers from different nests can also be found between neighbouring colonies within populations. We analysed the composition of trail pheromones from labial gland secretions extracted from workers from nine colonies collected at three different populations in Costa Rica. The differences in pheromone composition were even more distinct between neighbouring nests within a population than between nests of different populations. This finding corroborates the hypothesis that nest specificity of trail pheromones serves to communicate the location of a food source exclusively to nestmates, thereby avoiding intraspecific competition at resources. Resource partitioning by avoiding conspecific non-nestmates is particularly adaptive for aggressive bee species, such as *T. corvina*.

Keywords Chemical communication · Trail pheromone · Nest specificity · Stingless bees · *Trigona corvina*

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Introduction

Stingless bees (Hymenoptera, Apidae, Meliponini) comprise a group of eusocial insects that is distributed over the tropical and subtropical areas throughout the world (Michener, 2000). One of the characteristics of eusocial insects is division of labour, which requires communication mechanisms to coordinate the many individuals of a colony (Wilson, 1971). Probably the most important way in which social insects communicate is by means of chemical compounds based on both specific pheromone signals and learnt cues (e.g. Blum and Brand, 1972; Chapman, 1998).

Foragers of social bees can recruit workers from their nest to a particular food source in order to efficiently gather food (Wilson, 1971). *Trigona corvina*, the stingless bee species investigated in the present study, uses scent trails to recruit nestmates to a food source (Slaa, 2003; Aguilar et al., 2005; Jarau et al., 2010), whereby recruiting foragers land on vegetation and deposit the pheromone produced and stored in their labial glands (Jarau et al., 2004, 2006, 2010, 2011; Schorkopf et al., 2007; Stangler et al., 2009; Lichtenberg et al., 2011).

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Jarau et al. (2010, 2011) found a nest-specific composition and effect in releasing trail following behaviour in the pheromones of two stingless bee species, *T. corvina* and *Scaptotrigona pectoralis*. Especially in *T. corvina*, the nest-specific effect in eliciting trail following behaviour in newly recruited workers proved to be very distinct. Jarau et al. (2010) found nine compounds in labial gland extracts from foragers of this species that were perceived by the antennae of workers. Eight of the compounds could be chemically identified (octyl hexanoate, octyl octanoate, octyl decanoate, decyl hexanoate, decyl octanoate, decyl decanoate, geranyl octanoate, and geranyl decanoate). The relative composition of these compounds differed significantly in workers taken from three nests that originated from different, spatially well-separated populations in Costa Rica. The study, therefore, had shown that the relative composition of the trail pheromone of *T. corvina* foragers from nests that are geographically separated by large distances differs. Nevertheless, Jarau et al. (2010) postulated that nest-specific trail pheromones may be adaptive in a way that minimizes competition at food sources by allowing recruited bees to identify resources that are exploited by their nestmates, thus avoiding resources visited by conspecifics of foreign colonies. To investigate this idea, however, neighbouring colonies within a population have to be studied. The present study, therefore, seeks to answer the following question: how does the composition of the trail pheromone of *T. corvina* differ in workers taken from different nests within a population as compared to workers from nests of different populations?

Materials and methods

Collecting sites

Foragers of *T. corvina* were collected from different nests in three geographically separated areas (different populations) in Costa Rica between November 2007 and March 2008. The exact position of each nest was determined with an eTrex Summit HC GPS (Garmin Deutschland GmbH, Gräfelfing, Germany). The distances between the population, as well as the distances between the nests within a population, are given in Table 1.

The first population was located in Heredia, province of Heredia, where bees from five *T. corvina* nests (1.1–1.5) were collected. Three nests (1.1–1.3) were located on the campus of the Universidad Nacional (UNA), and the remaining two nests (1.4 and 1.5) were found approximately 4 km away from that site, in the INBioparque. The second population was located near the tropical field station in La Gamba, Puntarenas Province, where bees from three nests (2.1–2.3) were collected. The third population was situated near Pozo Azul de Abangares, Guanacaste Province, where bees from only one nest (3.1) could be collected.

Table 1 Distances (in meters) between the studied populations (a), as well as between the nests within population 1 (b) and population 2 (c)

	Population 1	Population 2	Population 3
a			
Population 1	0	171,316	92,091
Population 2	171,316	0	249,021
Population 3	92,091	249,021	0

	Nest 1.1	Nest 1.2	Nest 1.3	Nest 1.4	Nest 1.5
b					
Nest 1.1	0	196	338	3,894	3,778
Nest 1.2	196	0	505	3,948	3,842
Nest 1.3	338	505	0	4,080	3,950
Nest 1.4	3,894	3,948	4,080	0	198
Nest 1.5	3,778	3,842	3,950	198	0

	Nest 2.1	Nest 2.2	Nest 2.3
c			
Nest 2.1	0	209	412
Nest 2.2	209	0	593
Nest 2.3	412	593	0

Collection of bees, gland dissection, and preparation of gland extracts

We collected between 14 and 34 bees per nest. We captured the bees with a butterfly net next to the nests' entrances. Only bees that were flying towards the nest from further away were captured to ensure that foragers were used. The labial gland extracts of 5–18 bees per nest were prepared. In total, 117 labial gland extracts were analysed. To prepare gland extracts, the bees were killed by freezing and their cephalic labial glands carefully dissected by separating them from any other tissues in a saline solution under a stereo microscope. The glands of each individual were then extracted separately in 100 µl hexane for 24 h at room temperature (about 24 °C on average).

Chemical analyses

The extracts were analysed by gas chromatography as described previously (Jarau et al., 2010). Elucidation of the structure of the trail pheromone compounds from *T. corvina* labial gland extracts by means of GC–MS analyses was reported elsewhere (Jarau et al., 2010). In the present study, we performed GC-runs (HP 5890 GC, Palo Alto, CA, USA) with pure reference compounds of octyl hexanoate, octyl

octanoate, decyl hexanoate, geranyl octanoate, decyl octanoate, octyl decanoate, geranyl decanoate, and decyl decanoate to identify them in the gland extracts by means of retention time comparisons. Quantitative analyses of the chromatograms were done with the program GC ChemStation (Agilent Technologies). For each sample, we determined the trail pheromone composition by calculating the relative amounts of its compounds as the percentage of the total amount of the eight selected labial gland components within the labial gland extracts.

Statistical analyses

We carried out statistical comparisons of the trail pheromones collected from foragers of different nests with the program IBM® SPSS® Statistics Version 20. A principal components analysis (PCA) was done with the relative amounts of the eight selected labial gland compounds in order to combine collinear variables into new independent variables, the principal components (PCs) (Field, 2009). This was necessary due to existing relationships between the original variables in our data set (Bartlett's test of sphericity: $\chi^2 = 396.256$, $df = 21$, $P < 0.05$). To reveal whether our pre-defined groups, i.e. the different colonies, can be distinguished from each other based on the composition of their foragers' trail pheromones, the PCs with eigenvalues >1 were used for canonical discriminant functions analyses (DFA). Calculated group classifications of the individual extracts were carried out using the "leave one out" method with the entire data set (9 colonies), as well as with the nests from population 1 and 2, separately.

To visualize the similarities among the trail pheromone compositions of the different nests from the three studied populations, an agglomerative hierarchical cluster analysis was performed with their group centroid functions calculated in the DFA. Clusters were assembled according to their squared Euclidean distances and the nearest neighbour method. We allowed the algorithm to decide the number of clusters.

In order to check for significant differences in the median values of the relative amounts of each compound, Kruskal–Wallis tests with subsequent pairwise comparisons (Dunn's Method) were performed.

Results

Trail pheromone specificity

Overall, the relative compositions of the trail pheromone blends collected from foragers of the different *T. corvina* nests showed great variability, both within and among populations (Table 2). Octyl octanoate was the predominant compound found in the labial gland extracts of *T. corvina* workers from nests 1.1 (47.9 ± 21.8 % of the entire blend), 1.3 (58.1 ± 6.6 %), 1.5 (43.5 ± 26.4 %), 2.1 (51.0 ± 21.2 %), and 2.3 (59.4 ± 11.1 %), whereas decyl hexanoate dominated the labial gland extracts of foragers from nests 1.2 (52.0 ± 34.9 %), 1.4 (47.2 ± 21.4 %), 2.2 (37.4 ± 19.2 %), and 3.1 (68.5 ± 20.3 %). Likewise, the identity of the second most abundant component in the foragers' gland extracts varied between the nests (decyl octanoate/octyl decanoate in nests 1.1, 1.4, 2.1, and 2.2; geranyl decanoate in nest 1.2; geranyl octanoate in nests 1.3, 1.5, and 2.3; octyl hexanoate in nest 3.1).

The most variable compound (18 significant differences between the extracts of foragers from different nests; Kruskal–Wallis tests and pairwise comparisons with Dunn's method, $P < 0.05$) was octyl octanoate, followed by decyl hexanoate and geranyl decanoate showing significantly

Table 2 Mean relative amounts and corresponding standard deviations (in %) of the eight trail pheromone compounds extracted from labial glands of *T. corvina* workers

Nest	Octyl octanoate	Decyl hexanoate	Decyl octanoate/ octyl decanoate	Decyl decanoate	Geranyl decanoate	Geranyl octanoate	Octyl hexanoate
1.1 ($N = 18$)	47.9 ± 21.8	3.9 ± 5.5	39.4 ± 17.9	2.8 ± 1.9	0.01 ± 0.04	1.8 ± 3.8	0.6 ± 0.5
1.2 ($N = 14$)	1.2 ± 1.5	52.0 ± 34.9	9.3 ± 7.9	3.1 ± 2.6	23.5 ± 23.8	9.4 ± 3.9	1.3 ± 0.6
1.3 ($N = 15$)	58.1 ± 6.6	0.1 ± 0.5	6.7 ± 3.9	0.4 ± 0.2	0.7 ± 0.3	32.1 ± 8.0	1.9 ± 0.9
1.4 ($N = 14$)	3.2 ± 7.4	47.2 ± 21.4	19.8 ± 8.9	12.0 ± 7.0	9.5 ± 7.7	4.6 ± 11.2	3.2 ± 2.2
1.5 ($N = 14$)	43.5 ± 26.4	5.4 ± 2.9	12.0 ± 16.0	0.7 ± 0.4	0.2 ± 0.3	33.8 ± 28.3	1.5 ± 1.7
2.1 ($N = 14$)	51.0 ± 21.2	9.6 ± 6.9	25.1 ± 16.7	0.7 ± 2.5	7.8 ± 22.8	1.0 ± 3.6	3.0 ± 2.5
2.2 ($N = 14$)	10.8 ± 6.3	37.4 ± 19.2	19.7 ± 9.7	10.6 ± 4.7	0.7 ± 1.1	8.7 ± 13.0	3.9 ± 2.7
2.3 ($N = 9$)	59.4 ± 11.1	1.8 ± 3.5	2.5 ± 2.2	3.9 ± 4.2	0.2 ± 0.2	25.0 ± 15.1	5.0 ± 3.0
3.1 ($N = 5$)	3.9 ± 7.9	68.5 ± 20.3	8.3 ± 8.0	2.6 ± 1.1	3.1 ± 1.6	2.0 ± 2.5	11.6 ± 5.7

The peaks for decyl octanoate and octyl decanoate could not be separated in the chromatograms

N number of individual extracts analysed per nest

different proportions in 15 and 12 comparisons, respectively. The relative proportions of decyl octanoate/octyl decanoate, geranyl octanoate, and octyl hexanoate significantly differed in eight nest comparisons and decyl decanoate in only six nest comparisons.

Differences within and between populations

The PCA based on the trail pheromone bouquets of foragers from the nine different nests revealed three principal components with an eigenvalue >1 ($PC_1 = 2.341$, $PC_2 = 1.521$, $PC_3 = 1.205$) that together accounted for 72.3 % of the data's variance (33.4, 21.7, and 17.2 %, respectively). A canonical DFA conducted with these three principal components resulted in three discriminant functions (DF_1: $\chi^2 = 355.005$, $df = 24$, $P < 0.001$; DF_2: $\chi^2 = 138.720$, $df = 14$, $P < 0.001$; DF_3: $\chi^2 = 47.805$, $df = 6$, $P < 0.001$). DF_1 was mainly weighted on geranyl decanoate and octyl octanoate, DF_2 on geranyl octanoate and decyl octanoate/octyl decanoate, and DF_3 on geranyl decanoate, octyl octanoate, as well as on octyl- and decyl-hexanoate. DF_1 and DF_2 together explain 93.2 % of the variance (Fig. 1). In the calculated group, classification done with all nine colonies; only 65.8 % of the originally grouped cases (individual bees) were correctly classified to their respective nest origin due to the relative composition of their trail pheromone compounds. The classification results were much better when the analyses were done separately for the nests of each population. Overall 86.7 % of the individuals from population 1 were correctly classified to their nests and in population 2, the respective number was 94.6 %.

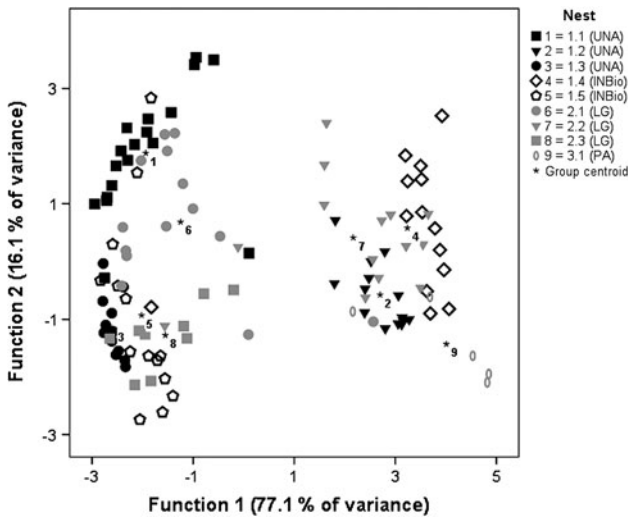

Fig. 1 Comparison of the trail pheromone composition of foragers from nine *Trigona corvina* colonies originating from three different populations in Costa Rica. The analysis (PCA followed by DFA; DF_1: $\chi^2 = 355.005$, $df = 24$, $P < 0.001$; DF_2: $\chi^2 = 138.720$, $df = 14$, $P < 0.001$) is based on the relative proportions of the pheromone's 8 components. Note that neighbouring nests within a (sub-) population are better separated than nests from different populations. *Black filled symbols*, nests from population 1, university campus subpopulation (UNA); *black open symbols*, nests from population 1, INBioparque subpopulation (INBio), *filled gray symbols*, nests from population 2 in La Gamba (LG); *open gray symbols*, nest from population 3 in Pozo Azul (PA)

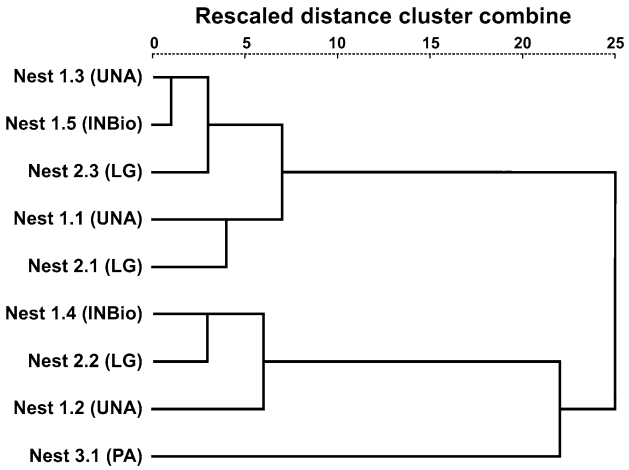

Fig. 2 Similarity in the trail pheromone composition of the *Trigona corvina* nests investigated in this study according to the relative amounts of the pheromone's components. The dendrogram was constructed using the squared Euclidean distances between the discriminant function scores' group centroids with the nearest neighbour method. The nests from the University Campus (*UNA*) and the INBioparque (*INBio*) from population 1 are geographically separated by a distance of ca. 4 km. *LG* La Gamba, *PA* Pozo Azul

In the hierarchical cluster analysis, which clustered nests according to the similarity in trail pheromone composition of their foragers, no neighbouring nests from the same sub-population were combined in the same sub-cluster (Fig. 2). Nests 1.3 and 1.5 as well as 1.2 and 1.4, clustered together, but each pair of nests was geographically separated by a distance of approximately 4 km (Table 1b). The pheromone composition of the foragers from each nest is more similar to that of foragers from a distant nest rather than to a neighbouring nest within its own sub-population (Fig. 2).

Discussion

In this study, we confirmed earlier findings by Jarau et al. (2010) that the trail pheromone of *T. corvina* foragers differs among individuals from different nests collected from separated populations in Costa Rica. More importantly, however, we also found that the trail pheromones are nest-specific for workers of neighbouring colonies within a population. The differences between the pheromone blends were mainly due to the relative proportions of octyl octanoate, decyl hexanoate and geranyl decanoate, as revealed by the large numbers of significant differences between the

nests in our analyses. Jarau et al. (2010) demonstrated that in *T. corvina*, such differences in the trail pheromone are sufficient for newly recruited bees that search for food to discriminate scent trails deposited by nestmates or by conspecific workers. The discrimination of the nest-specific pheromones, which allows to avoid the trails deposited by foragers from foreign colonies (Jarau et al., 2010), may contribute to resource partitioning and avoidance of competition by foragers of different nests. Within a given area, food sources are limited, which may lead to competition between bees of neighbouring nests (Slaa, 2003). *T. corvina* is an aggressive bee species (Johnson and Hubbell, 1974; Johnson, 1983; Wille, 1983; Slaa, 2003; Biesmeijer and Slaa, 2004), and encounters between workers from more than one colony at a food source usually result in fights and the deaths of up to several hundreds of individuals (Johnson and Hubbell, 1974). Avoiding such encounters could considerably limit the negative effects on the fitness of a colony due to the loss of workers. Thus, it is particularly advantageous for foragers of neighbouring colonies within a population to deposit nest-specific trail pheromones that only guide their own nestmates to an encountered food source (Jarau et al., 2006, 2010). Our analyses of nest-specific trail pheromone blends in *T. corvina* indeed revealed larger differences in the composition of pheromones produced by foragers from nearby nests within a particular population as compared to nests from different (sub-)populations. This conclusion is corroborated by the finding that the classification of individuals to their colony done with all nine nests assigned a much smaller number of bees correctly (ca. 66 %) as compared to classifications done solely with the nest of the single populations (87 and 95 %). Furthermore, in the dendrogram constructed by the cluster analysis, all sub-clusters contained nests from different populations, indicating that certain nests from different populations are more similar in the trail pheromone compositions produced by their foragers than the nests located in the same population. This is even true for nests 1.3 and 1.5 as well as for nests 1.2 and 1.4 from population 1 that clustered together, but are geographically isolated from each other by about 4 km. This distance is much farther than the expected maximum foraging flight range of medium-sized bees like *T. corvina* (ca. 1,100–1,700 m; Araújo et al., 2004). Thus, it is unlikely that bees from the INBioparque subpopulation (nests 1.4 and 1.5) compete for the same food sources with bees from the campus of the Universidad Nacional (nests 1.1, 1.2, and 1.3). We also detected large intra-colonial variation in the pheromone of some of the nests in our study. These differences may reflect different gland contents due to age differences of the single workers. We did not use bees of a specific age. Rather, we collected workers upon their return to the colony, thus they all were in the final stage of their lives, working as foragers. In sum, our results corroborate the hypothesis that nest-specific trail pheromones are important for competitor avoidance and resource partitioning among stingless bee colonies that forage at the same set of food sources in a particular foraging area.

An interesting but so far unresolved question arising from our results is how the pronounced nest specificity in the pheromones of neighbouring *T. corvina* colonies is achieved. Since mating is expected to be more likely between virgin queens and males from nests located close to each other within a population, genetic similarity between individuals of neighbouring nests should be greater than between individuals from different populations. In addition, new nests are founded by a queen's daughters within a few hundred meters of their mother colony (Sakagami, 1982) and, thus, are genetically related to each other. Accordingly, the foragers' trail pheromone should be more similar in nearby nests as compared to nests from different populations. However, we found the contrary in our study. Possibly, competition between colonies arising from similar trail pheromones contributed to selection for mating of queens with males originating from distant nests, which may be sufficient for the observed variation in trail pheromone composition. Alternatively, newly started colonies that do not differ from colonies already present within their flight range in terms of the foragers' trail pheromone may suffer severe losses of workers at food sources and die before becoming large, established nests. Provided that worker force in old colonies is much larger than in young ones, this may eventually lead to the extinction of such new and small nests. However, both mating biology and the mechanisms involved in nest foundation remain to be studied in *T. corvina* and the question of how this species achieves the observed nest-specific trail pheromone compositions remains elusive.

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References

Aguilar I., Fonseca A. and Biesmeijer J.C. 2005. Recruitment and communication of food source location in three species of stingless bees (Hymenoptera, Apidae, Meliponini). *Apidologie* **36**: 313-324

Araújo E.D., Costa M., Chaud-Netto J. and Fowler H.G. 2004. Body size and flight distance in stingless bees (Hymenoptera: Meliponini): inference of flight range and possible ecological implications. *Braz. J. Biol.* **64**: 563-568

Biesmeijer J.C. and Slaa E.J. 2004. Information flow and organization of stingless bee foraging. *Apidologie* **35**: 143-157

Blum M.S. and Brand J.M. 1972. Social insect pheromones: their chemistry and function. *Am. Zool.* **12**: 553-576

Chapman R.F. 1998. *The Insects: Structure and Function*. 4th ed. Cambridge University Press, Cambridge

Field A. 2009. *Discovering Statistics using SPSS*. 3rd ed. SAGE Publication Ltd. London

Jarau S., Hrncir M., Zucchi R. and Barth F.G. 2004. A stingless bee uses labial gland secretions for scent trail communication (*Trigona recursa* Smith 1863). *J. Comp. Physiol. A* **190**: 233-239

Jarau S., Schulz C.M., Hrncir M., Francke W., Zucchi R., Barth F.G. and Ayasse M. 2006. Hexyl decanoate, the first trail pheromone compound identified in a stingless bee, *Trigona recursa*. *J. Chem. Ecol.* **32**: 1555-1564

Jarau S., Dambacher J., Twele R., Aguilar I., Francke W. and Ayasse M. 2010. The trail pheromone of a stingless bee, *Trigona corvina* (Hymenoptera, Apidae, Meliponini), varies between populations. *Chem. Sens.* **35**: 593-601

Jarau S., Hemmeter K., Aguilar I. and Ayasse M. 2011. A scientific note on trail pheromone communication in a stingless bee, *Scaptotrigona pectoralis* (Hymenoptera, Apidae, Meliponini). *Apidologie* **42**: 708-710

Johnson L.K. 1983. Foraging strategies and the structure of stingless bee communities in Costa Rica. In: *Social Insects in the Tropics* (Jaisson P., Ed), Presses de l'Université Paris XIII, Paris. pp 31-58

Johnson L.K. and Hubbell S.P. 1974. Aggression and competition among stingless bees: Field studies. *Ecology* **55**: 120-127

Lichtenberg E.M., Hrncir M., Turatti I.C. and Nieh J.C. 2011. Olfactory eavesdropping between two competing stingless bee species. *Behav. Ecol. Sociobiol.* **65**: 763-774

Michener C.D. 2000. *The Bees of the World*. Johns Hopkins University Press, Baltimore London

Sakagami S.F. 1982. Stingless bees. In: *Social Insects, Vol. III* (Hermann H.R., Ed.), Academic Press, New York. pp 361-423

Schorkopf D.L.P., Jarau S., Francke W., Twele R., Zucchi R., Hrncir M., Schmidt V.M., Ayasse M. and Barth F.G. 2007. Spitting out information: *Trigona* bees deposit saliva to signal resource locations. *Proc. R. Soc. B* **274**: 895-898

Slaa E.J. 2003. *Foraging Ecology of Stingless Bees: From Individual Behaviour to Community Ecology*. Dissertation thesis, Utrecht University

Stangler E.S., Jarau S., Hrncir M., Zucchi R. and Ayasse M. 2009. Identification of trail pheromone compounds from the labial glands of the stingless bee *Geotrigona mombuca*. *Chemoecology* **19**: 13-19

Wille A. 1983. Biology of the stingless bees (Meliponinae, Apidae). *Annu. Rev. Entomol.* **28**: 41-64

Wilson E.O. 1971. *The Insect Societies*. Belknap Press, Cambridge