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Effect of temperature on the chemical profiles of nest materials of social wasps

Kamylla B. Michelutti[a,b,*], Raul C. Piva[b], Sidnei E. Lima-Junior[b], Claudia A.L. Cardoso[b], William F. Antonialli-Junior[a,b]

[a] Laboratório de Ecologia Comportamental (LABECO), Universidade Estadual de Mato Grosso do Sul, Dourados, Mato Grosso do Sul, 79804-970, Brazil
[b] Centro de Estudos em Recursos Naturais (CERNA), Programa de Pós-Graduação em Recursos Naturais, Universidade Estadual de Mato Grosso do Sul, Dourados, Mato Grosso do Sul, 79804-970, Brazil

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ABSTRACT

Social insects depend on their nests for protection against predation and abiotic threats. Accordingly, the chemical compounds present in the material wasps use to build their nests can both facilitate communication and repel predators. It is herein hypothesized that different wasp species build their nests with different structure and substrate materials and that such materials consist of chemical compounds related to unique wasp behavior and outside temperature variation. To test this hypothesis, nests were collected from three species of social wasps, the samples of which were subjected to temperature variation under laboratory conditions. The compounds present in the substrate were analyzed by gas chromatography coupled to mass spectrometry. Chemical compounds identified in the nest material of the three species responded differently to temperature variation. Chemical compounds from *Polybia* nests were altered significantly when subjected to temperature variation, whereas the nests of *Polistes versicolor* did not significantly change in relation to the control. The differences found between *Polistes* and *Polybia* nests may be related to genetic factors, but also to the type of nest they construct. It is possible that divergent evolutionary strategies for maintaining colony temperature, as a function of the chemical composition of the nests, may have appeared between wasps that have open and closed nests. In relatively small colonies, nest substrate is more resistant to temperature variation because it is composed of a greater diversity of elements and thus capable of holding heavier, longer carbon chains. Our results suggest that chemical compounds in the nest material of the three wasp species analysed responded differently to fluctuating ambient temperatures and that such variation could result from the biochemical differences of unique wasp species or from thermoregulation strategies of colonies.

1. Introduction

Among social insects, the nest is divided between a part dedicated to the development of larvae and a part where socializing takes place. Offspring are reared and protected by adults in the nest, which, consequently, represents a decisive factor in the origin and evolution of social life (Turillazzi, 2012). To build their nests, wasps scrape wood fibers with their jaws, mixing it with water and saliva, forming wood pulp that enables them to construct cells in hexagonal cross sections (Wenzel, 1998). These wasps are popularly known as “paper wasps” by their use of plant material to build nests (Wenzel, 1998). Apart from its phylogenetic, behavioral, ecological and evolutionary significance, the architecture of the nests of social wasps has high taxonomic value (Hunt and Carpenter, 2004).

The evolution of wasp nest structure is influenced by a variety of factors, including the physical environment, structural requirements of the nest, energy requirements for construction and maintenance, predation by vertebrates and arthropods, parasitism and social requirements of the colony (Jeanne, 1975). Stelocyttarous nests have combs attached to the substrate by means of peduncles. For Phragmocyttarous nests, the initial comb is fixed directly to the substrate, and a protective casing is constructed around the sides of the combs (Richards and Richards, 1951; Carpenter and Marques, 2001). Nests are used for many functions, one of which is to provide containers in the form of

* Corresponding author. Programa de Pós-graduação em Recursos Naturais, Universidade Estadual de Mato Grosso do Sul, Dourados, Mato Grosso do Sul, 79804-970, Brazil.
E-mail addresses: kamylla_michelutti@yahoo.com.br (K.B. Michelutti), raul.c.piva@hotmail.com (R.C. Piva), selimajunior@hotmail.com (S.E. Lima-Junior), claudia@uems.br (C.A.L. Cardoso), williamantonialli@yahoo.com.br (W.F. Antonialli-Junior).

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individual cells for the development of offspring (Jeanne, 1975). Nests also provide storage for protein (Michelutti et al., 2017a) and shelter to protect individuals against predation (Jeanne, 1975; Espelie and Hermann, 1990), especially ants and birds (Jeanne, 1975). In addition, nests provide a propitious microclimate that protects individuals from variations in the external environment, such as relatively extreme temperatures (Jeanne, 1975; Jones and Oldroyd, 2007). Diverse mechanisms are used to regulate the temperature in colonies of social wasps (see Jones and Oldroyd, 2007).

Importantly, the various functions of nest substrate are related to chemical composition, and a relatively well-studied relationship is that between nestmates by the exchange of chemical signals (Gamboa et al., 1986; Espelie et al., 1990; Singer and Espelie, 1996; Michelutti et al., 2017b). Gamboa et al. (1986) suggest that the queen emits a scent that can be indirectly acquired by other adults of the colony. Indeed, it is possible to identify high similarity between the cuticle of wasps in a given colony and their nest material, an identification already made in the colonies of *Polistes metricus* (Espelie et al., 1990). Thus, a close relationship exists between the cuticle compounds of social wasps and the material used to build the nest of which they are a part (Gamboa et al., 1986; Espelie et al., 1990; Singer and Espelie, 1996; Michelutti et al., 2017b; Sguarizi-Antonio et al., 2017). Accordingly, we may infer that the chemical compounds in the substrate material of social wasp nests play a role in communication, but also in maintaining thermostability.

Although exposure to temperature variation can directly affect the success of a colony (Käfer et al., 2012), no study has so far evaluated its effects on the chemical composition of the nest material and, indirectly, its ability to maintain the stability of the nest in order to, in turn, maintain homeostatic regulation of temperature within the colony. The chemical compounds identified in the nest of social wasps may vary among alkanes, both linear and branched (Espelie et al., 1990; Espelie and Hermann, 1990; Silva et al., 2016), and alkenes (Michelutti et al., 2017b), and they are mostly found as a characteristic chemical signature of the colony (Singer and Espelie, 1996).

The role of these chemical classes in nest substrate is unknown, but their presence in wasps is already known, and the class of linear alkanes and methyl alkanes is considered to be fundamental for waterproofing, while the dimethyl alkanes and alkenes are very important for chemical communication (Menzel et al., 2017). However, studies have proven the efficiency of linear alkanes as signaling intermediates, acting effectively in communication (Lorenzi et al., 2004; Tannure-Nascimento et al., 2007; Ferreira et al., 2012; Michelutti et al., 2018a). In addition to the class of compounds, carbon chain length (Gibbs and Pomonis, 1995; Gibbs et al., 1997; Menzel et al., 2017; Michelutti et al., 2018b) and the position of the methyl and unsaturated groups (Gibbs and Pomonis, 1995) determine the efficiency of chemical compounds to function in the cuticle for both communication and waterproofing (Chung and Carroll, 2015).

Polistes versicolor, Polybia paulista and *Polybia ignobilis* belong to the *Vespidae* family (Richards, 1971), subfamily Polistinae, the first species belonging to the tribe Polistini and the last two species to the tribe Epiponini (Carpenter and Marques, 2001). *P. versicolor* builds an open-type nest with a single comb, while species of the genus *Polybia* build nests covered with an envelope with several combs suspended one below another (Richards, 1971; Carpenter and Marques, 2001).

Central to our hypothesis, many compounds identified in the cuticle also occur in the material used to build the nest (Espelie et al., 1990; Singer and Espelie, 1992; Michelutti et al., 2017b). Also, Michelutti et al. (2018b) reported that the cuticle of social wasps can undergo alteration of its composition as a result of variation in temperature. In line with the above inference and these findings, we herein investigate the possible effects of temperature variation on the composition of nest substrate. It is hypothesized that different wasp species build their nests in correspondingly different structures by also using different substrate materials to maintain thermal homeostasis and protect against extreme outside temperature variation. To test this hypothesis, nests were collected from three species of social wasps noted above, the samples of which were subjected to temperature variation under laboratory conditions and thereafter analyzed for the chemical components.

2. Material and methods

2.1. Collection and experimental design

Active colonies of *P. versicolor, P. ignobilis* and *P. paulista* were collected around the municipality of Dourados/MS, Brazil (22°13′16″S; 54°48′20″W) in April 2016. Since each colony presents its own chemical signature (Dani et al., 2004; Cotoneschi et al., 2007) and since compounds present in the nest also reflect this variation (Sumana et al., 2005; Espelie et al., 1990; Singer and Espelie, 1992; Michelutti et al., 2017b), we chose to use samples from only one colony of each species to reduce the effects of intraspecific variation and examine the effects of temperature variation on them.

After collecting colonies of the three species, nest material (~0.05 g) was collected from two different parts. One from the interior part (0.025 g) and the other from the external part (0.025g) were analysed together.

To evaluate the chemical composition of samples under the effect of thermal variation, nest samples were subjected to the following temperatures: 15 °C, 20 °C, 30 °C, 35 °C and 40 °C for 24 h at each temperature. Ten samples were analysed for each temperature and for each species. These temperatures were selected according to the average temperature of the two main seasons of the previous year. In the dry and cold season, the minimum average was 15.6 ± 3.4 °C, reaching 6 °C on the day with the lowest temperature, while in the hot and humid season, the maximum average was 31.5 ± 3.9 °C, reaching 38 °C on the day of the highest temperature (data from the Embrapa Agropecuária Oeste, a local weather station).

Following the methodology described by Michelutti et al. (2018b), five 500 mL plastic containers containing nest samples of each species were placed in a biochemical oxygen demand (BOD) chamber (Fanem, Model 347 CD, São Paulo – SP–Brazil) with temperature variation within the range of ± 2 °C differences from the treatment temperature and light-dark cycle of 12:12 h. The samples were initially subjected to a temperature of 15 °C for 24 h. Then one plastic container with the nest material for each one of the three species was removed to extract the compounds. The temperature was then raised to 20 °C, and the remaining plastic containers were left at this temperature for a further 24 h, after which another plastic container containing nest samples for each of the three species was collected for a second round of compound analysis. This sampling protocol was repeated for all temperature treatments until 40 °C. As a control, after the colonies had been initially collected, samples were collected immediately as described above and extracted as a baseline prior to placing in the BOD.

2.2. Extraction and analysis of the compounds of the nest material by GC-MS

Ten samples were extracted for each treatment. For the extraction, each sample was immersed in a glass vessel with 2 mL of hexane (Tedia, HPLC grade) for 2 min. After the solute was removed, the samples were dried in a fume hood and frozen for a maximum of 30 days. For chromatographic analysis, each extract was solubilized in 200 μL of hexane (Tedia, HPLC grade). Samples were analysed using a gas chromatograph (GC-2010 Plus) coupled to a mass spectrometer (MS Ultra, 2010; Shimadzu, Kyoto, Japan). Analytical conditions included programming follows: the oven to start initially at 150 °C and then ramp up to 280 °C at 3 °C min with a hold time of 20 min at the final temperature. Helium was used as the carrier gas at a flow rate of 1 mL/min, and a one μL volume of samples was injected in the splitless mode. The temperatures for the injector, detector and transfer lines were 250 °C, 290 °C and 290 °C respectively. All analyses were carried out in the electro impact ionisation mode (EI), 70 eV scanning for 0.3s within the range of 40–600 mz.

Identification of compounds was performed with retention indices (Van den Dool and Kratz, 1963) of linear alkanes with chain lengths C_7–C_{40} (Sigma Aldrich with purity ≥ 90%) in comparison to retention indices found in the literature (Brophy et al., 1983; Espelie and Hermann, 1990; Bonavita-Cougourdan et al., 1991; Espelie et al., 1994; Lorenzi et al., 1997; Yusuf et al., 2010; Tokoro and Makino, 2011; Moore et al., 2014; Weiss et al., 2014). Individual peak identification was achieved by fragmentation pattern comparison with the NIST mass spectra database. The method for normalizing GC-MS data was obtained by dividing the area of each peak in a profile by the total sum of all peaks. The normalized data were multiplied by 100, and expressed in terms of their percent contribution to the total area (total relative area).

Compounds less than 0.1% of relative area were considered too low and were not presented in the tables. The major compounds were considered those that represented at least 3% of the total relative area.

2.3. Statistical analysis

To determine any variation of the chemical compounds of the nest material subjected to the different temperatures relative to the control, a permutational multivariate analysis of variance (PERMANOVA) was used to test the existence of significant differences among the temperatures. A post hoc test (Bonferroni-corrected p-values) was performed in order to compare these temperatures (R Development Core Team, 2017). The Bray-Curtis index was used to obtain the similarity matrix, and significance of comparisons was calculated from the randomization of the original matrix (9999 permutations). In order to complement this analysis and to visualize the ordering of nest profiles as a function of temperature, a non-metric multidimensional scaling (nMDS) was used with Bray-Curtis index (R Development Core Team, 2017).

3. Results

In the control samples of *P. versicolor*, the major compounds were heptacosane, nonacosane, 15-methylnonacosane, 3-methylnonacosane, hentriacontane, 13-methylhentriacontane, 5,15-dimethylhentriacontane and 15-methyltritriacontane. These comprise branched alkanes, linear alkanes, alkenes, alkadienes and lactone (Supplementary Table 1). Fig. 1 shows a greater number and contents of branched alkanes, followed by linear alkanes and alkenes. Only small semi-quantitative variations were found between the composition of the control samples and those subjected to different temperatures in *P. versicolor* species (Fig. 1a); PERMANOVA analysis revealed statistical difference (pseudo-$F_{(5,4602)}$ = 2.77; $p < 0.001$). However, comparing the control relative to the other temperatures, none of the paired comparisons showed significant differences (Table 1).

The major compounds in the control samples of *P. ignobilis* were pentacosane, heptacosane, x,y-dimethyltriacontane and x,y-dimethyldocotriacontane. Branched alkanes, linear alkanes, alkenes and isocoumarines were identified (Supplementary Table 2). Fig. 1b shows the three most representative classes in number and percentage. Variations were found between the composition of the control samples and those subjected to the different temperatures of *P. ignobilis* (Supplementary Table 2 and Fig. 1b). PERMANOVA analysis revealed statistical difference (pseudo-$F_{(5,2934)}$ = 9.54; $p < 0.001$). However, relative to the control, only the sample subjected to the 40 °C temperature showed significant difference (Table 1).

The major compounds in the control samples of *P. paulista* were heptacosane, nonacosane, 14-methylnonacosene, x-methylnonacosane and 11- or 13-methylhentriacontane (Supplementary Table 3). According to Supplementary Table 3 and Fig. 1c, differences were found between the composition of the control samples and the composition of samples subjected to the different temperatures. PERMANOVA analysis revealed statistical difference (pseudo-$F_{(5,2198)}$ = 10.10; $p < 0.001$). The *a posteriori* test showed that all the samples differed significantly from the control (Table 1).

The nMDS analysis with Bray Curtis similarities shows how each nest sample of each species differs under different temperature regimes and the compounds responsible for the separation (Fig. 2; Supplementary Tables 1–3).

4. Discussion

Chemical compounds identified in the nest material of the three species responded differently to temperature variation. Composition of *P. versicolor* nest samples did not vary significantly from the control sample, whereas compositions of the two *Polybia* wasps varied significantly. Compositions of the samples of the 3 species showed differences (Supplementary Tables 1–3). This result was expected since nest compounds are also those compounds comprising the chemical signature of the colony, acting effectively in chemical communication (Gamboa et al., 1986; Espelie et al., 1990; Espelie and Hermann, 1990; Singer and Espelie, 1996; Sumana et al., 2005); therefore, these values should vary intra- and interspecifically.

In general, the nest houses individuals of the colony (Jeanne, 1975; Espelie and Hermann, 1990), protecting them from variations in ambient temperature (Jones and Oldroyd, 2007). Thus, the nest can be considered a structure that maintains colony homeostasis (Jeanne, 1975; Jones and Oldroyd, 2007; Yamane et al., 2009; Souza et al., 2015). Wasp cuticles contain chemical compounds essential for maintaining internal stability, thereby avoiding desiccation (Howard and Blomquist, 2005). Therefore, we have hypothesized the likelihood that these same compounds have similar functions in the nest when subjected to different environmental pressures, either remaining intact or undergoing some kind of change in their composition in order to suit to changing ambient conditions, in particular temperature fluctuations.

Based on our results, it seems that the composition of *P. versicolor* nest is less subject to variation under exposure to different temperatures. On the other hand, the compositions of *Polybia* nests seem to be more sensitive. These different responses may be related to the chemical composition characteristic of the nest of the species, or they may be tied to factors directly linked to thermoregulation strategies of colonies.

The higher number of compounds, in particular branched alkanes, in *P. versicolor* nests is consistent with the complexity found by Michelutti et al. (2018b) for the cuticle of this species. Such compound complexity is linked to the greater complexity of information content (Lorenzi et al., 2014; Michelutti et al., 2018b), mainly because it has more branched alkanes (Bonavita-Cougourdan et al., 1991; Dani et al., 1996, 2001; Hefetz, 2007; Lorenzi et al., 1997; Murakami et al., 2015). However, it may also be related to thermoregulation. The differences found between *Polistes* and *Polybia* nests may be related to genetic factors, but also to the type of nest they construct. Thus, the multilayered envelope of *Vespinae* nests has evolved in response to temperature fluctuations to which their colonies may have been subjected through time, as well as other protective functions (Jeanne, 1975). In fact, nests of the genus *Polybia* have a relatively fragile structure, which can affect the conduction of heat inside the nest; thus, *Polybia* wasps protect against overheating of their phragmocyttarous-type nest by combining particular nest architecture with the choice of nesting in shady locations (Hozumi et al., 2005). This could explain why the compounds of their nests may be relatively more susceptible to temperature variation than those of *Polistes*, the nests of which are more exposed to ambient conditions.

The selection of less exposed sites for nesting is a strategy of wasps of both genders because the nests are not as exposed to daily temperature variations, thus considerably reducing the cost of cooling activities (Hozumi et al., 2005; Höcherl et al., 2016). Therefore, in

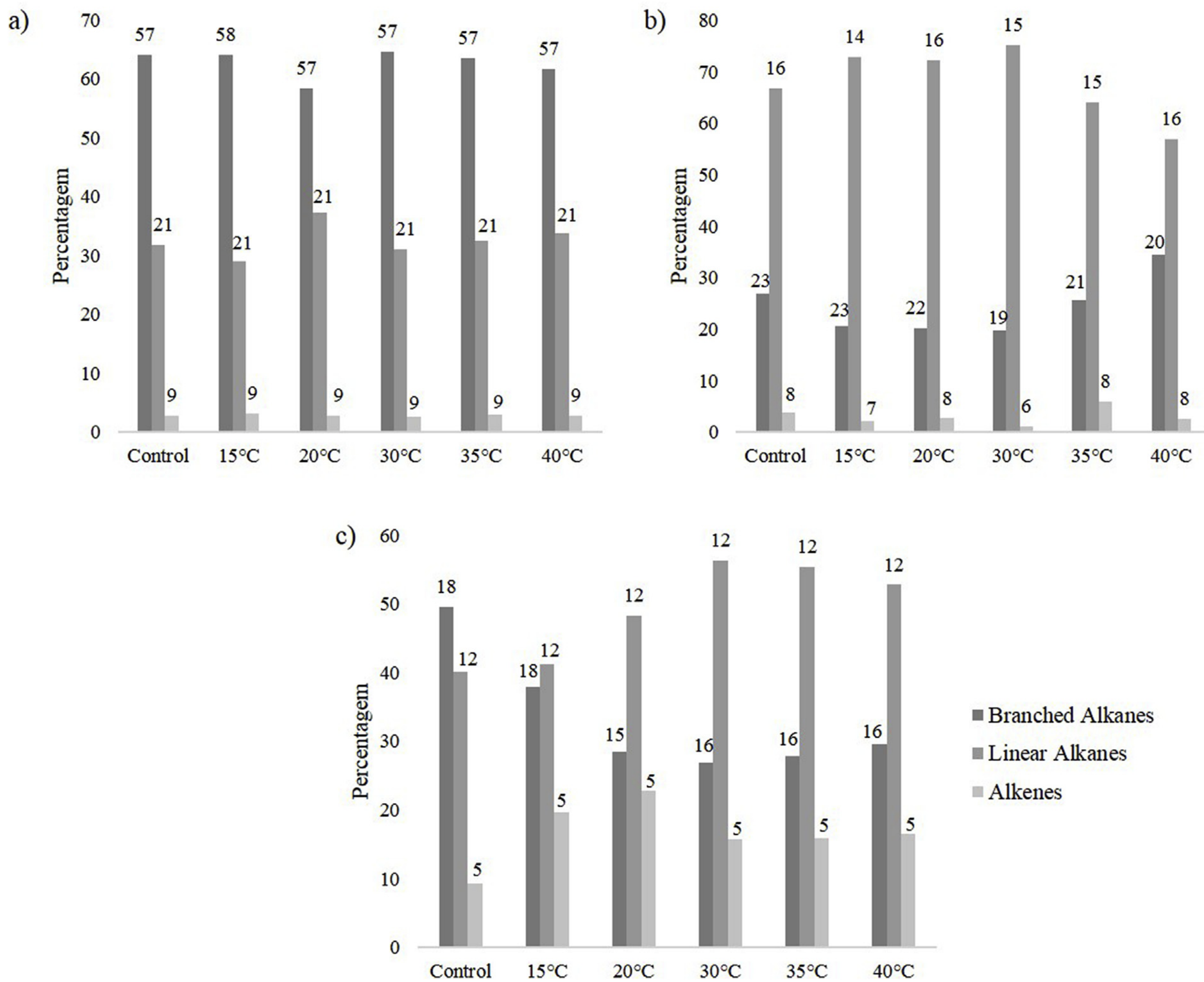

Fig. 1. Number of compounds and percentage of the 3 main classes of compounds present in the nest material of a) *Polistes versicolor,* b) *Polybia ignobilis* and c) *Polybia paulista* under different temperature regimes.

Table 1
P values of the PERMANOVA analysis of the hydrocarbons in the samples under the effect of the different temperature regimes, when compared among *Polistes versicolor, Polybia ignobilis* and *Polybia paulista*.

	Polistes versicolor	*Polybia ignobilis*	*Polybia paulista*
	Control	Control	Control
15 °C	0.09	1.00	0.04
20 °C	0.11	1.00	< 0.001
30 °C	0.85	1.00	< 0.001
35 °C	1.00	0.07	< 0.001
40 °C	1.00	0.03	< 0.001

colonies of *P. dominula*, wasps carefully select the site since temperature variations can lead to high energy costs (Höcherl et al., 2016). Indeed, Kovac et al. (2017) reported that *Polistes* species with different ability to tolerate temperature variation choose their nesting sites accordingly. *Apoica* wasps often select tree branches where the microclimate is kept relatively constant by numerous leaves used for cover (Yamane et al., 2009).

Both groups apply saliva in the nest; this behavioral strategy can help protect the nest from the elements, i.e., climatic factors such as temperature and precipitation (Yamane and Itô, 1994; Kudô et al., 1998; Erturk, 2017). Studying *Dolichovespula saxonica*, Yamane and Itô (1994) detected entire surfaces of nests to be coated with salivary secretion, noting that the coat was thicker in tree-built nests compared to those built in protected sites. Furthermore, Kudô et al. (1998), studying *Polistes chinensis*, demonstrated that wasps can regulate the amount of oral secretion applied in the nest in response to environmental conditions. Thus, in periods of higher precipitation, correspondingly higher levels of saliva are detected in the nest of these wasps. In this way, the individuals of the colony may contribute to maintaining the composition of the nest, even changing the profile of the nest, in order to respond to variation in temperature. However, the experiments here were performed in the absence of wasps.

The nests built by *P. versicolor* do not have a protective envelope and are, therefore, much more susceptible to changes in temperature or other ambient conditions. In this case, the chemical composition of the nest is essential to maintaining the internal homeostasis of the cells, which, in turn, would explain the relatively weak change of chemical nest composition with temperature fluctuation of this species. On the other hand, it is possible that the chemical composition of nests may be similar to the chemical composition found of the wasps‘ cuticle and, thus, can be expected to respond to the same cues in the same way. Michelutti et al. (2018b) evaluated the cuticle of *P. versicolor, P. paulista* and *P. ignobilis* wasps, and they found that these wasps may undergo adjustments in response to temperature fluctuation. In this sense, wasps may actively change the composition of their nests, allowing the substrate materials to serve as a buffer against temperature variations much like a wasp’s cuticle.

Protective strategies differ according to the different characteristics of the colony. For example, wasps that construct open combs occupy a

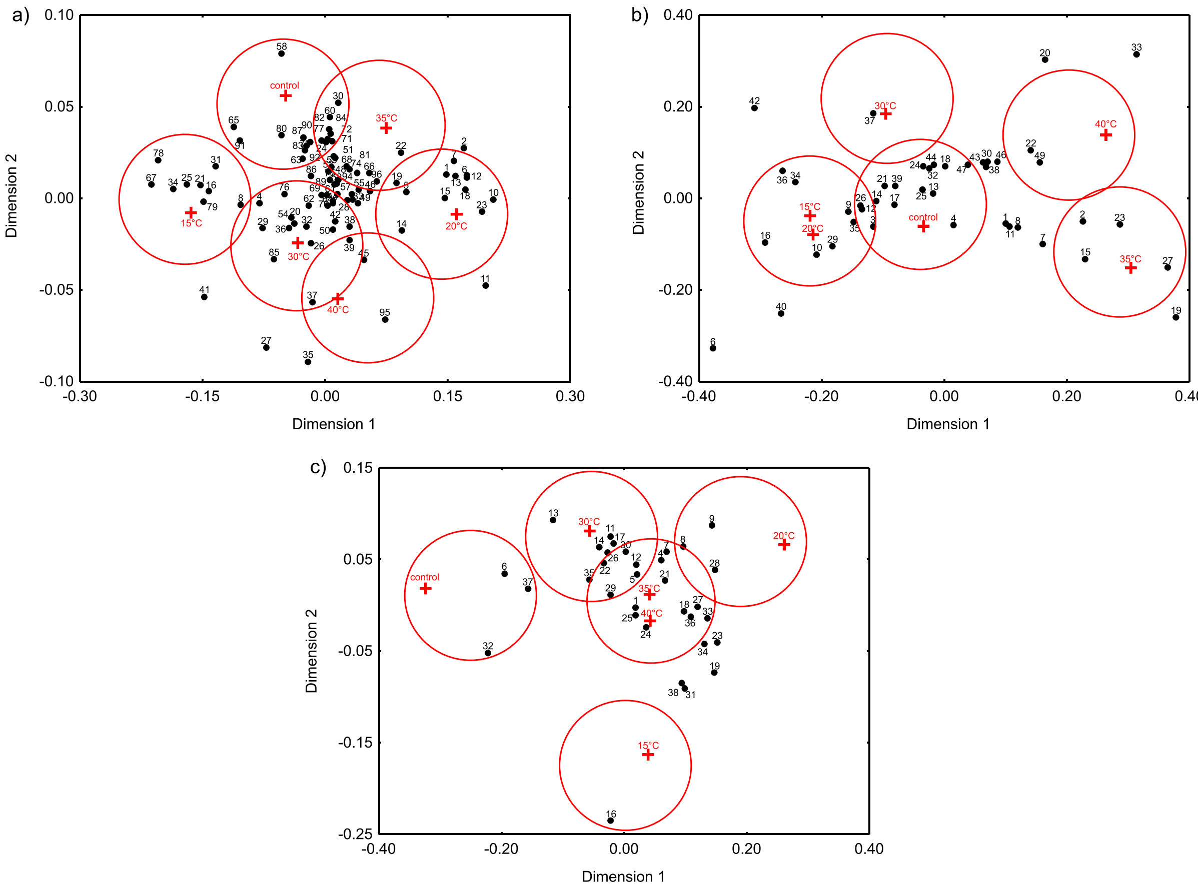

Fig. 2. NMDS with Bray Curtis similarities analysis indicating variability of chemical compounds in the nest substrate material of a) *Polistes versicolor,* b) *Polybia ignobilis* and c) *Polybia paulista* subjected to different temperatures. The numbers in each figure represent the compounds shown in Supplementary Tables 1–3.

smaller, less crowded, space, but also one that is more susceptible to environmental fluctuations. Those wasps that build covered combs have a larger colony, but also more physical protection. According to Richards and Richards (1951), for covered comb wasps, colony size is also a factor that will determine proper internal temperature since both the production and maintenance of heat depends on the number of individuals in the colony, while the loss of heat depends on the surface. This means that large colonies will conserve more heat. Hozumi et al. (2010) reported that the maintenance of colony temperature also depends on the thermogenesis of individuals in colonies of *Polybia scutellaris*, in which the workers perform incubation behavior.

The temperatures in the nests of the Epiponini will depend on physical properties and modifications of the enclosure, especially the construction of additional layers separated by air (Richards and Richards, 1951). In addition, Souza et al. (2015) argue that the occurrence of wasps, especially the genus *Polybia*, at high altitudes is possible because of the presence of the envelope, which might help keep the internal temperature of the nest more constant. Studying colonies of *Apoica flavissima*, Yamane et al. (2009) have shown that immature and adult wasps resting on the underside of the comb seem to function as an effective insulator, suggesting that immature heat capacity, a thick blanket of adults, and the production of metabolic heat by the inhabitants all play a role in temperature stabilization. The related *Apoica* and *Polybia* wasps belonging to the Epiponini tribe (Carpenter and Marques, 2001 may share a resemblance in their survival strategies.

The survival strategy for the genus *Polistes* would be to have nests, whose chemical composition do not undergo alteration but instead are relatively resistant to temperature variations, thus tending to maintain chemical stability independent of temperature fluctuations. Nests of *P. versicolor* possess a greater number of compounds compared to *P. ignobilis* and *P. paulista*, and most of these compounds have relatively long chains. Thus, the greater complexity of the chemical composition combined with a high concentration of compounds with long carbon chains can promote greater compositional integrity. Among the major compounds, three have relatively long chains: 15-methylnonacosane (2930), 13-methylhentriacontane (3131) and 15-methyltritriacontane (3329) (Supplementary Table 1), which have also been identified as major compounds in nests of *P. metricus* (Espelie et al., 1990). Michelutti et al. (2017b) also identified these same compounds in samples of *Mischocyttarus consimilis*. Compounds with longer chains, as well as compound classes such as linear alkanes, tend to resist higher temperatures (Gibbs, 1998), providing a solid foundation for the hypothesis driving the present work.

The ability of *Polistes* wasps to maintain intact nest compounds has been described by Sumana et al. (2005), who demonstrated that the compounds present in the *P. dominula* nest remain intact after winter, allowing wasps to return to their birth nest and regain its colonial profile. Natal homing is common in *Polistes* wasps (Reeve, 1991) and may be a means of finding nestmates after winter, thereby reducing the chance of forming associations with unrelated individuals and increasing the chance that individuals will reclaim their specific hydrocarbon signature from the colony, which was maintained throughout the winter by nest substrate (Sumana et al., 2005). This homing to natal nest reinforces the importance of maintaining the chemical signature in the face of changing temperatures (Sumana et al., 2005).

It seems plausible that divergent evolutionary strategies for maintaining colony temperature, between wasps that have open and closed nests, function as drivers of differences in the chemical composition of the nests. Maintaining a constant temperature in the colony may also be a matter of both nest substrate/structure and the activity of wasps themselves. In relatively small colonies, nest substrate is more resistant to temperature variation because it is composed of a greater diversity of elements, which would also be capable of holding heavier, longer carbon chains, thus providing better insulation.

Throughout the course of evolution, wasps have made many adaptations to temperature that have permitted their survival (Hozumi et al., 2005; Jones and Oldroyd, 2007; Yamane et al., 2009; Höcherl et al., 2016). The role wasps play in contributing to the chemical compounds present in the nest is unclear; nonetheless, to the best of our knowledge, this is the first study to assess the functionality of compounds present in nest material. It associates those compounds with differences between paper wasps in the strategies of adaption to fluctuating ambient temperatures.

Conflicts of interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jtherbio.2019.07.009.

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Kamylla Balbuena Michelutti: Graduation in Biological Sciences (2013) and master's degree in Natural Resources (2015) by State University of Mato Grosso do Sul (UEMS). During the master course, developed research on cuticular hydrocarbons of the developmental stages of social wasps. Doctor by the program in Natural Resources of State University of Mato Grosso do Sul (UEMS) (2019). Has experience in General Biology, analytical chemistry with emphasis on Entomology.

Raul Cremonezi Piva: Master of the Pós-graduação em Química da Universidade Federal da Grande Dourados (UFGD). Graduated in Industrial Chemistry from the Universidade Estadual de Mato Grosso do Sul (UEMS). He was a Fellow of CNPq, FUNDECT and PETROUEMS of scientific initiation in the area of Analytical Chemistry, with emphasis on natural resources and chromatographic techniques.

Sidnei Eduardo Lima Junior: Bachelor, Master and PhD in Biological Sciences by the State University of São Paulo (Unesp), Campus of Rio Claro. He is an Adjunct Professor (level V, on an exclusive dedication basis) at the State University of Mato Grosso do Sul (UEMS) since August 2003. He is a member of the Permanent Nucleus of the Postgraduate Program in Natural Resources - UEMS since its implementation in March 2010. He has experience in the areas of Zoology and Ecology, with emphasis on Ichthyology - Biology and Fish Ecology. He was UPR Rector of Research and Post-Graduation between September 2007 and June 2011. He was coordinator of the Graduate Program in Natural Resources - UEMS between May 2013 and May 2015.

Claudia Andrea Lima Cardoso: Graduation in Chemistry by Federal University of Mato Grosso do Sul(1993). Master's degree in Chemistry by Institute of Chemistry of Araraquara -UNESP (1996) and doctorate in Chemistry (2000) by the same institution. Professor at State University of Mato Grosso do Sul in the graduate and postgraduate. Experience in the field of Chemistry, with emphasis on chromatographic techniques applied to the analysis of samples of plant, animal and environmental origin.

William Fernando Antonialli Junior: Graduation in Biological Sciences by Paulista State University Júlio de Mesquita Filho, Rio Claro, Brazil (1996). Master's degree, doctorate and post-doctorate in Zoology by Paulista State University Júlio de Mesquita Filho (2003). Professor at State University of Mato Grosso do Sul (UEMS). Experience in the field of Behavioral Ecology of social Hymenoptera.