ROLE OF CUTICULAR HYDROCARBONS AS POTENTIAL CUES OF POPULATION ORIGIN IN THE STINGLESS BEE *MELIPONA MARGINATA* LEPELETIER, 1836 (HYMENOPTERA, APINAE, MELIPONINI)

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INTRODUCTION

Stingless bees are widely distributed by different Neotropical biomes (Sakagami, 1982). Currently, morphometric analysis is used to study population structure and geographical variation between races, or even between populations of *Apis mellifera* (Francoy *et al.*, 006; Mendes *et al.*, 007). In addition, studies on mitochondrial DNA polymorphism (Torres *et al.*, 009) and cuticular hydrocarbons (Francisco *et al.*, 008) have been used to identify differences between species.

Hydrocarbons are very important for social insects. They constitute a protection barrier against water loss (Lockey, 1988; Nelson & Blonquist, 1995). In evolutionary process, these compounds have been used as communication cues (Le Conte & Hefetz, 2008). Howard & Blomquist (2005) consider this secondary function of hydrocarbons as a reason of cuticular compounds diversity found among insect groups.

Recently, some bioassays have shown that surface hydrocarbons are important tools for recognition systems for solitary insects but above all, for social insects were the chemical communications between individuals are complex (Provost *et al.*, 008). Hydrocarbons also showed differentiation as colony specificity, age, caste or sex of individuals (Blonquist *et al.*, 998; Monnin & Peters, 1999; Sledge *et al.*, 001; Provost *et al.*, 008).

In stingless bees, there are relatively few studies that investigate intra and inter - specific biodiversity. In this study we showed that is possible to differentiate population of bees according to the individual profile of cuticular hydrocarbons.

OBJECTIVES

The aims of the study were to investigate the differences between cuticular hydrocarbon profiles of workers of different populations in the stingless bee *Melipona marginata*.

MATERIAL AND METHODS

1 - Study species and colonies

Three colonies of *Melipona marginata* were collected in Cunha (two colonies) and Itanhaém (one colony), placed in wooden boxes and kept at the meliponary of Universidade de São Paulo, Ribeirão Preto. From each colony we collected 10 newly emerged workers, 10 workers with brood cell activity and 10 foragers.

2 - Sample collection

The epicuticular compounds were extracted in hexane (1ml per individual, 1 minute). After eliminating the solvent, the apolar extract was suspended in 50 μl of hexane for the analysis by combined gas chromatography - mass spectrometry (GC - MS: SHIMADZU, model QP2010) with 70 eV ionization. Separation was achieved on a DB - 5MS column 30m and the gas carrier was helium at 1.0 ml min - 1. The oven temperature was initially 150oC, and raised by 3oC min - 1 to 280oC. Analyses were performed in splitless mode.

3 - Analysis of data

The data were analyzed with GCMS solutions for Windows (Shimadzu Corporation). The chemical compounds were identified based on their mass spectra, by comparison with Wiley Library data and with a standard solution of different synthetic hydrocarbons.

The areas under the peaks of the chromatograms were transformed according to Reyment's formula ($Z = \ln[Ap/g(Ap)]$), where Ap is the area of the peak and g(Ap) is the geometric mean peak area (Aitchison, 1986). The subsequent statistical analysis was applied only to those substances with three or more samples for each group. Principal components analysis (PCA) was used to define the main peaks of compounds to be compare. Only those peaks with highest factorial weight on the first two roots were selected. After,

the data were analyzed by a stepwise discriminant analysis to verify segregations between the groups of the analyzed individuals. The statistical analyses were performed using the software Statistic 7.0 for Windows (Statsoft, inc).

RESULTS AND DISCUSSION

The chemical analysis of the cuticular hydrocarbons of M. marginata identified a total of 25 hydrocarbons. These compounds varied between C22 and C31, and were classified as linear alkanes, linear alkenes and alkadienes.

The cuticular of workers from Cunha-SP showed 23 and 25 hydrocarbons, the most alkanes and alkenes. The compounds with a great representatively in the course of worker life were: tricosane, pentacosane and heptacosane. The compounds that showed increase was Z - pentacosene (Retention Time 28.45 min), while compounds that had yours relative concentration decrease were Z - heptacosene (Retention Time 33.29 min) and Z - hentriacontene (Retention Time 42.35 min).

The cuticle of workers from Itanhaém-SP showed less variety of hydrocarbons with relation to colonies from Cunha. We found 19 hydrocarbons and the most abundant compounds in the course of this worker's life were: tricosane, pentacosane, Z - heptacosene (Retention Time 33.29min), heptacosane and Z - nonacosene (Retention Time 38.19min). Z - heptacosene (Retention Time 33.29min), octacosane, Z - nonacosene (RT 38.192) and Z - hentriacontene (Retention Time 42.44min) were the compounds whose relative concentrations decrease in the course of age.

The results showed that cuticular hydrocarbon profiles of colonies from Cunha were more closed that profile found in the colony from Itanhaém. Factor 1 of the principal components analysis (PCA) using the analyzed 8 peaks described 53.44% of the observed variance. Factor 1 + Factor 2 described 72.82% and Factor 1 + Factor 2 + Factor 3 described 84.98% of the total variance.

The discriminant analyses separated workers according their colony origin (< 0.001) in all cases. Mahalanobis Distances results were: Newly emerged workers: Colony Cunha 1 x Colony Cunha 2 = 16.81; Colony Cunha 1 x Colony Itanhaém = 101.13; Colony Cunha 2 x Colony Itanhaém = 130.16. Workers with brood cell activity: Colony Cunha 1 x Colony Cunha 2 = 187.86; Colony Cunha 1 x Colony Itanhaém = 194.08; Colony Cunha 2 x Colony Itanhaém = 177.63. Foragers workers: Colony Cunha 1 x Colony Cunha 2 = 20.51; Colony Cunha 1 x Colony Itanhaém = 210.62; Colony Cunha 2 x Colony Itanhaém = 191.57.

The cuticular hydrocarbons varied with regard to origin of colonies. Colonies from Cunha showed, according to their cuticular profiles, higher similarity to the colony from Itanhaém, except by group of workers with activity at brood cell. One reason for this divergence in chemical profile could be the great exposition for these individuals with the nest material. The workers closed in cell don't have a continuous exposition with nest material even their emergence and the foragers are more susceptible to loose compounds for volatilization when they are outside colony, beyond to be in contact with the same resources.

CONCLUSION

Our investigation confirms the existence of differences in the cuticular hydrocarbon profiles among population of Melipona marginata. These differences may be the basis for olfactory recognition of the population origin. The discriminant analysis revealed that, even to be fitting to three different colonies, the workers showed characterized by their tasks. The newly emerged workers were the most distant among the worker groups. The higher similarity occurred between workers with brood cell activity and foragers. That is, cuticular profiles are strongly influenced by ambient, mainly by the comb waxes.

Acknowledgements

The authors thank CNPq and Capes for financial support.

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