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Variation of cuticular chemical compounds in three species of *Mischocyttarus* (Hymenoptera: Vespidae) eusocial wasps

Eva Ramona Pereira Soares[a,b,*], Nathan Rodrigues Batista[b], Rafael da Silva Souza[c], Viviana de Oliveira Torres[b], Claudia Andrea Lima Cardoso[c], Fabio Santos Nascimento[d], William Fernando Antonialli-Junior[a,b]

[a] *Universidade Federal da Grande Dourados, Programa de Pós-graduação em Entomologia e Conservação da Biodiversidade, Dourados, MS, Brazil*
[b] *Universidade Estadual de Mato Grosso do Sul, Laboratório de Ecologia Comportamental, Dourados, MS, Brazil*
[c] *Universidade Estadual de Mato Grosso do Sul, Centro de pesquisa em biodiversidade, Dourados, MS, Brazil*
[d] *Universidade de São Paulo, Faculdade de Filosofia Ciências e Letras de Ribeirão Preto, Departamento de Biologia, Ribeirão Preto, SP, Brazil*

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ABSTRACT

The social wasps have a remarkable system of organization in which chemical communication mediate different behavioral interactions. Among the compounds involved in this process, cuticular hydrocarbons are considered the main signals for nestmate recognition, caste differentiation, and fertility communication. The aims of this study were to describe the cuticular chemical compounds of the species *Mischocyttarus consimilis*, *Mischocyttarus bertonii*, and *Mischocyttarus latior*, and to test whether these chemical compounds could be used to evaluate differences and similarities between *Mischocyttarus* species, using gas chromatography coupled to mass spectrometry (GC–MS). Workers from these three species presented a variety of hydrocarbons ranging from C_{17} to C_{37}, and among the compounds identified, the most representative were branched alkanes, linear alkanes and alkenes. The results revealed quantitative and qualitative differences among the hydrocarbon profiles, as confirmed by discriminant analysis. This study supports the hypothesis that cuticular chemical profiles can be used as parameters to identify interspecific and intercolony differences in *Mischocyttarus*, highlighting the importance of these compounds for differentiation of species and populations.

Introduction

Social insects have a sophisticated colony organization system regulated mainly by chemical signals or pheromones, which are involved in all social activities (Wilson, 1965). Among these insects are the social wasps, which belong to the Vespidae family, divided in six subfamilies. Among these subfamilies is Polistinae, which englobes the genus *Mischocyttarus* Saussure (1853). This genus is the only representative of the tribe Mischocyttarini (Carpenter, 1993) and is the largest genus of social wasps, with more than 240 species distributed in nine subgenera (Carpenter and Wenzel, 1988; Silveira, 2008).

Their colonies are established by independent foundresses varying from one to a few queens (reproductive females) that start to build the nests (Jeanne, 1980; Von Ihering, 1896). A typical nest consists of a single comb attached to the substrate by a peduncle (Gadagkar, 1991; Jeanne, 1972; Wenzel, 1991). *Mischocyttarus* is an essentially Neotropical genus, with exception of a few species that occur in northern Mexico and Florida, USA (Hermann and Chao, 1983; Silveira, 1998, 2008), and has been considered of great importance in studies regarding the sociobiology (Jeanne, 1970, 1972; O'Donnell, 1999; Strassmann et al., 1995).

An important trait that plays a role in the cohesion of insect societies is the ability to distinguish between nestmates and non-nestmates (Singer et al., 1998). Chemical communication is very important for this purpose (Billen, 2006), as these insects use information provided by chemical compounds known as pheromones. Karlson and Luscher (1959) define pheromones as chemical signals produced by an organism that, even released in small quantities, may induce behavioral and/or physiological changes in another individual of the same species. These pheromones are generally divided into two types: light and volatile substances secreted by the exocrine glands and heavy hydrocarbon molecules found in the cuticle (Howard, 1993).

Cuticular hydrocarbons (CHCs) are compounds that essentially consist of carbon and hydrogen (Blomquist and Bagneres, 2010) and compose part of the lipid layer covering the cuticle of insects. They are essential to the survival of the individuals, because their

* Corresponding author.
E-mail: evapereirasoares@gmail.com (E.R. Soares).

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primary function is to prevent dehydration (Lockey, 1988); at the same time, they form a protective barrier against microorganisms (Provost et al., 2008). The CHCs are synthesized by secreting cells derived from epidermal cells (Lockey, 1988) and are transported to the cuticle via hemolymph by lipophorin proteins (Bagneres and Blomquist, 2010).

Among the hundreds of CHCs identified in insects, there are three groups that stand out: n-alkanes, methyl branched alkanes, and unsaturated hydrocarbons (Blomquist, 2010). These compounds also act as signals during communication among insects, especially in social insects, enabling the recognition of conspecific individuals, nestmates, age, sex, and caste (Howard, 1993; Singer et al., 1998; Vander Meer and Morel, 1998). Variation can occur in the CHCs composition, depending on genetic and exogenous factors (Arnold et al., 1996; Blomquist and Bagneres, 2010; Dahbi et al., 1996; Gamboa et al., 1996; Kather and Martin, 2012; Page et al., 1991).

Another function of CHCs that has been previously explored in social wasps is their use as a complementary tool to assess variations among insect populations (Calderón-Fernández et al., 2005; Dapporto et al., 2004), as in the case of the species *Polistes dominula* (Christ, 1791) (Dapporto et al., 2004). Furthermore, previous studies have used the CHCs to differentiate species of termites (Kaib et al., 1991), ants (Martin et al., 2008), and Stenogastrinae wasps (Baracchi et al., 2010). Despite the recognized importance of these compounds as signaling or biochemical markers, there have been few studies with social wasps of the genus *Mischocyttarus*.

Mischocyttarus has been considered important for the study of sociobiology in wasps due to the incipient social organization and reproductive totipotence (Jeanne, 1970, 1972; Strassmann et al., 1995). However, the success of these studies depends on parallel efforts to rebuild relationships within this highly diverse genus (Silveira, 2008). Therefore, the aims of this study were 1) to describe the cuticular chemical compounds of the species *Mischocyttarus consimilis* (Zikan 1949), *Mischocyttarus bertonii* (Ducke, 1918), and *Mischocyttarus latior* (Fox, 1898), and 2) to test whether these chemical compounds could be used to evaluate differences and similarities between *Mischocyttarus* species.

Materials and methods

Collection

Twenty colonies were collected in different areas of the municipalities, two in Ivinhema (22°21′22.5″ S; 53°45′26.1″ W), 14 in Mundo Novo (23°56′23″ S; 54°17′25″ W), and three in Dourados (22°13′16″ S; 54°48′20″ W) in Mato Grosso do Sul State; and one in the municipality of Palotina (24°16′23″ S; 53°52′38″ W) in Paraná State, Brazil. Colonies of three species were collected: *Mischocyttarus (Kappa) bertonii*, *Mischocyttarus (Kappa) latior*, and *Mischocyttarus (Monocyttarus) consimilis*. All the adults were killed by freezing and stored until the moment of extraction of the compounds. Only workers were used, because differences between castes could influence the individual chemical composition. Castes were distinguished by behavioral observation prior to collection using the behavioral repertoire described by Torres et al. (2012) in *M. consimilis*. In addition, all colonies collected were in the post-emergence phase, according to the classification of Jeanne (1972).

Extraction of cuticular compounds for chemical analysis

The cuticular compounds were extracted by washing each individual for 2 min in 2 mL of hexane (Vetec, HPLC grade). The extracts were dried in an exhaustion chapel and stored until the day of the analysis, when the samples were solubilized in 200 μL of hexane. The CHCs were extracted from each female and the variation depended on the number of individuals in the colony, the colonies collected, and the species. The analyses were performed using a gas chromatograph coupled to a mass spectrometer (Model 2010 GCMS-QP, Shimadzu). A DB5-MS column was used (30.0 m length × 0.25 mm internal diameter, 0.25 μm film thickness), with heating from an initial temperature of 150 °C to 280 °C, at a rate of 3 °C/min, and maintaining the final temperature for 25 min. Helium (99.99%) was used as carrier gas, at a flow rate of 1 mL/min, and 1 μL sample volumes were injected in splitless mode. The temperatures of the injector, detector, and transfer line were 250 °C, 250 °C, and 290 °C, respectively. The mass spectrometer parameters included electron impact ionization voltage of 70 eV, mass range of 40–600 m/z, and scan time of 0.3 s.

The cuticular compounds were identified using the retention indexes calculated using a series of linear alkanes (Van den Dool and Kratz, 1963), the library of the equipment (NIST21 and WILEY229), and analysis of the mass spectra. In the case of the linear alkanes (C_8–C_{40}), standards of the compounds were also used.

The peak area for each compound was determined by manual integration of the total ion chromatogram (TIC). All the areas were then transformed into relative percentage areas.

Statistical analysis

We used a discriminant analysis to separate the groups defined previously according to their chemical profiles of inter- and intraspecific differences. Wilks' Lambda was employed as a measure of the contribution of each variable. In these multivariate analyses, the percentage values were calculated from the chromatogram peak areas used as the independent variables.

Results

Interspecific variation among the three species of Polistinae wasps

The chemical compounds identified in the analyses of samples from the three species ranged from C_{17} to C_{37} (Table 1), corresponding to over 47% of the compounds detected and representing a relative proportion exceeding 76%. The three species presented 10 common compounds: 3-methyloctadecane, pentacosane, heptacosane, 3-methylheptacosane, octacosane, X-methyloctasane, 3-methyloctacosane, nonacosane, 13-methylnonacosane, and 3-methyltriacontane (Fig. 1). However, the use of relative proportions of these compounds in the chromatograms permitted the identification of quantitative differences between the species. For example, the relative percentage of 3-methyloctacosane was significantly higher in *M. latior* than in the two other species, and there were important contributions of 3-methylheptacosane and nonacosane in *M. bertonii* and *M. consimilis*, respectively (Fig. 1).

The compounds 6-methylpentacosane, 1 heptacosane, nonacosene, and 13,17-dimethyltriacontane only occurred in workers of the *M. bertonii* species. Workers of *M. latior* did not show any unique compounds (Table 1).

M. consimilis showed the highest number of detected (163) and identified (60) compounds, of which 13 were exclusive to this species: 4,8-dimethyloctacosane, 13,17-dimethylhentriacontane, 11-methyldotriacontane, 11,21-dimethyltritriacontane, 7-methyltritriacontane, X-methyltetratriacontane, X-methylpentatriacontane, 11,21-dimethylpentatriacontane, X,Y-dimethylpentatriacontane, 7,11-dimethylpentatriacontane, hexatriacontane, X-methylheptatriacontane and 11,21-dimethylheptatriacontane.

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