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Colony membership is reflected by variations in cuticular hydrocarbon profile in a Neotropical paper wasp, *Polistes satan* (Hymenoptera, Vespidae)

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ABSTRACT. Nestmate recognition is one the most important features in social insect colonies. Although epicuticular lipids or cuticular hydrocarbons have both structural and defensive functions in insects, they also seem to be involved in several aspects of communication in wasps, bees and ants. We analyzed and described for the first time the cuticular hydrocarbons of a Neotropical paper wasp, *Polistes satan*, and found that variation in hydrocarbon profile was sufficiently strong to discriminate individuals according to their colony membership. Therefore, it seems that small differences in the proportion of these compounds can be detected and used as a chemical-based cue by nestmates to detect invaders and avoid usurpation.

Key words: Chemical identity, Cuticular hydrocarbons, Kin selection, Social wasps

INTRODUCTION

Social insects are frequently efficient in discriminating resident and kin from strangers (Wilson, 1971; Arnold et al., 1996, 2000). Chemical cues play a significant role in nestmate recognition, which are defined by both genetic (Arnold et al., 1996) and environmental components (reviewed in Gamboa, 1996). The main compounds involved in the process of intracolonial recognition are the epicuticular hydrocarbons (Espelie and Hermann, 1990; Howard and Blomquist, 2005), and previous studies have demonstrated that the blends covering the cuticle of these insects are acquired from the nest material (Singer and Espelie, 1992; Breed, 1998).

In *Polistes* wasps, analyses of cuticular extracts showed that the main hydrocarbons, *n*-alkanes, methyl-branched alkanes, and alkenes, are involved in interspecific and individual recognition (Bonavita-Cougourdan et al., 1991). Studies in *P. dominulus* showed that young wasps obtained their chemical profiles within 24 h after emergence while older ones did not incorporate additional hydrocarbons (Lorenzi et al., 2004). Therefore, these compounds provide a kind of chemical signature which is recognized by the members of colony (Gamboa et al., 1986; Gamboa, 1996). Other reports revealed that hydrocarbon profiles also varied significantly between alpha, subordinate and worker individuals in the same species (Dani et al., 2001; Sledge et al., 2001).

Compared to other social wasps from temperate areas, the composition of epicuticular hydrocarbons and the mechanisms underlying nestmate recognition in Neotropical species are virtually unknown. Because these wasps have both distinct colonial cycle and ecological constraints (Gobbi and Zucchi, 1980; Gonzales et al., 2002; Tannure-Nascimento et al., 2005), different proximate mechanisms of recognition may have evolved, which could be reflected in their cuticular hydrocarbon profiles. Therefore, the aims of the present study were 1) to describe the cuticular hydrocarbons of *Polistes satan* females, and 2) to test whether intercolonial identity could be discriminated using these compounds.

MATERIAL AND METHODS

Study site, species and observations

Polistes satan builds one to twenty satellite nests in dark and sheltered places (Richards, 1978; Tannure-Nascimento et al., 2002). This study was carried out in a sugarcane farm surrounded by patches of natural vegetation located in Cajuru, São Paulo State, Brazil. In such a place, colonies are founded in abandoned farm houses and the nests are built on doors, windows or any wooden furniture left in the houses.

We selected randomly four pre-emergence colonies (colonies 1 to 4) to carry out the behavioral, physiological and chemical analyses. Adult individuals were marked with paint dots on the thorax. Each newly emerged wasp was also marked during the study period. Behavioral analyses were performed for three months to determine whether individuals actually belonged to the colonies, as well as to determine the dominance and the functional worker roles in each colony. After the period of observations, colonies were captured and individuals were anesthetized and killed in a freezer. We dissected each female under a stereomicroscope to determine ovary condition and insemination. Individuals showing mature oocytes and sperm in the spermateca were classified as queens. Workers invariably showed the lack of oocytes and non-inseminated conditions.

Chemical analyses and statistics

To determine the differences between hydrocarbon profiles of *Polistes satan* females, we extracted the epicuticular hydrocarbons by washing each individual for 1 min in hexane. Further, we performed a GC-MS spectral analysis of the hexane extracts. The extracts were taken to dryness under a stream of nitrogen. The remaining compounds were suspended in 50 µL hexane and analyzed on an HP 5890A gas chromatograph linked to an HP 5971A mass selective detector (using 70 eV). The temperature protocol used was as follows: 70-150°C at a rate of 30°C/min (held for 5 min), and 150-320°C at 5°C/min (held for 13 min). Analyses were performed in splitless mode and individual mass spectra were compared with the Wiley library data and standard alkanes.

The areas of 23 peaks, representing one or more cuticular compounds, were subjected to both univariate and multivariate statistical analyses. For multivariate analysis, peaks were directly analyzed with stepwise discriminant analysis. Discriminant analysis was performed to determine whether the predefined colony groups could be discriminated according to their profiles, and Wilk's lambda values were used to infer the contribution of each variable to the discrimination. Before multivariate tests, each peak area was transformed according to the following formula: $Z = \ln[Ap/g(Ap)]$ (Aitchison, 1986). Where Ap is the area of the peak, $g(A\mathrm{p})$ is the geometric mean of the peak in that female and Z is the transformed peak area. All statistical analyses were performed using Statistica software.

RESULTS

Colony composition, ovary conditions and behavior

During the three months of study, we observed that 32 emerged individuals and former individuals through behavioral interactions formed a dominance hierarchy in the colonies (Table 1). A single satellite nest was built in two colonies (A and B). Observations confirmed that the queens performed the most aggressive acts and laid most of the eggs. Colonies had a single reproductive and inseminated female (queen), while other females performed worker activities such as foraging, nest maintenance and brood care. Therefore, the results confirmed that the queens were the mothers of all newly emerged females.

Table 1. Composition of the studied colonies of *Polistes satan* after collection.

Colony	Number of females (number of founding individuals)	Number of nest + satellite nests	Cells	Eggs	Larvae	Pupae
1	13 (4)	1 + 0	32	4	10	10
2	9 (3)	1 + 0	15	10	6	2
3	13 (5)	1 + 1	39 + 28	19 + 2	20 + 19	19 + 0
4	15 (6)	1 + 1	32 + 24	9 + 5	10 + 7	5 + 0

Chemical analyses

Odd chain linear and methyl-branched alkanes were dominant on the surfaces of individuals belonging to the four studied colonies (Figure 1). Several dimethyl-chained alkanes were also found in lower quantities. Some hydrocarbons (*n*-C27; 11-, 12-, 13-, 14-, 15-methyl C27; 11-, 13-, 15-methyl C29; 11,15-, 13,17-dimethyl C29, and 11-methyl C31) showed a higher level of relative abundance (>10% of total) than did other compounds.

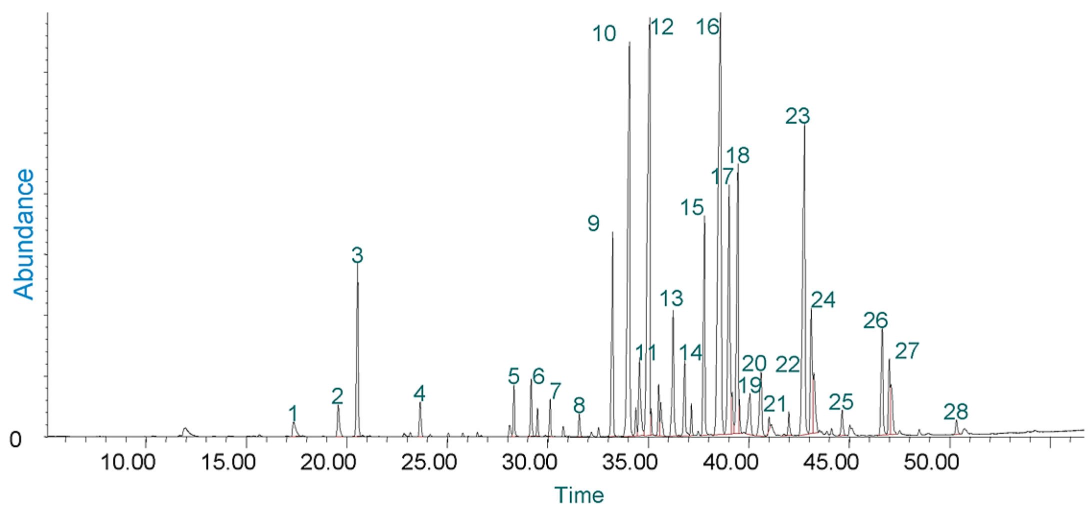

Figure 1. Gas chromatography showing the hydrocarbons present in the cuticle of *Polistes satan*. The numbers refer to the following compounds: **1.** 1-octadecene; **2.** acid (unknown); **3.** *n*-C22 (synthetic compound); **4.** acid (unknown); **5.** *n*-C25; **6.** 11-, 13-Me C25; **7.** 3-Me C25; **8.** 11-, 12-, 13-, 14- and 15-Me C26; **9.** *n*-C27; **10.** 11-, 12-, 13-, 14- and 15-Me C27; **11.** 11-, 15-DiMe C27; **12.** 3-Me C27; **13.** 13- and 14-Me C24; **14.** 4-Me C28; **15.** *n*-C29; **16.** 11-, 13- and 15-Me C29; **17.** 11,15- and 13,17-DiMe C29; **18.** 3-Me C29; **19.** 3,9-DiMe C29; **20.** 10-, 11-Me C30; **21.** 13,17-DiMe C30; **22.** *n*-C31; **23.** 11-Me C31; **24.** 13,17-DiMe C31; **25.** 11,12-DiMe C32; **26.** *n*-C33; **27.** 13,15- and 13,19-DiMe C33; **28.** 11,12-DiMe C34.

Discriminant analysis showed that all females could be separated according to their chemical profiles (Figure 2). Colony membership was significantly discriminated providing 100% correct classifications for three colonies (1, 2 and 3) and 93% of classifications for a single colony (colony 4). The most important hydrocarbons separating individuals according to their colonies were *n*-C28, *n*-C27, 11-methyl C31, and *n*-C33 (discriminant analysis: Wilks' lambda: 0.007; $F_{51,42} = 21.44$; P < 0.0001). Squared Mahalanobis' distance showed that individuals belonging to colony 1 had chemical profiles closer to that of individuals from colony 4 than did other individuals, but the distance between individuals of different colonies was sufficiently high to separate the groups (Figure 2; Mahalanobis distance = 82.02; $F = 8.10$; P < 0.001).

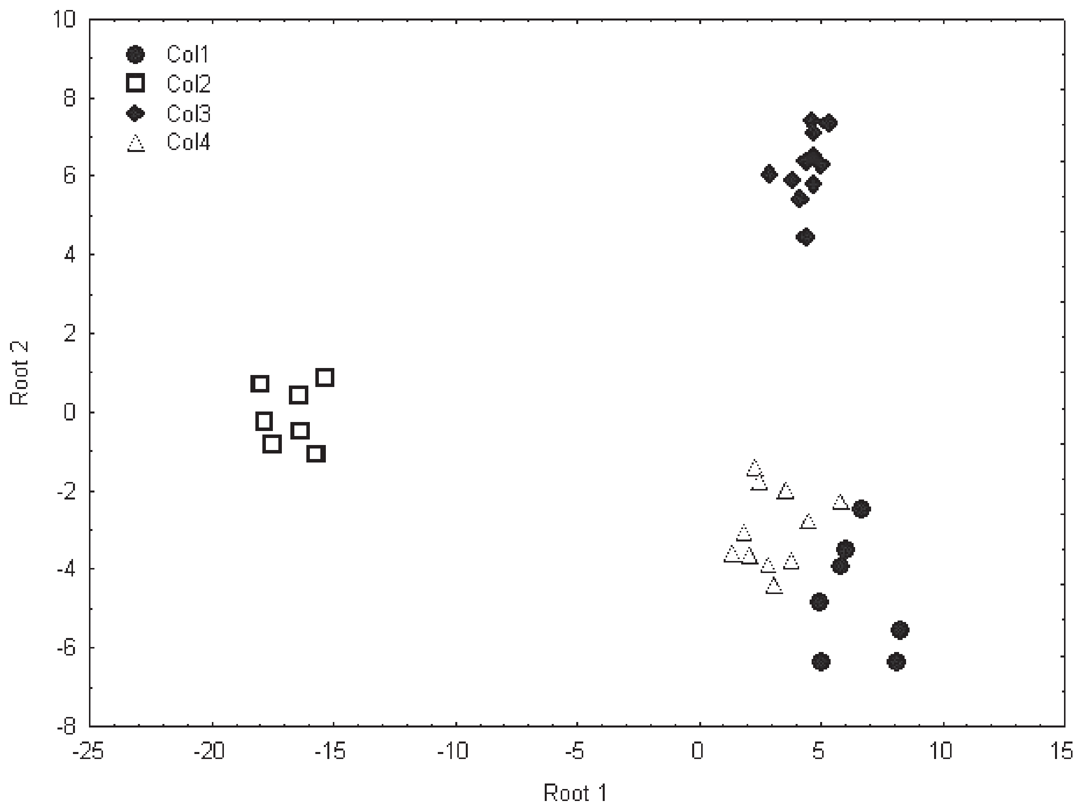

Figure 2. Discrimination of individuals from the four studied colonies based on their cuticular hydrocarbon compounds. Classification of all females was correct according to their colonies.

DISCUSSION

The composition of epicuticular hydrocarbons has been reported for only four American and seven European species (reviewed in Dani, 2006). This is the first study concerning the role of these compounds in a Neotropical species of *Polistes*. Previous studies suggest that linear alkanes are not used as recognition cues in temperate *Polistes* spp (Singer et al., 1998; Dani et al., 2001). On the other hand, methyl-branched alkanes and single alkenes are considered better chemicals to provide information for nestmate discrimination. As in other species, *Polistes satan* had a high relative abundance of both linear and methyl-branched hydrocarbons, and a low proportion of alkenes. Surprisingly, linear alkanes (*n*-C27, *n*-C29 and *n*-C33) were more significant than methyl-branched alkanes in both relative proportion and multivariate analysis.

Our data support the occurrence of a colonial signature based on cuticular hydrocarbons in *Polistes satan*. The reproductive structure of colonies was similar among the colonies comprised by a single queen, associated workers and their daughters. This is important to consider due to the ability of workers to acquire epicuticular profiles characteristic of foundresses since the reproductive dominance is based on interactions between queen and workers. In fact, cuticular hydrocarbons could provide sufficient information for nepotism in colonies with different matrilines (Dani et al., 2004; reviewed in Monnin, 2006). In addition, subordinate females develop a similar chemical profile of dominant foundresses right after their experimental removal (Sledge et al., 2001).

Variation in the chemical profiles was sufficiently strong to discriminate individuals according to their colony membership. Therefore, it seems that small differences in the proportion of cuticular hydrocarbons could be detected by nestmates and used as a chemical-based cue by colony guards to avoid usurpation. Sledge et al. (2001) verified that cuticular hydrocarbons were also significantly distinct among colonies in the European species *Polistes dominulus*. Interestingly, experimental studies showed that this same species can use any chemical substance variation besides that of hydrocarbons (Pickett et al., 2000). In fact, wasps use small differences in colony odors for discrimination. Therefore, *P. satan* females probably use these differences for recognition. Although we did not perform bioassays in this study, field observations confirm that wasps are aggressive toward non-resident individuals.

In conclusion, this paper corroborates previous studies concerning the potential role of cuticular hydrocarbons as mediators of nestmate recognition in social insects. However, this is the first step toward a better understanding of the importance of cuticular hydrocarbons in Neotropical social wasps. Further bioassays are necessary, however, to determine how variation in the proportions of these compounds affects nestmate recognition.

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