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Intraspecific Variation of the Composition of Linear Alkanes in Social Wasp *Mischocyttarus consimilis*

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Abstract

An important attribute of the evolution and maintenance of sociality in insects is their ability to distinguish members of their own colonies by means of chemical signals during their interactions. From this type of signals, the cuticular hydrocarbons, is responsible for intraspecific recognition stand out, among other functions. Linear alkanes are indicated as the class of compounds that would be most involved in water retention in the body of insects, however, some studies have investigated their role as mediators of interactions. Thus it is possible that there is significant intraspecific variation of its composition, so the objective of this study was to test the hypothesis that linear alkanes vary significantly among populations, castes and sexes, as well as between newly emerged females of different ages of the *Mischocyttarus consimilis* Zikán 1949 wasp. The samples were analyzed by gas chromatography with flame ionization detector and gas chromatography coupled to mass spectrometry. The results show that there is a significant variation between the chemical cuticular profiles of samples from different populations, as well as between castes, sex and newly emergent workers of different ages. Therefore, it is possible to infer that this class of compounds may vary according to genetic differences between populations, but also by different environmental conditions. The differences between castes, sex and ages suggest that these compounds may also be involved in mediating interactions between nestmates.

Introduction

Colonies of social insects that carry out various tasks requires interactions between individuals and the availability of mechanisms of nestmates recognition (Singer et al., 1998). The ability to recognize nestmates is extremely important to the success of these insects, so it prevents exploitation by individuals from other colonies (Hefetz, 2007).

During the evolution, the social insects developed a signals mechanism which is based on the variation of the cuticular chemical composition on its exoskeletons that also protect and support its bodies (Ross & Matthews, 1991). These compounds, besides the primary function of protection against microorganisms, toxins and desiccation (Lenoir et al., 2001), also play a determining role in inter and intraspecific recognition as already investigated in social bees (Nunes et al., 2009; Abdalla et al., 2003); ants (Antonialli-Junior et al., 2007, 2008) and social wasps (Butts et al., 1995; Sledge et al., 2001; Ferreira et al., 2012; Neves et al., 2012, 2013).

Thus, in social insects within the same species, the qualitative profile of CHs is similar among colonies, varying only in their relative proportions according to their origin (Bonavita-Cougourda et al., 1987), which functions as a “chemistry signature” colonial. Thus, through the chemical signals and due to the contact not only with the adult individuals between themselves and with the off-spring, but also with the nest substrate, the social insects can identify their nestmates from others (Gamboa, 1996).

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Ruther et al. (2002) supported the hypothesis that colony specific cuticular hydrocarbon profiles are involved in the phenomenon of nestmate recognition among social insects. According to Shellman and Gamboa (1982), newly emerged females acquire this ability trough the exposition with its own nest material, and Espelie et al. (1990) noticed that the nest substrate is rich in hydrocarbon compounds produced by colony's members, so to Gamboa (1996), the nest odor is like a colonial signature to be a reference to nestmate recognition.

Lorenzi et al. (1999) observed that newly wasps of *Polistes biglumis* (Linnaeus, 1758) do not yet have the colonial odor after their emergence. Panek et al. (2001) noticed that the cuticular hydrocarbons present at the *Polistes fuscatus* (Fabricius, 1793) newly emerged wasps differ in composition from those present at the older wasps with 3 days of emergence, the same was observed by Lorenzi et al. (2004) in *Polistes dominula* (Christ, 1791) wasps. The cuticular surface of *P. dominula* is most susceptible to acquire chemical components from the environment at the initial stage after emergence and this ability is reduced by the time, what means that the older wasps do not incorporate new chemical substance in their cuticles, so their colonial signature can not be altered (Lorenzi et al., 2004).

In this context, social insects recently emerged can be transferred with success between co-specified colonies (Gamboa, 2004). *Polybia occidentalis* Olivier, 1791 with 24 hours of emergence are easily accepted by unknown colonies (O'Donnell & Jeanne, 1992). The newly emerged wasps are treated as nestmates by members of the others colonies because they do not have colonial odor from their original colony (Gamboa, 2004).

Studies such as those by Dani et al. (1996) and Lorenzi et al. (2014) investigated the importance of branched alkanes among other cuticle compounds as the main responsible for signaling intraspecific interactions. However, although studies have identified linear alkanes as those most involved in the waterproofing of the cuticle to avoid insect dehydration, Tannure-Nascimento et al. (2007) verified in their study with *Polistes satan* Bequaert, 1940 that linear alkanes had a greater abundance in relation to the other compounds to mediate the interactions.

Therefore, since there are few studies that have already investigated the intraspecific variation of linear alkanes in social wasps, the objective of this study was to test the hypothesis that linear alkanes vary significantly among populations, caste and sex, as well as among newly emerged females of different ages of the species *Mischocyttarus consimilis*

Material and methods

Sample collection

For the analysis of population variation of *M. consimilis* were collected 12 colonies from 6 different populations sampled, in different types of environment in the state of Mato Grosso do Sul, Brazil (Fig 1 and Table 1). All colonies were collected with the aid of plastic bags wrapped around the nest were then detached from substrates.

After collect, all wasps were sacrificed and preserved by freezing, thus avoiding the use of any type of fixative or chemical preservative which could react with the chemicals of the cuticle.

To evaluate the variation of the profile of linear alkanes between sex and caste were used eight queens, 19 males and 77 workers of different colonies. At a later time, there was an analysis to determine the chemical profile of the cuticle

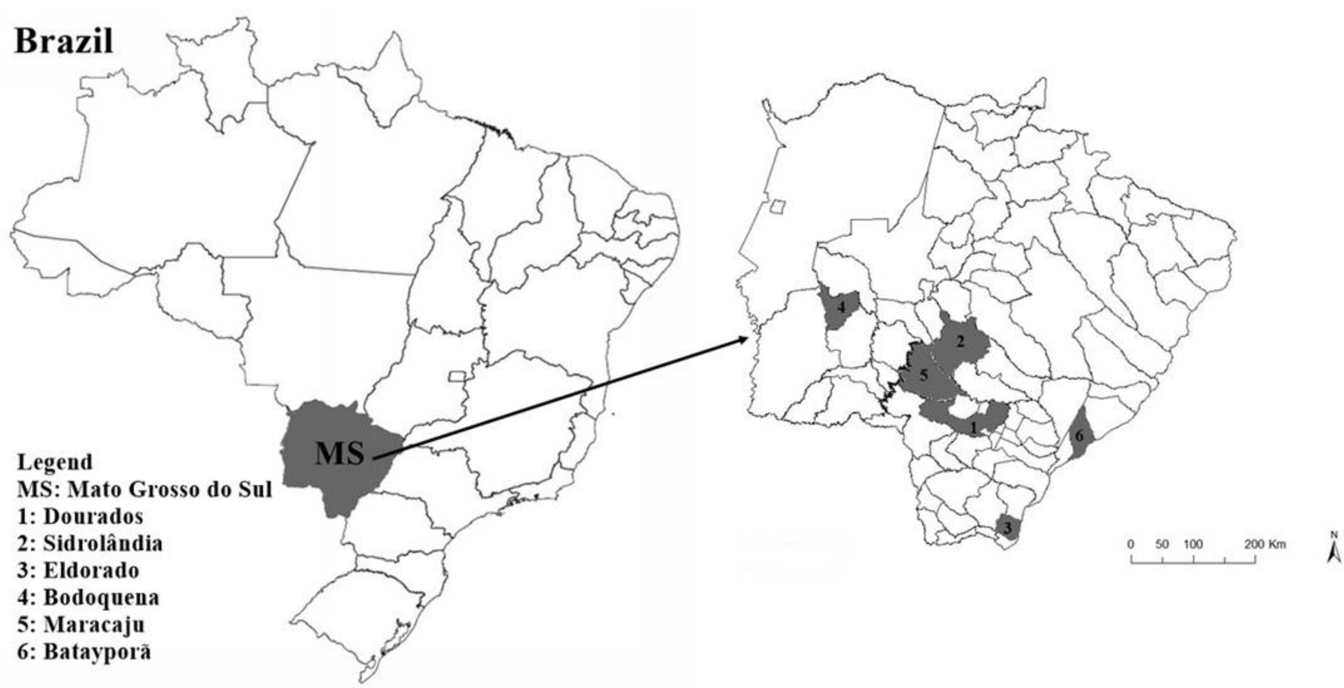

Fig 1 Map of Brazil highlighting the municipalities of Mato Grosso do Sul where populations of *Mischocyttarus consimilis* wasps were collected.

according to the worker caste and gynes, and the workers were separated into two groups; workers 01 (pre-emergency) which were the workers first in the colony; and workers 02 that were produced in the middle of the colony development (phase known as ergonomic) and gynes that are produced in the final stage of the colony; so were collected 13 females of the group workers 1; 12 individuals of the group workers 2 and 13 gynes, all collected in a single municipality, at the case Dourados-MS, to minimize environmental differences.

To evaluate when the composition of linear alkanes is completed after emergence were evaluated 10 workers of different age, from newly emerged up to 5 days after emergence, based on the results achieved by Panek et al. (2001). To minimize the possible environmental variations all the workers were collected from different colony nesting in the city of Dourados, MS.

To determine wasps' age, the colonies of *M. consimilis* were daily mapped according to the methodology proposed by Giannotti (1998). The newly emerged wasps were marked on the thorax with non-toxic ink in order to monitor their age. During these daily observations was also possible to see which individuals presented behaviors of dominance and subordination, according to the study by Torres et al (2012) with division of labor in this species.

Chemical and statistical analyses

All the insects used for both analyses were anesthetized, killed, and preserved by freezing. For the analyses of the cuticular hydrocarbon profile, the gaster was extracted from each individual, individual, it was standardized using the gaster, since the intention of the study was to evaluate the variations between samples and not describe the compounds.

The cuticular hydrocarbons of gaster were extracted in 2 mL hexane (HPLC grade, Vetec - Química Fina Ltda., Rio de Janeiro, Brazil) in an ultrasonic bath for 30 min. This process was performed in duplicate. In sequence, the 2 fractions were united and the solvent dried. The dried extract was dissolved in 100 µL hexane for analysis using gas chromatography with flame ionization detector (Focus, GC, Thermo Scientific - San Jose, USA).

The gas chromatography with flame ionization detector (GC–FID) was equipped with an OV-5 capillary column of Ohio mark (Ohio, USA) with a composition of 5% phenyl-dimethyl-polysiloxane in a fused silica capillary with dimensions of 30 m in length x 0.25 mm in diameter x 0.25 µm film thickness. Nitrogen was used as the carrier gas at a constant pressure of 0.8 bar. The analyses were performed in splitless mode with 1µL injection, injector temperature of 250°C, and detector temperature of 320°C. The oven was programmed to reach a final temperature of 320°C, starting from an initial temperature of 50°C and rising at a rate of 5°C/min to 85°C, then rising at a rate of 8°C/min to 300°C, which was held for 15 min, and then raised to 320°C at a rate of 10°C/min and held for 35 min.

Table 1. Collection sites of *Mischocyttarus consimilis*, indicating the geographical coordinates and types of environment.

Population	Sample collection	Geographical coordinates	Type of environment
01	Dourados	22º13’6”S, 54º48’20”W	urban areas with rural surroundings
02	Sidrolândia	20º55’55”S, 54º57’41”W	urban areas with rural surroundings
03	Eldorado	23º47’13”S, 54º17’01”W	urban areas with rural surroundings
04	Bodoquena	20º32’19”S, 56º42’54”W	rural area farm
05	Maracaju	21º36’52”S, 55º10’06”W	forest area
06	Batayporã	22º17’96”S, 53º77’02”W	urban area

The chromatograms were registered using the Chrom Quest 5.0 program and analyzed with the Workstation Chrom Data Review program. The chemical compounds were identified based on linear hydrocarbon standards of C_7 to C_{40} (4 µg/mL in hexane) through comparison with retention times. The concentration of each compound was calculated using relative area, in percentage, referring to the peak areas of alkanes C_7 to C_{40}. The analyses of linear alkanes present on the *M. consimilis* cuticle were performed on the CG-FID and the differentiation analysis to these studies was completed using the peak areas of the corresponding C_{15} to C_{30} hydrocarbons, which are common to all of the individuals.

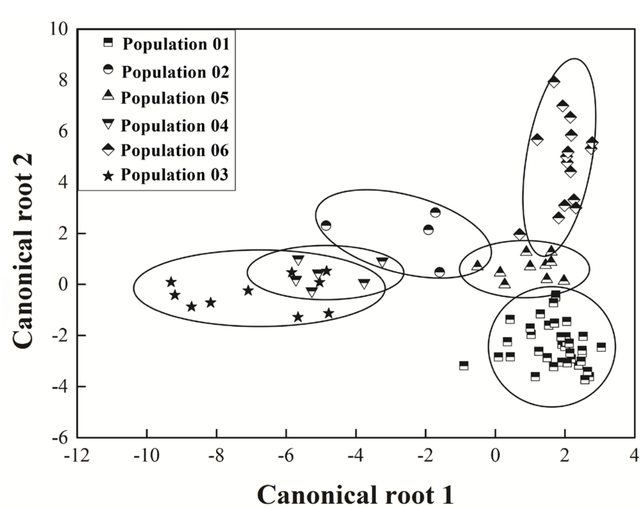

Fig 2. Scatter plot using the peak areas obtained from linear alkanes by GC-FID present in the cuticle of the species of wasps *Mischocyttarus consimilis* in colonies of different populations.

For the statistical analyzes the values of the percentage relative area of linear alkanes, analyzed by GC-FID, were subjected to stepwise discriminant function analysis of Statistica 7 program and indicated by selecting a set of variables if the analyzed groups differ or not, the Wilk's lambda statistic is used as a measurement of the difference between the groups, in which values close to 0 indicate that the groups do not overlap, whereas values close to 1 indicate high overlap among groups and a lack of significant difference (Quinn & Keough, 2002).

Results

The discriminant analysis shows that there are significant differences in the composition of linear alkanes among populations with Wilk's Lambda = 0.003, F = 70.313 and p<0.0001, the first canonical root explains 52.25% of the results and the second canonical root 90.90%. Linear alkanes are most significant for this discriminant analysis are shown in table 2.

The compounds significant for separation of populations on the scatter plot were: n-octadecane for population 01; n-pentadecane and n-octacosane for the population 02; n-nonadecane to population 03; n-tricosano to population 04; n-hexacosane to population 5 and n-heptadecane for population 6 (Table 2).

The results show that there are significant differences between the composition of linear alkanes between the sex and castes of this species (Fig 3 and Table 3), the analyzes were significant with Wilk's lambda = 0.065, F = 18.606, p<0.001, canonical root 1 of 83.4% and canonical root 2 of 16.6%. The linear alkanes responsible for that are C_{15}, C_{17}, C_{19}, C_{21}, C_{23}, C_{27}, C_{28}, C_{29} and C_{30} (Table 3).

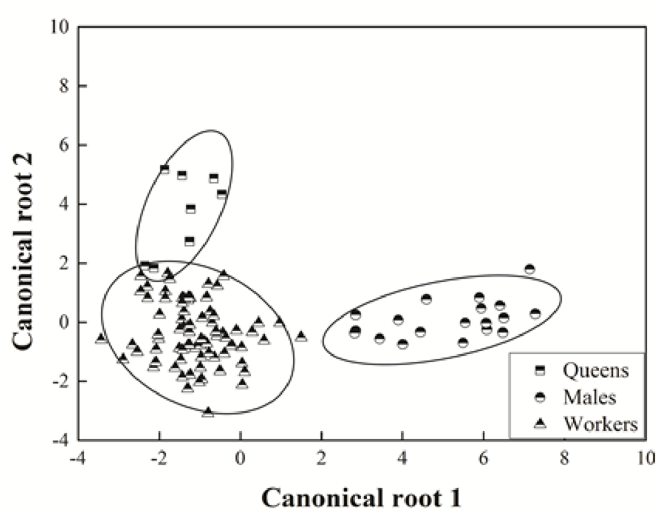

Fig 3. Scatter plot using the peak areas obtained from linear alkanes by GC-FID present in the cuticle of the species of wasps *Mischocyttarus consimilis* for caste and sex.

There are significant differences between the composition of linear alkanes of cuticle of different categories of females (Fig 4) with Wilks's lambda = 0.000, F = 32.40, p<0.0001, with the canonical root 1 explaining 86% of results and the canonical root 2 14%. The linear alkanes responsible for the separation among the gynes and the workers can be see in table 4.

The discriminant analysis in function of the values found on linear alkanes on wasps with 1 to 5 days after emergence showed Wilk's Lambda=0.029 and F=5.525 significant differences between the hydrocarbon profiles (p<0.0001), with canonical root 1 explaining 72% and along with root 2, 94% of the data. The Fig 5 shows the 3 separated groups of individuals, one with 1 to 3 days and the others with 4 and 5 days after emergence. The Table 5 shows the statistical values of each linear alkane evaluated and the responsible ones for this distinction among the newly wasps.

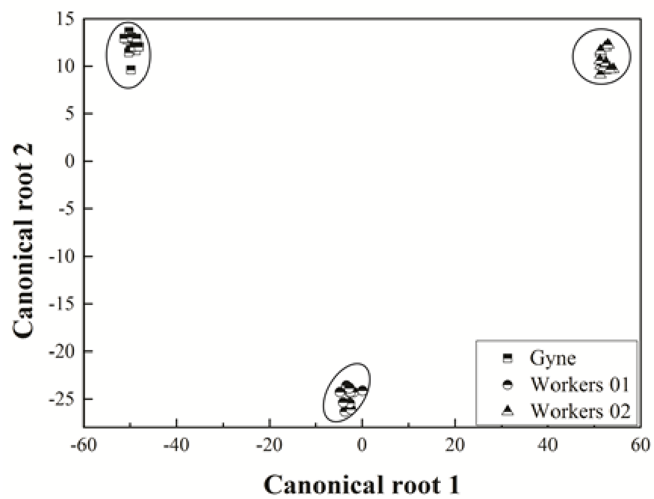

Fig 4. Scatter plot using the peak areas of linear alkanes obtained by GC-FID present in the cuticle of wasps of the species *Mischocyttarus consimilis* identified for gynes and workers.

Discussion

The results show that there is significant variation in the composition of linear alkanes between the different populations (Fig 2 and Table 2).

To explain the variations found, the authors (Liang & Silverman 2000; Sorvari et al., 2008; Zweden et al., 2009) discuss the importance of genetic factors, but above all the environmental to compose the cuticle of these insects. Ridley (2006), in fact, discusses that the geographic variation exists in all species and tend to be caused by adaptations to the environment, which suggests that environmental conditions are relevant for analyzing population differences. Therefore, in addition to genetic factors determine part of the chemical profile, exogenous factors also play an important role.

According to the results (Fig 2), in some cases the grouping appears to be geographic distance, but on the other hand, others may be due to the sharing of similar nidification environments. Therefore, as already discussed in other studies (Gamboa et al., 1986; Singer et al., 1998; Dapporto et al., 2004a; Dapporto et al., 2004b), it appears that both genetic and environmental factors are important to explain the variation of these compounds in these samples.

Table 2. Statistical values of the discriminant analysis and mean concentration values in % relative area for the linear alkanes that distinguishing populations *Mischocyttarus consimilis*.

CH	Wilk's Lambda	p<	Canonical coefficients		% Relative area					
			Root 1	Root 2	Population 01	Population 02	Population 03	Population 04	Population 05	Population 06
C_{15}	0.004	0.000	-0.176	0.592	0.123 ±0.085	0.180 ±0.060	0.095 ±0.035	0.132 ±0.039	0.197 ±0.090	0.120 ±0.117
C_{16}	0.003	NS	-	-	-	-	-	-	-	-
C_{17}	0.007	0.000	0.612	1.888	0.384 ±0.182	0.456 ±0.152	0.423 ±0.241	0.506 ±0.187	0.608 ±0.319	0.643 ±0.279
C_{18}	0.004	0.000	0.837	-6.321	0.550 ±1.053	0.162 ±0.057	0.205 ±0.110	1.900 ±4.215	0.681 ±1.424	0.182 ±0.085
C_{19}	0.005	0.000	-0.667	-0.601	0.322 ±0.274	0.403 ±0.143	0.683 ±0.416	0.261 ±0.155	0.456 ±0.244	0.421 ±0.201
C_{21}	0.003	NS	-	-	-	-	-	-	-	-
C_{23}	0.016	0.000	-1.321	0.266	6.853 ±10.487	12.845 ±16.340	34.616 ±9.759	42.742 ±3.262	2.527 ±2.517	1.270 ±0.707
C_{24}	0.003	NS	-	-	-	-	-	-	-	-
C_{25}	0.003	NS	-	-	-	-	-	-	-	-
C_{26}	0.004	0.000	2.840	-0.646	1.470 ±1.331	0.415 ±0.260	0.592 ±0.284	2.065 ±4.669	2.117 ±2.313	1.579 ±1.421
C_{27}	0.003	NS	-	-	-	-	-	-	-	-
C_{28}	0.004	0.000	-2.221	0.715	3.579 ±1.527	4.052 ±2.018	1.075 ±0.512	2.757 ±5.977	4.708 ±1.404	3.089 ±1.848
C_{29}	0.003	NS	-	-	-	-	-	-	-	-
C_{30}	0.003	NS	-	-	-	-	-	-	-	-

CH = Cuticle Hydrocarbon; NS = not significant.

Daportto et al. (2004a) observed quantitative and qualitative differences between the cuticular chemical profiles between three populations of the species *P. dominula*; this study also found greater similarity between these compounds in colony nesting nearest and attributed these results to both environmental components and genetic similarities.

Gamboa et al. (1986), studying *P. fuscatus*, discusses the importance of exogenous factors to determine the CHs profile, especially the diet. Furthermore, studies performed with ants show that the quantity and quality of food is also responsible for the changes of CHs, since influences the chemical recognition during intraspecific interactions in ants (Le Moli et al., 1992; Richard et al., 2004; Liang & Silverman, 2000; Sorvari et al., 2008), suggesting that diet is one of the factors that certainly contribute to the construction of the chemical signature of the colony. Bernardi et al. (2014) also observed a significant influence of external factors on the variation of CHs among ants under natural diet and controlled in the specie *Ectatomma brunneum* Smith, 1858.

Table 3. Statistical values of discriminant analysis of the peak relative areas of linear alkanes obtained by GC-FID presents on the cuticle of *Mischocyttarus consimilis* wasps identified by castes and sex.

CH	Wilk's Lambda	p<	Canonical coefficients		% relative area		
			Root 1	Root 2	Queens	Males	Workers
C_{15}	0.074	0.001	-0.563	0.111	0.084 ± 0.043	0.092 ± 0.053	0.159 ± 0.112
C_{16}	0.065	NS	-	-	-	-	-
C_{17}	0.105	0.001	1.561	-0.972	0.319 ±0 .135	0.550 ± 0.159	0.562 ± 0.371
C_{18}	0.068	NS	-	-	-	-	-
C_{19}	0.113	0.001	-1.350	0.151	0.233 ± 0.149	0.270 ± 0.068	0.420 ± 0.338
C_{21}	0.070	0.028	-0.253	-0.296	0.930 ± 0.261	12.675 ± 10.921	5.028 ± 9.651
C_{23}	0.223	0.001	1.235	0.314	1.706 ± 2.284	36.416 ± 8.423	6.592 ± 10.506
C_{24}	0.069	NS	-	-	-	-	-
C_{25}	0.066	NS	-	-	-	-	-
C_{26}	0.068	NS	-	-	-	-	-
C_{27}	0.101	0.001	-0.433	1.778	38.108 ± 3.528	17.441 ± 7.510	27.55 ± 13.12
C_{28}	0.095	0.001	1.351	-3.832	2.148 ± 0.800	1.379 ± 0.731	3.736 ± 2.258
C_{29}	0.070	0.021	0.211	0.659	47.563 ± 4.545	17.741 ± 5.772	43.737 ± 16.601
C_{30}	0.084	0.001	-1.945	0.968	0.829 ± 0.522	0.456 ± 0.157	1.979 ± 1.885

CH = Cuticle Hydrocarbon; NS = not significant.

Another aspect to be taken into account are the nest compounds that help to compose the chemical signature of the colonies (Singer et al., 1992; Singer & Espelie, 1996; Lenoir et al., 2009). Thus, as part of the composition of the nest depends on plant material collected by the wasps in plants around the nest, it is expected that there would be a variation of these compounds depending on the material provided by the different plant materials available in each type of environment.

Specifically as the variation of linear alkanes, according to other studies, these compounds appear to have a significant role in water loss prevention in the insects (Armold & Regnier, 1975; Gibbs, 1998, 2002). Therefore, the composition of linear alkanes is directly related to prevailing conditions, as for example, in hot and dry climates there is an increased production of long-chain n-alkanes, which prevents desiccation (Chapman et al., 1995; Wagner et al., 2001). In general, the alkanes have a function more directed the prevention of desiccation, due to their high melting points, different from alkenes which are more related to chemical communication (Gibbs, 2002). Thus, it is also possible that the differences and similarities found in our results are at least in part due to the small climatic differences between the microhabitats of the nesting sites.

The results show that there are significant differences between the composition of linear alkanes between workers, queens and males (Fig 3), therefore, there is a variation according to the sex, but also according function that they realize in their colony. The variation according to gender is probably because females originate from fertilized eggs while the males originate from unfertilized eggs being haploid. The compound responsible for the separation of males in the dispersion diagram, occurring in higher contents is the n-tricosane (Table 3).

This linear alkane was also found in a higher concentration in the cuticle of the bee males *Melipona quadrifasciata* Lepeletier, 1836 being one of the compounds responsible for the separation of the groups, and as in our results this compound appeared in a lower concentration in the queen's cuticle (Borges et al., 2012). In fact, there should be sex flags in colonies of social wasps, since Cotoneschi et al. (2009) observed the capability of workers to distinguish the sex of their nest mates through cuticular hydrocarbons in *P. dominula.*

Kudô and Zucchi (2006) studying colonies of *P. paulista* assessed that newly emerged males were accepted in other colonies, until a certain age, when they begin to acquire specific chemical odors from their own colony.

The compounds responsible for distinguishing queens and workers foram n-heptacosane (C_{27}) and n-nonacosane (C_{29}), which are more abundant in the cuticle of queens (Table 3). These compounds were also found by Hora et al. (2008) in the cuticle of *Ectatomma tuberculatum* (Olivier, 1792) and the authors suggested that the content of these compounds may signal the insemination of queens. In fact, Van Zweden et al. (2013) found the n-heptacosane compound in the highest concentration in the cuticle of the wasps of the *Dolichovespula saxonic* (Fabricius, 1793) wasp.

The differences between these chemical signals in the cuticle of the two castes are directly related to the physiological and behavioral differences. The queens differ from the workers in the ovarian activity and in the repertoire of tasks realizing in the colony (Billen & Morgan, 1998), such as physical dominance,

Table 4 Statistical values of discriminant analysis of the peak relative areas of linear alkanes obtained by GC-FID presents on the cuticle of *Mischocyttarus consimilis* wasps identified by gynes and workers.

CH	Wilk's Lambda	p<	Canonical coefficients		% relative area		
			Root 1	Root 2	Gynes	Workers 02	Workers 01
C_{15}	0.000002	0.293970	-0.030468	-0.290215	0.71923±0.09768	1.6525±0.10001	0.46077±0.10332
C_{16}	0.000003	0.013018	-0.132770	-0.181498	1.46615±0.09751	1.51167±0.12386	0.23231±0.044
C_{17}	0.000002	0.270816	0.016698	-0.057446	0.88538±0.04446	1.22333±0.09059	1.09538±0.17309
C_{18}	0.000002	0.022139	0.050672	0.025981	1.02231±0.0744	1.36667±0.22876	2.26769±0.34562
C_{19}	0.000003	0.013462	0.004956	0.016801	2.03462±0.08412	1.91417±0.30318	2.18154±0.43437
C_{20}	0.000002	0.322367	-0.054703	0.050502	2.63077±0.10444	1.64417±0.09913	1.36923±0.35323
C_{21}	0.000002	0.062814	0.037402	0.175244	1.69154±0.07559	1.11167±0.10232	2.12±0.16921
C_{22}	0.000002	0.034956	0.059754	0.084648	2.14769±0.10826	2.09917±0.15078	3.02385±0.17624
C_{23}	0.000004	0.000646	0.013099	0.112682	4.26846±0.22248	2.82667±0.17068	4.72692±0.63617
C_{24}	0.000003	0.008305	0.039653	0.064805	5.85385±0.39536	5.58833±0.3865	7.79231±0.64778
C_{25}	0.000008	0.000000	-0.378256	0.085745	9.57385±0.13295	5.66917±0.16071	2.34385±0.24602
C_{26}	0.000002	0.217125	0.011529	-0.046340	2.31±0.08803	3.03417±0.52153	2.71462±0.21046
C_{27}	0.000003	0.011908	-0.044572	0.020945	8.44385±0.29202	6.42667±0.74986	5.21769±0.92065
C_{28}	0.000002	0.220214	0.032369	-0.098701	5.55385±0.53285	8.24583±0.26834	7.36615±0.66523
C_{29}	0.000002	0.032951	0.012388	0.108955	7.08923±0.37682	5.33667±0.38382	7.63±0.69082
C_{30}	0.000002	0.120229	0.020553	0.080349	6.72615±0.5637	5.5475±0.40779	7.87±0.73228

CH = Cuticle Hydrocarbon.

food solicitation and oviposition realized by the queens, adult-adult trofhallaxis, alarm, foraging realized by the workers, so the queens remain more time in the nest developing the activities of dominance hierarchy and oviposition while the workers were engaged in maintenance activities, defense and success of the colonies (Montagna et al., 2009; Torres et al., 2012).

Sledge (2001) noted that when removed the dominant females of *P. dominula* colonies, the subordinate assumed this position and, consequently, had changes in their ovarian developing and in their cuticular hydrocarbons what became similar to those removed, whose chemical profiles were rich in alkanes and metyl-alkanes.

Gynes and workers also showed significant differences between the composition of linear alkanes your cuticles. Gynes and workers 01 presented higher contents of n-tricosane compared to workers 02, (Fig 4 and Table 4). As there are also differences between the categories of workers, since the n-docosane compound occurs in a higher concentration in the workers 01, whereas the n-pentadecane and n-hexadecane compounds appear in higher concentration in the cuticle of the workers 02 (Fig 4 and Table 4), probably for the same reason, since their physiological conditions and their functions in the colony may be distinct, although here we have not performed physiological and behavioral analyzes.

According to Dapporto et al. (2004a) in *P. dominula* there are variations qualitative and quantitative between the cuticular composition of the alphas founding compared at subordinate, probably due to the same reasons discussed above. Therefore, the cuticular linear alkanes are important compounds used for the identification of individuals (Singer et al., 1998), which can vary according to their tasks in the colony (Leal, 2005).

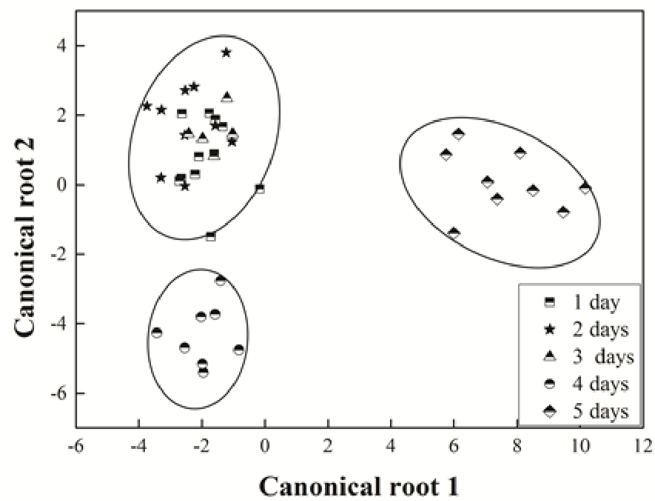

Fig 5. Scatter plot using the peak areas of linear alkanes obtained by GC-FID present in the cuticle of wasps of the species *Mischocyttarus consimilis* with 1 to 5 days after emergence.

However, despite our results show that linear alkanes too may signal the different caste and sex, Lommelen et al. (2006) reports that branched alkanes appear to be more involved in signaling during interspecific interactions, as this class has a high complexity molecular, showing a high potential to encode information (LeConte & Hefetz, 2008; Blomquist & Bagnères, 2010), and linear alkanes would be more involved in building a barrier to prevent water loss (Armold & Regnier, 1975).

According to Fig 5, it is possible to affirm that the emerged wasp of the genus *M. consimilis* acquires more complete chemical composition of linear alkanes after the fourth day after the emergence. The compounds which differ

Table 5. Statistical values of discriminant analysis of the peak relative areas of linear alkanes obtained by GC-FID presents on the cuticle of *Mischocyttarus consimilis* wasps with 1 to 5 days after emergence.

CH	Wilk's Lambda	p<	Canonical coefficients		% relative area				
			Root 1	Root 2	1 day	2 days	3 days	4 days	5 days
C_{15}	0.01	0.000	-0.60	-2.50	0.08 ± 0.03	0.11 ± 0.06	0.15 ± 0.07	0.19 ± 0.05	0.06 ± 0.02
C_{16}	0.00	NS	-	-	-	-	-	-	-
C_{17}	0.00	NS	-	-	-	-	-	-	-
C_{18}	0.00	NS	-	-	-	-	-	-	-
C_{19}	0.00	NS	-	-	-	-	-	-	-
C_{20}	0.01	0.00	1.18	0.03	7.63 ± 7.68	3.46 ± 1.58	3.65 ± 1.08	19.90 ± 5.25	47.91 ± 9.51
C_{21}	0.00	NS	-	-	-	-	-	-	-
C_{23}	0.01	0.03	0.75	1.28	37.21 ± 7.27	35.26 ± 10.26	42.14 ± 12.56	24.70 ± 11.74	18.49 ± 12.45
C_{24}	0.01	0.01	0.52	2.04	0.71 ± 0.16	0.77 ± 0.41	0.91 ± 0.27	0.58 ± 0.27	0.18 ± 0.05
C_{25}	0.01	0.01	-0.57	-2.70	10.61 ± 2.25	10.81 ± 2.03	10.67 ± 1.08	10.42 ± 1.33	5.86 ± 1.97
C_{26}	0.00	NS	-	-	-	-	-	-	-
C_{27}	0.01	0.01	0.96	1.31	11.00 ± 4.53	13.92 ± 6.15	14.90 ± 5.63	17.37 ± 5.69	14.06 ± 6.00
C_{28}	0.00	NS	-	-	-	-	-	-	-
C_{29}	0.00	NS	-	-	-	-	-	-	-
C_{30}	0.00	NS	-	-	-	-	-	-	-

CH = Cuticle Hydrocarbon; NS = not significant.

the age groups are n-pentadecane (C_{15}); n-eicosane (C_{20}); n-tricosane (C_{23}); n-tetracosane (C_{24}); n-pentacosane (C_{25}) and n-heptacosane (C_{27}). At the firth day emerged, the cuticular hydrocarbon profile of the wasp is distinct, showing in higher concentration of the compound n-eicosane (C_{20}) and in lower concentration of the alkanes C_{23}, C_{24} and C_{25} than the others age-groups (Table 5).

Neves et al. (2012) had shown that the workers of this specie take the same time to complete a cuticular chemical composition, however, they used another technique, FTIR-PAS (Fourier Transform Infrared-Photoacoustic Spectroscopy), which evaluates in general the variation all compounds found in the cuticle of insects. So, part of this significant variation between workers of different ages also refer to linear alkanes.

In addition, Kudô 2007 suggests that in colonies of *P. paulista* the newly emerged workers acquire chemical signatures specific to their colonies within a couple of days after emergence and, in experiments of transfer of individuals between colonies, the acceptance rate of young wasps depends on the stage of the receiver colony (Kudô et al. 2010). In the same species, workers are able to distinguish larvae from their own colony from intruders, suggesting that even larvae recognition by workers might be mediated by specific colonial signals (Kudô et al., 2017).

In fact, while the colonial signature was not yet defined, the wasps can probably be accepted by members of others colonies, as observed with *P. fuscatus* wasps which not rejected strange newly individuals, but threw the foreign older ones with 3 days of emergence out their nests (Panek et al., 2001). The same was noticed with the genus *Ropalidia marginata* Lepeletier, 1836, but with the age of 6 days after their emergence, more than that the individuals were rejected (Arathi et al., 1997). The change in composition of linear alkanes, in this sense, demonstrates again how are relevant, as well as other compounds to mediate the interactions of social wasps' colonies.

Thus, our results allow us to confirm the hypothesis that there is a significant variation between the composition of linear alkanes among populations, castes and sexes, as well as between newly emerged females of different ages. Part of this variation may be a function of genetic factors, such as in the case of samples from different populations, between the sexes and the environment, as well as in the case of populations. The differences between the composition of females of different castes and categories, as well as ages, are probably related to the physiological differences and tasks they perform in their colonies. Therefore, in these cases, the results allow to infer that this category of compound may also be involved in the mediations of the interactions between nestmates.

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