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Relationship Between Hydrocarbon Composition on the Cuticle of *Melipona quadrifasciata* (Hymenoptera: Apidae) Workers and the Secretion of the Cephalic Salivary Glands

SB Poiani[1], ED Morgan[2], FP Drijfhout[2], C Cruz-Landim[1]

1 - Departamento de Biologia, Universidade Estadual Paulista, Instituto de Biociências, Rio Claro, São Paulo, Brazil

2 - Chemical Ecology Group, Lennard Jones Laboratories, Keele University, United Kingdom

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Corresponding author
Silvana B. Poiani
Universidade Estadual Paulista - UNESP
Departamento de Biologia
Instituto de Biociências
Avenida 24A, 1515, Bela Vista
CEP 13506-900, Rio Claro-SP, Brasil.
E-Mail: silbeani@gmail.com

Abstract

Since chemical communication is pivotal for social insect success, the present paper aimed to quantify and qualify the chemical compounds that might have pheromonal role in both cephalic salivary gland and epicuticle of workers of *Melipona quadrifasciata* Lepeletier using gas chromatography-mass spectrometry (GC/MS). The results indicated that the hydrocarbons were the main compounds in both cephalic salivary gland and epicuticle, followed by esters. Positive Mantel correspondence analysis suggests that the glands could contribute to replenishment of surface compounds as an auxiliary source. Discriminant analysis also pointed out that gland and epicuticle chemical profiles were phase-related.

Introduction

Insects produce chemicals of several natures that act in communicative interactions among individuals of different species, named allelochemicals, and among individuals of same species, known as pheromones (Morgan, 2010). In social insects these compounds are found in the secretion of several exocrine glands and also in the epicuticle's surface mediating social interactions among the nestmates (Jarau et al., 2004, 2006; Howard & Blomquist, 2005; Cruz-Landim, 2009). The epicuticle, the outermost layer of the insect cuticle, is constituted of 90% of hydrocarbons. Along evolution, epicuticle acquired informational roles about physiological state, age, sex, species and caste of individuals as well as mating and discrimination of nestmates (Howard, 1993; Abdalla et al., 2003; Poiani et al., 2014; Nunes et al., 2017; Vollet-Neto et al., 2017).

Cuticular compounds are lost in a few weeks, suggesting that cuticular compounds must be constantly renewed (D'Ettorre et al., 2006). Oenocytes are of ectodermal origin and they are pointed out as the main endogenous source of surface compounds (Kramer & Wigglesworth, 1950; Gu et al., 1995). Epidermal cells are also of ectodermal origin, however, in the adult, they are inactive except in certain glandular regions. Thus, in addition to the class III exocrine glands, all glands of ectodermal origin such as tegumental class I gland and exocrine glands structured as organs (Cruz-Landim, 1994) have the potential to contribute to secretion and formation of surface compounds. In fact, cephalic salivary glands (GSC) and Dufour's have been pointed out as source of these hydrocarbons (Kullenberget al., 1973; Bergman & Bergström, 1997; Poiani & Cruz-Landim, 2017).

In general, bee colonies are formed by adult and immature individuals. Adult workers and queens are females

and comprise different castes. The division of labor among workers presents age-correlated patterns of task performance (temporal polyethism) (Wilson, 1985). Worker bees generally pass through following phases/stages: callow/newly emerged (incubation and repairs of the brood chambre), nurse bee (construction and provisioning combs, cleaning the nest, and feeding larvae, young adults and the queen), housekeeper (further cleaning the nest, reconstruction of the involucrum, reception and ripening of nectar, and guard duty at the entrance of the nest) and forager (foraging for pollen, nectar, propolis and other materials) (Wille, 1983). To perform most of the tasks, the worker bee also present developmental state and secretion content of glands changing according to phase or task (Katzav-Gozansky et al., 2001; Poiani & Cruz-Landim, 2009, 2010a, b, c).

The chemical profile of CSG has been investigated among bees. Hydrocarbons are present in newly emerged, nurse and forager workers of *Apis mellifera* Linnaeus and some males of *Bombus* species (Kullenberg et al., 1973; Katzav-Gozansky et al., 2001; Poiani & Cruz-Landim, 2017). Esters are most likely found in newly emerged, nurse and forager workers of Meliponini bees (Jarau et al., 2006; Schorkopf et al., 2007; Stangler et al., 2009; Poiani et al., 2015) and a mixture of oxygenated compounds and hydrocarbons were observed in virgin and old queens and workers of *Bombus terrestris* (Linnaeus) (Amsalem et al., 2014).

The function of CSG varies among bee species. In Meliponini, CSG produces a trail pheromone to communicate food source to nestmates (Jarau et al., 2006; Schorkopf et al., 2007; Stangler et al., 2009). Also, it aids to handle resins collected in the field (Santos et al., 2009). In *A. mellifera* and some *Bombus* species, the hydrocarbons produced by CSG are also present in the cuticle of workers (Kullenberg et al., 1973; Arnold et al., 1996; Poiani & Cruz-Landim, 2017; Martin et al., 2018). In *A. mellifera*, CSG may take part as an auxiliary source of cuticular hydrocarbons (Poiani & Cruz-Landim, 2017). Males of some *Bombus* species use CSG content for territorial marking and female attraction (Terzo et al., 2007; Amsalem et al., 2014).

Melipona quadrifasciata Lepeletier is a Brazilian eusocial stingless bee. The body size of worker measures 10 to 11 mm. The population of this species ranges from 300-400 bees within the colony (Lindauer & Kerr, 1960). The present research aimed to analyze and to identify the chemical profile of CSG and epicuticle of newly emerged, nurse and forager workers of *M. quadrifasciata*. The ultimate purpose is to verify if CSG is a candidate for auxiliary source of epicuticle hydrocarbon by comparing their profiles.

Material and Methods

Samples preparation

Workers of *M. quadrifasciata* were collected in different phases of life. Newly emerged (NE) at the time of emergence from their brood cells; workers provisioning brood combs (CA); and forager workers returning to the nest with pollen (FO). All workers were taken from a single colony. The colony is located at the meliponary of Sao Paulo State University UNESP, Institute of Biosciences, Rio Claro, Brazil.

Twenty pairs of wings and 20 glands from NE, CA and FO were used. Wings were chosen because they have the same chemical composition found on the body surface. Also, they are less subject to contamination from tegumentary exocrine gland discharge (Cruz-Landim et al., 1965).

Chemical analysis

Chemical analyses were conducted on an Agilent 6890 gas chromatograph (Agilent, Santa Clara, CA, USA) equipped with HP-5MS column (30 m long, 0.25 mm diam; Agilent). The gas chromatograph is coupled to an Agilent 5973 mass detector with electron impact ionization at 70 eV.

Solid sample injection (Morgan, 1990) was used. The glands and pair of wings from each individual were dissected and inserted into a thin glass capillary. Glands and wings were analyzed separately. The capillary measures approximately 2–3 cm and was sealed at one end. The capillary was inserted into the solid sampler in the heated GC inlet, and crushed to release the compounds. Samples were analyzed in splitless mode. The oven temperature program was held at 40 °C for 1 min then increased to 320 °C at 15 °C/min., and then held for 10 min. Helium was used as carrier gas at a constant flow of 1.0 ml/min.

Relative quantification was based on the peak areas in the total ion chromatograms obtained (Singer & Espelie, 1992). The compounds were identified according to their mass spectra, comparing them with a database (MS-Database), and consulting the Wiley Library.

Statistical analysis

Mantel test statistic was performed to verify correspondence between gland and cuticle surface compounds in each worker group. The relative amounts of each compound in CSG and cuticle of each individual worker was used. The amount is related to the area of each peak in the chromatogram. The test statistic is the Pearson product-moment correlation coefficient (r). The coefficient r falls in the range of –1 to +1. The r value close to –1 indicates strong negative correlation. The r value close to +1 indicates strong positive correlation. Mantel tests performed here were conducted using the R package ade4 by running the function mantel.rtest (gla.dist, cut.dist, nrepeat = 9999) and at $\alpha = 0.05$, where gla.dist is the distribution of distances between the relative quantity of chemical compounds in the gland for a given group – either NE, CA, FO); and cut.dist is the distribution of distances between the relative quantity of chemical compounds in the cuticle for the equivalent group Yet, the distribution of distances was calculated with the command dist() and stored in the variables gla.dist and cut.dist.

Discriminant analysis (DA) and Fisher distance were used to verify whether the phases were correctly classified within the pre-established groups (NE, CA and FO) according to their chemical compounds in CSG and cuticle surface. Statistical significance for DA was established at $p < 0.0001$. After running DA, the confusion matrix generated from a cross-validation (method Jackknife) was checked in order to assess the overall accuracy of original data and estimation of error rates for every group. For all groups we found correct classification rate of 100%.

Moreover, hierarchical cluster analysis (HCA) and Rho distance similarity was performed to identify which group of workers is more similar/closer to each other for both gland content and cuticle chemical profile. Both HCA and DA were performed using average percentage original data and the software PAST.

Results

Chemical profile of cephalic salivary glands

A total of 33 different compounds were found in the glands being 10 esters and 23 hydrocarbons. The hydrocarbons include alkanes, methyl-branched alkanes, alkenes and alkadienes. The hydrocarbons chain ranges from *n*-C21 to *n*-C31 (Table 1).

The quality and relative quantity of each compound varied according to worker phase (NE, CA and FO). However, the alkenes (Z)-pentacosene and (Z)-heptacosene were the major compounds in the NE, CA and FO glands. NE workers presented 20 compounds in their glands: three esters and 17 hydrocarbons. Hexadecyl dodecanoate, (ZZ)-nonacosadiene, 11 and 13-methylnonacosane and 11 and 13-methylhentriacontane are exclusive in NE glands. The glands of CA workers presented 29 compounds being nine esters and 20 hydrocarbons. The exclusive compounds found in CA workers were alkanes: 3-methyltetracosane and 11-methylhexacosane. The glands of FO workers presented 25 compounds being nine esters, and 16 hydrocarbons (Table 1).

Chemical profile of cuticle surface

A total of 28 different compounds were found in the cuticle surface being one ester, two acids, and 25 hydrocarbons. The hydrocarbons include alkanes, methyl-branched alkanes, and alkenes ranging from *n*-C21 to *n*-C31. The quality and relative quantity of each compound varied according to worker phase. The alkenes (Z)-pentacosene and (Z)-heptacosene were the major compounds in the NE, CA and FO cuticle surface.

NE workers presented 24 compounds in their cuticle: one ester, two acids, and 21 hydrocarbons. The cuticle of NE workers contains exclusive compounds that were all alkanes: *n*-heneicosane, 5-methylheptacosane, 11 and 13-methylnonacosane, 5-methylnonacosane and 11 and 13-methylhentriacontane. The cuticle of CA workers contains 22 compounds being one ester, two acids and 19 hydrocarbons. The exclusive compound found in CA workers was 3-methylhentriacontane. The cuticle of FO workers presented 21 compounds being one ester, two acids, and 18 hydrocarbons (Table 1).

Table 1. Mean and standard deviation (SD) of relative concentration (%) of the compounds from cephalic salivary gland secretion and cuticle of workers of *Melipona quadrifasciata*.

RT	Compounds	Cephalic Salivary Gland			Cuticle		
		NE (n = 20) Mean ± SD	CA (n = 20) Mean ± SD	FO (n = 20) Mean ± SD	NE (n = 20) Mean ± SD	CA (n = 20) Mean ± SD	FO (n = 20) Mean ± SD
	Acids						
13.60	Hexadecanoic acid	-	-	-	0.47 ± 1.02	0.95 ± 1.02	0.44 ± 1.21
14.94	(*Z,Z*)-9,12-Octadecadienoic acid	-			0.08 ± 0.01	0.09 ± 0.05	0.06 ± 0.13
	Esters						
14.79	Ethyl-9,12-octadecadienoate	-	-	-	3.82 ± 7.11	1.75 ± 1.53	0.21 ± 0.57
15.29	Hexadecan-1-yl acetate	-	0.30 ± 0.41	0.16 ± 0.20	-	-	-
15.82	Methyl octadecanoate	0.38 ± 0.53	0.13 ± 0.11	0.37 ± 0.23	-	-	-
16.17	Ethyl oleate	-	0.32 ± 0.94	0.16 ± 0.25	-	-	-
16.27	(*Z*)-13- Octadecen-1-yl acetate	-	0.18 ± 0.25	0.19 ± 0.18	-	-	-
16.40	Octadecyl acetate	-	0.23 ± 0.32	0.33 ± 0.31	-	-	-
20.13	Octadecyl dodecanoate	1.81 ± 2.00	0.21 ± 0.25	0.47 ± 0.48	-	-	-
19.89	Hexadecyl dodecanoate	2.48 ± 2.22	-	-	-	-	-
20.84	Eicosyl decanoate	-	*	0.51 ± 0.71	-	-	-
22.10	Docosyl decanoate	-	0.07 ± 0.11	0.66 ± 1.28	-	-	-
22.60	Tetracosyl decanoate	-	0.15 ± 0.15	1.37 ± 3.06	-	-	-
	Hydrocarbons						
15.53	(*Z*)-Heneicosene	-	0.16 ± 0.20	0.12 ± 0.10	-	-	-
15.67	Heneicosane (C21)	-	-	-	1.02 ± 1.14	-	-

Table 1. Mean and standard deviation (SD) of relative concentration (%) of the compounds from cephalic salivary gland secretion and cuticle of workers of *Melipona quadrifasciata*. (Continuation)

RT	Compounds	Cephalic Salivary Gland			Cuticle		
		NE (n = 20) Mean ± SD	CA (n = 20) Mean ± SD	FO (n = 20) Mean ± SD	NE (n = 20) Mean ± SD	CA (n = 20) Mean ± SD	FO (n = 20) Mean ± SD
16.65	(*Z*)-X-Tricosene	0.08 ± 0.15	2.32 ± 1.37	2.62 ± 1.53	0.41 ± 0.38	0.19 ± 0.14	*
16.76	Tricosane (C23)	2.46 ± 1.15	1.29 ± 0.74	0.94 ± 0.50	9.28 ± 2.92	1.21 ± 0.62	0.60 ± 0.64
16.94	9-Methyltricosane	-	0.12 ± 0.09	0.22 ± 0.06	-	-	-
17.30	3-Methyltricosane	-	3.03 ± 1.46	3.20 ± 1.67	0.38 ± 0.32	0.52 ± 0.22	0.31 ± 0.29
17.32	Tetracosane (C24)	-	-	-	0.48 ± 0.36	0.51 ± 0.28	0.25 ± 0.21
17.59	11-Methyltetracosane	-	0.46 ± 0.54	0.27 ± 0.41	-	-	-
17.61	3-Methyltetracosane	-	0.49 ± 0.53	-	-	-	-
18.06	(*Z*)-Pentacosene	19.33 ± 7.99	40.73 ± 2.99	38.77 ± 7.48	21.27 ± 4.60	19.85 ± 2.62	18.53 ± 5.20
18.37	Pentacosane (C25)	4.99 ± 1.80	4.72 ± 3.43	4.22 ± 1.51	12.58 ± 4.20	16.34 ± 2.29	15.59 ± 5.38
18.41	9-Methylpentacosane	1.45 ± 0.59	1.23 ± 0.43	1.24 ± 1.04	1.80 ± 0.42	0.44 ± 0.22	0.29 ± 0.23
17.58	5-Methylpentacosane	2.41 ± 0.92	1.16 ± 0.57	-	1.89 ± 1.05	0.53 ± 0.26	0.82 ± 0.30
18.56	3-Methylpentacosane	2.07 ± 0.56	5.29 ± 1.05	5.53 ± 1.53	2.26 ± 0.88	1.89 ± 0.46	1.82 ± 0.54
	Hexacosane (C26)	-	-	-	0.16 ± 0.26	1.08 ± 0.38	0.81 ± 0.31
17.79	11-Methylhexacosane	-	0.54 ± 0.50	-	-	-	-
18.17	(*Z*)-Heptacosene	30.73 ± 5.95	27.85 ± 4.28	26.95 ± 5.56	18.84 ± 3.59	21.17 ± 3.43	24.78 ± 6.14
18.22	Heptacosane (C27)	3.04 ± 1.15	1.07 ± 2.30	-	4.02 ± 1.21	14.01 ± 1.52	17.68 ± 4.66
18.44	11 and 13-Methylheptacosane	2.86 ± 0.38	1.32 ± 0.57	2.07 ± 1.08	1.74 ± 0.65	-	-
17.72	7 and 9-Methylheptacosane	-	-	-	-	0.92 ± 0.26	1.23 ± 0.44
17.78	5-Methylheptacosane	-	-	-	2.90 ± 1.27	-	-
18.00	3-Methylheptacosane	3.59 ± 1.29	1.25 ± 0.62	0.92 ± 0.36	-	-	-
18.53	(*Z*)-Octacosene	-	-	-	-	1.12 ± 0.18	1.23 ± 0.29
18.72	(*Z,Z*)-Nonacosadiene	0.47 ± 0.63	-	-	-	-	-
19.72	(*Z*)-Nonacosene	14.51 ± 7.47	4.85 ± 2.25	7.37 ± 3.84	10.92 ± 3.59	6.92 ± 1.40	8.45 ± 2.45
19.78	Nonacosane (C29)	-	-	-	1.45 ± 1.42	4.73 ± 1.00	5.74 ± 3.10
19.91	11 and 13-Methylnonacosane	2.39 ± 1.21	-	-	1.25 ± 0.56	-	-
19.93	5-Methylnonacosane	0.48 ± 0.83	0.16 ± 0.17	0.28 ± 0.16	0.39 ± 0.41	-	-
20.45	(*Z*)-Hentriacontene	0.23 ± 0.50	0.12 ± 0.15	0.41 ± 0.52	0.79 ± 0.55	0.39 ± 0.25	0.39 ± 0.30
20.49	Hentriacontane (C31)	-	-	-	-	1.18 ± 0.97	0.60 ± 0.60
20.59	11 and 13-Methylhentriacontane	0.34 ± 0.57	-	-	0.54 ± 0.49	-	-
21.16	3-Methylhentriacontane	-	-	-	-	0.44 ± 0.27	-

NE = newly emerged; CA = workers from comb area; FO = foragers; RT = retention time
*The compounds with relative concentration less than 0.05% were considered as traces.

Correlation among chemical profiles

The correspondence analysis Mantel *r* showed that there is positive correlation between CSG and cuticle in NE ($r = 0.61$), CA ($r = 0.47$) and FO ($r = 0.48$). For all groups $p < 0.0001$ indicates that the results are statistically significant at $\alpha = 0.05$.

DA analysis of chemical content of both glands (Fig 1A) and cuticle surface (Fig 1B) has shown that the three groups NE, CA and FO were completely segregated. The Fisher distances among all groups were $p < 0.001$.

HCA dendrogram of CSG content (Fig 2A) separated the workers into three well defined groups, as expected, and showed that nurses and foragers are closest. HCA dendrogram of cuticle surface compounds (Fig 2B) also showed that nurses and foragers are closest. It was possible to observe nurse and forager individuals out of their predicted groups.

Discussion

Chemical compounds on body surface and exocrine glands are important chemical cues used for social interactions and community homeostasis in social insects (Jarau et al., 2004, 2006; Howard and Blomquist 2005; Cruz-Landim, 2009). The present study provided information about quality and quantity of chemical profile in CSG and epicuticle of workers of *M. quadrifasciata* in different phases of life comparing the results in order to seek for correspondence between both compartments.

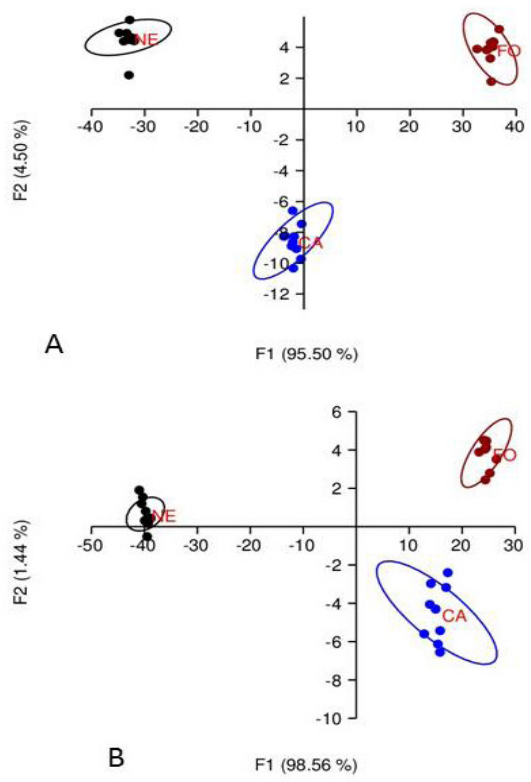

Fig 1. Discriminant analysis graphs of chemical contents of cephalic salivary glands (A) and cuticle surface (B) of workers of *Melipona quadrifasciata*. All groups differed from each other. The individuals were 100% allocated correctly (F1+F2=100%). NE = newly emerged; CA = working in comb area; FO = forager.

In stingless bees, CSG has been pointed out as a producer of esters that act as scent trails to guide their nestmates to a food source (Jarau et al., 2004, 2006; Schorkopf et al., 2007; Stangler et al., 2009; Poiani et al., 2015). However, workers of *M. quandrifasciata* use sound pulses to recruit nestmates to food source (Hrncir et al., 2000) suggesting that CSG in *M. quadrifasciata* might has function other than scent trail.

It is known that oenocytes are the main responsible for cuticle hydrocarbon production. However, some epidermal cells that function as tegumental glands are also involved in cuticle hydrocarbon synthesis (Kramer & Wigglesworth, 1950; Abdalla et al., 2003) and such compounds can be fully or partially stored in glands after being released as secretion (Bagnères & Blomquist, 2010). The present research showed that hydrocarbons were the major compounds in CSG indicating that the studied gland may be an auxiliary source of cuticle hydrocarbons as observed in *A. mellifera* (Poiani & Cruz-Landim, 2017; Martin et al., 2018). In ants, the post-pharyngeal gland (PPG) contains similar hydrocarbons found on cuticle surface (Bagnères & Morgan, 1991; Kaib et al., 2000). The content of PPG can be transferred to cuticle by grooming (Meskali et al., 1995; Morgan, 2010). In honeybees, self-grooming enables bees to remove ectoparasites, dust, and pollen from their own bodies and helps disperse pheromones (Boecking & Spivak, 1999). It is described as involving biting and licking with the mouthparts, as well as movement of the pro- and/or mesothoracic legs (Danka & Villa, 2005). The grooming behavior displayed by *M. quadrifasciata* workers could contribute to spread the content of CSG on the body surface. The final duct opening of CSG on the glossa allows the content of these gland reach the mouthparts and be spread on the body surface using the pro- and/or mesothoracic legs.

The present study has shown that the chemical profile of the glands of NE, CA and FO contained mainly hydrocarbons but esters were also present in small amount. In cuticle, esters were practically absent. The hydrocarbons composition was similar in both compartments. The positive Mantel correspondence analysis for all groups indicated that there were correspondences between gland and cuticle compounds. These results indicate that CSG of *M. quadrifasciata* may play a role as an auxiliary source of hydrocarbons replenishment in the epicuticle. Similar results were found for *A. mellifera* (Arnold et al., 1996; Poiani & Cruz-Landim, 2017) and

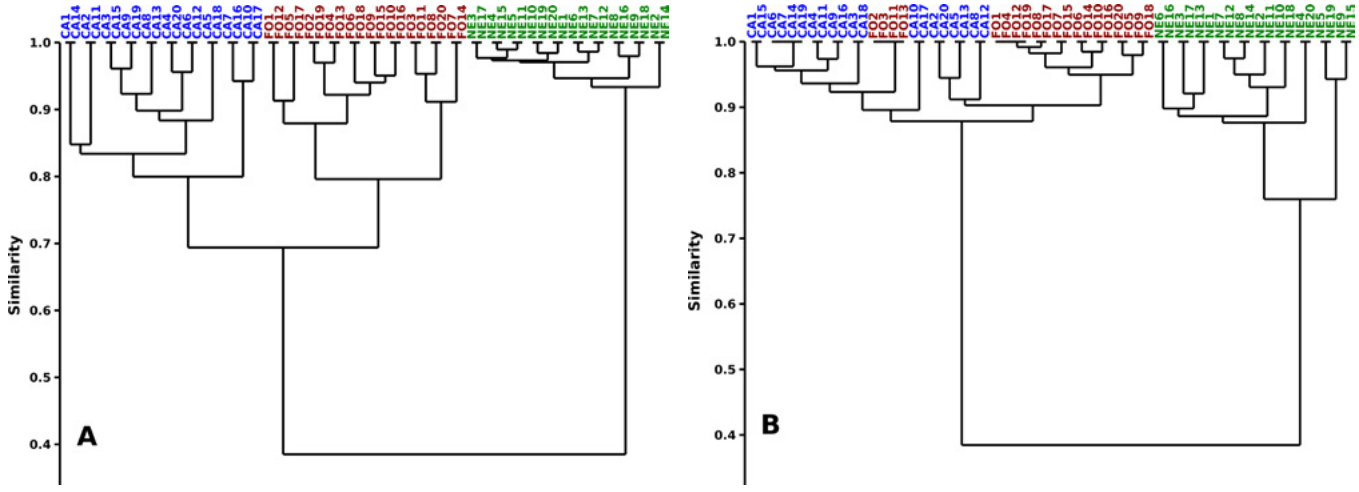

Fig 2. Dendrograms obtained from a hierarchical cluster analysis (HCA). The groups were formed according to similarities in the cephalic salivary gland secretion (A) and cuticle surface (B) chemical profiles of *Melipona quadrifasciata*. Both dendrograms showed that nurses (CA) and foragers (FO) are closest. NE = newly emerged workers.

some *Bombus* species (Kullenberg et al., 1973; Bergman & Bergström, 1997; Terzo et al., 2007).

CSG starts to produce lipid-based secretion (Simpson, 1960; Poiani & Cruz-Landim, 2016) soon as the worker emerge from brood comb (Poiani & Cruz-Landim, 2009). Then, additional use of secretion cannot be completely ruled out. The presence of small amounts of esters mixed with hydrocarbons may be used also to perform internal task in parallel of an auxiliary source of cuticle compounds. Santos et al. (2009) suggested that the CSG secretions of *Plebeia emerina* (Friese) nurses aid in the handling resins collected in the field.

CSG of NE, CA and FO contained most of the same compounds. However, the quantity of them was enough to distinguish the groups, as demonstrate by discriminant analysis. Despite hydrocarbons were majority, the presence of esters increases in CA and FO and suggest that CA and FO are more related with each other than with NE. Also, HCA dendrogram supported that CA and FO are closest groups. Such result is expected since the CSG of NE in bees are starting to produce secretion while in CA these glands present alveoli almost full of secretion and in CSG of FO were observed the peak of the secretion accumulation (Poiani & Cruz-Landim, 2009; 2010a,b,c). In *A. mellifera*, the content of CSG also showed more similarities between nurses and foragers (Poiani & Cruz-Landim, 2017) while in *Scaptotrigona postica* (Latreille) nurses are subdivided and there are individuals close to newly emerged and others that are similar to foragers suggesting progressive change in CSG content in this species (Poiani et al., 2015).

The most common cuticle lipids in insects are the hydrocarbons and they vary from C17 to C49, including alkanes, methyl-branched alkanes, and alkenes (Morgan, 2010). In this sense, *M. quadrifasciata* does not differ of the other insect species and presented hydrocarbons ranging from C21 to C31. The proportion of alkanes, alkenes and methyl-branched alkanes vary within insect species (Blomquist & Bagnères, 2010). Both alkanes and alkenes are related to chemical communication such as nestmate recognition, individual's fertility, sex, gender, caste, sex pheromone (Blomquist & Bagnères, 2010). Moreover, alkanes form an impermeable layer at cuticle that protects the insect against desiccation (Gibbs, 2002). The most compounds in epicuticle of *M. quadrifasciata* workers were alkenes.

The epicuticle chemical profile of workers of *M. quadrifasciata* provides information about the individual status as observed in castes or sex of other bee' species (Nunes et al., 2017; Vollet-Neto et al., 2017). Since almost all compounds were present in NE, CA and FO workers, the quantity variation of the compounds was pivotal to separate the workers groups according to their phase of life using discriminant analysis. The exclusive compounds in NE and CA also act as markers that indicate to which group the workers belong to. HCA dendrogram showed that nurse and forager workers share more similarities in their cuticle surface compounds in comparison with newly emerged. The acquisition of compounds increases according to age, contact with wax comb (Breed et al., 1995) and possibly to CSG development that may contribute to surface chemical profile.

Despite hydrocarbons were major compounds in cuticle, there were little amounts of oxygenated compounds. The presence of oxygenated compounds has been described for several species of insects (Buckner, 1993) and for some species of bees (Abdalla et al., 2003; Nunes et al., 2010). In bees, it was related to wax contact in the nest and playing a role in nestmate recognition (Breed et al., 1995). Then, it is possible that some contact with the nest material was responsible for the acquisition of oxygenated compounds in *M. quadrifasciata*.

In conclusion, CSG secretion and the cuticle chemical profile undergo significant chemical changes that correspond to a worker's phase of life suggesting a pheromonal role. The statistical test indicated positive correlation between CSG and cuticle surface compounds in NE, CA and FO, suggesting that there is a possibility for the CSG to be an auxiliary source of cuticular hydrocarbons.

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Authors' Contribution

All authors designed the experiments; S.B.P. collected bees, dissected the glands and wings, and performed the chemical experiments; S.B.P., E.D.M. and F.P.D. analysed the chemical data; S.B.P. carried out statistical analyses; S.B.P. and C.C.-L. analysed the statistical results; S.B.P. and C.C.-L. wrote the paper.

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