



RESEARCH ARTICLE - WASPS

Reproductive Status of the social wasp *Polistes versicolor* (Hymenoptera, Vespidae)

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Abstract

A fundamental feature in the evolution of social insects is the separation of castes, and the presence of wide differentiation between castes indicates a more advanced degree of sociability. In this study, we evaluated factors indicating the reproductive status of females in colonies of the social wasp *Polistes versicolor*. The reproductive status of each female was examined by measuring nine morphometric characters, by tracing the cuticular chemical profile, by evidence of insemination and by recording the relative age. We conclude that *P. versicolor* colonies present 3 female groups according to cuticular chemical profile difference. The first group is made of females with filamentous ovarioles, typical of workers; the second one is females with intermediate ovarioles; and the third group is the group of the queens, which are older females, already inseminated and with the greatest degree of ovarian development. No significant external morphological differences were found among these female groups. Therefore, despite the lack of significant morphological differences among females, there are other factors such as the chemical composition of the cuticula, which are indicative of the reproductive physiological condition of the female in the colony.

Introduction

An important feature for the ecological success of social insects is the division of labor among individuals in their colonies (Wilson, 1985). For this reason, many investigators have devoted their efforts to elucidate the parameters that determine this division, especially the distinction and determination of the caste (Robinson, 1992; O'Donnell, 1995; O'Donnell, 1998).

The subfamily Polistinae has characteristics that are important to understand how the social behavior has evolved in the wasps (Ross & Matthews, 1991). The degree of morphological differences among castes in this group can range from total absence (Richards, 1971; Strassmann et al., 2002) to sharp differences among castes (Jeanne, 1991). This, indeed, may be a key feature in the evolution of social insects, since the presence of wide differentiation among castes indicates a higher degree of sociality (Bourke, 1999).

In the basal Polistinae such as *Mischocyttarus* and *Polistes*, females are distinguished by their behavior, dominance hierarchy, degree of ovarian development and/or their

reproductive physiology (Röseler et al., 1985). The dominance status of the individual apparently initiates a physiological response that directly affects their ovarian development (Wheeler, 1986). The queen shows the highest degree of ovarian development and, by using behavioral strategies to dominate all of the other females, she largely monopolizes reproduction while avoiding energy-consuming tasks such as foraging (Jeanne, 1972; Strassmann & Meyer, 1983).

Evidently, on the lack of visible external traits, some other kind of detection mechanism, used by each member of the colony, is needed for the establishment and recognition of this hierarchy, and chemical communication is the most effective way to accomplish this recognition. Among the compounds involved in this process are the cuticular hydrocarbons (CHCs), which are a constituent of the lipid layer that coats the cuticle of insects, and have the primary functions of preventing desiccation (Lockey, 1988) and creating a barrier against microorganisms (Provost et al., 2008). CHCs also act as contact pheromones, allowing conspecific individuals to identify each other, thus assisting in maintaining the colony structure, separating individuals according to their function



in the colony, their physiological status and their hierarchical rank (Provost et al., 2008), functioning, therefore, as a specific chemical signature of the individuals.

Sledge et al. (2001) and Monnin (2006) noted that there is a strong correlation between reproductive status and CHC profile of each individual in a colony of wasps. According to Dapporto et al. (2005), in colonies of *Polistes dominula* (Christ) founded by a single female, the CHC profile of the queen and its brood are different and, when one of those colonies becomes orphaned, a worker assumes the queen position, its ovaries develop, and acquires a CHC profile similar to that of the original queen.

Maintenance of a reproductive monopoly by a queen is one of the goals reached by many social insects. In independent-founding species, it was believed that the queen maintains her reproductive status by using aggression toward other females; however, in recent decades many studies have demonstrated the importance and role of CHCs in the communication among members of the colonies and in maintaining the status of the queen (Bonavita-Cougourdan et al., 1991; Peeters et al., 1999; Liebig et al., 2000; Sledge et al., 2001; Dapporto et al., 2005).

This study is focused on analyzing the reproductive status of females of *Polistes versicolor* (Olivier) through examining morphological and reproductive physiological features and by tracing the chemical profiles of the cuticula.

Material and Methods

We collected 10 colonies of *P. versicolor* in the southern region of the state of Mato Grosso do Sul, in the cities of Dourados (22°13'16" S 54°48'20" W) and Mundo Novo (23°56'23" S 54°17'25" W). All of the females from each colony were evaluated for morphological, physiological and cuticular chemical profile analyses. The classification of the colonial stage are done according to the system proposed by Jeanne (1972).

After collection, the gaster of each female was individually fixed in an Eppendorf containing absolute ethyl alcohol (99.8% PA) for later analysis of ovarian development, insemination and relative age. The remainder of the body was preserved by freezing, for subsequent morphometric measurements and analysis of the cuticular chemical profile.

We performed nine morphometric measurements, modified from Shima et al. (1994) and Noll et al. (1997), in order to detect morphological differences: Head: width (HW), minimum interorbital distances (IDx); Mesosoma: width, length and height of mesoscutum (MSW, MSL and MSH, respectively); Metasoma: basal and apical heights of tergite 2 (T2BH and T2AH), length of tergite 2 (T2L); Wing: partial length of the forewing (WL).

The gaster was dissected under a Zeiss binocular stereomicroscope for evaluation of the degree of ovarian development, insemination and relative age. The ovaries were

classified according to the stage of development of the ovarioles, based on the observations of Baio et al. (2004).

For each female, the spermatheca was removed and put on a slide in a 1:1 solution of acid fucsina (1%) in order to determine the presence of sperm cells under a light microscope.

The relative age of all adult females was determined, according to the pigmentation of the transverse apodeme across the hidden base of the fifth sternum, as follows: LY (light yellow), LB (light brown), DB (dark brown) and BA (black). According to Richards (1971) and West-Eberhard (1973), this color sequence indicates a progression in the age of individuals, from younger (LY) to older females (BA).

For analysis of the cuticular chemical profile, the thorax of each female was submitted to optical spectroscopy by Fourier Transform Infrared Photoacoustic Spectroscopy (FTIR-PAS), after 48 hours in a vacuum oven, in order to minimize the water content. This technique was used by Antonioli-Junior et al. (2007 and 2008) and Neves et al. (2012) and has proved reliable for assessing the CHCs profiles of ants and wasps, even when compared to gas chromatography (Ferreira et al., 2012).

The FTIR-PAS technique measures the radiation absorbed by the sample. It is advantageous for application on very fragile objects, such as biological materials, because the low-intensity radiation does not destroy the sample. FTIR-PAS uses the infrared spectrum from 400 to 4000 cm^{-1} (Silverstein et al., 2000; Skoog et al., 2002), which is sensitive to the vibrations and rotations of molecular chemical groups, so it can identify and distinguish molecular radicals and some kinds of chemical bonds in the samples (Smith, 1999).

The resulting spectrum for each thorax was obtained from the mean of 64 spectra with a resolution of 8 cm^{-1} , which were separated in absorption lines between 400 and 4000 cm^{-1} , mostly those related to vibrations of CHCs.

The degree of ovarian development, morphometric data and the cuticular chemical profile were evaluated by stepwise discriminant analysis, which reveals the group of variables that better explain the groups evaluated in case of a difference. This is indicated by Wilk's Lambda, a measure of the difference, if any, among the groups (Quinn & Keough, 2002). The chi-square test was performed to test the association between the relative age and the three groups of females (workers 1, workers 2 and queens). For all analyses, the variable was considered significant when the level reached was <0.05.

Results and discussion

Four kinds of ovarian development (Fig. 1) were found in females of this species: type A, filamentous ovarioles without visibly developed oocytes; type B, ovarioles containing some oocytes in the initial stage of development; type C, ovarioles with moderately developed oocytes, some in the final phase of vitellogenesis; and type D, well-developed, longer ovario-

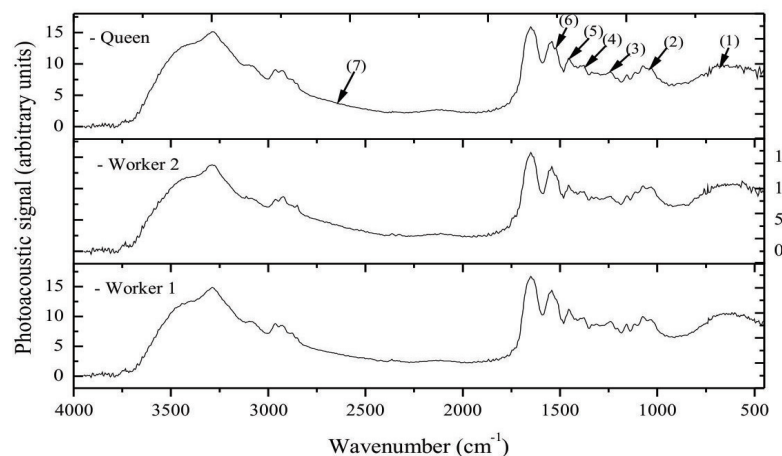


Fig 1. Different degrees of ovarian development found in females of *Polistes versicolor*. A) type A, filamentous ovarioles without visible developed oocytes; B) type B, ovarioles containing some oocytes in the initial stage of development; C) type C, ovarioles with moderately developed oocytes, some in the final phase of vitellogenesis; D) type D, well-developed, longer ovarioles, each containing two to several producing oocytes.

les, each containing two to several producing oocytes. Type D females were always inseminated.

These four physiological conditions were described by Baio et al. (2004) for *Brachygastra augusti* (Saussure). Noll et al. (2004) also described these same conditions in the species *Apoica pallens* (Fabricius), *Charterginus fulvus* (Fox) and *Nectarinella championi* (Dover). In all of these species, the females with the most advanced ovarian deve-

Fig 2. Mean curve for each group of mid-infrared absorption spectra of the thorax of *Polistes versicolor* females, grouped according to cuticular chemical profile and indicating the significant peaks for separation of the groups.



lopment were also the inseminated ones. However, Giannotti and Machado (1999), Gobbi et al. (2006) and Murakami et al. (2009) analyzed several independent founding species, *Polistes lanio* (Fabricius), *P. versicolor* and *Mischocyttarus cassununga* (von Ihering) among them, and found six, five and five ovarian development patterns, respectively.

Other studies evaluating the relationship between the degree of ovarian development and the reproductive position occupied by the females were performed on *Parachartergus smithii* (Saussure) (Mateus et al., 1997), *Pseudopolybia vespiceps* (Ducke) (Shima et al., 1998), *Chartergellus communis* (Richards) (Mateus et al., 1999), *Brachygastra lecheguana* (Latreille) (Shima et al., 2000), *Parachartergus fraternus* (Gribodo) (Mateus et al., 2004) and *Protopolybia chartegoides* (Gribodo) (Felippotti et al., 2007), all of them species of Etiponini.

The colonies of *P. versicolor* contained on average 2.9 ± 1.72 inseminated females, demonstrating the potential of other females to replace the queen; however, only one female had an ovarian condition typical of a laying individual (Fig. 1D). Giannotti & Machado (1999) and Murakami et al. (2009) also reported more than one inseminated female in colonies of *P. lanio* and *M. cassununga*, respectively. In fact, the presence of more than one inseminated female in the colony, capable of reproducing, is a common feature in independent-founding species, as is the case for *Polistes* and *Mischocyttarus*. This fact shows that the distinction between reproductive and non-reproductive females is quite flexible and complex, depending on physiological, behavioral and ecological factors (Murakami & Shima, 2006).

Insemination of two or more females in the same colony can be a strategy to overcome problems encountered during the colony cycle, such as predation or parasitism, as suggested by Murakami et al. (2009) for *M. cassununga*. Gobbi et al. (2006) found that 75% of *P. versicolor* females in the aggregate and 85% in the foundation association were inseminated; therefore, insemination must occur before the formation of aggregates.

The results from the analyzes of the cuticular chemical profile show that the 4 different types of ovarian development

degree are distributed among three distinct categories of females: a) Workers 1, with filamentous ovarioles, type A; b) Workers 2, with partially developed ovarioles, types B and C; and c) Queens, with fully developed ovarioles, type D (Fig. 1 and 2). These differences were significant (Wilks' Lambda = 0.476, $F = 6.303$, $P < 0.001$) (Fig. 3).

The spectra analyzed by FTIR-PAS showed significant differences among the cuticular chemical profiles, indicating seven significant peaks for the separation of females groups (Fig. 2 and Table 1). These compounds were linked to chitin (1238 , 1523 e 2634 cm^{-1}) and CHCs (667 , 1030 , 1377 and 1450 cm^{-1}) present in the female cuticle (Table I). Antonialli-Junior et al. (2007) and Neves et al. (2012) discuss the importance of these peaks for distinguishing the groups analysed. However, the most significant peaks for those groups were mainly those corresponding to the hydrocarbon band (Fig. 2 and Table 1).

The first canonical root explained 93% of the results, and the second one the remaining 7%, explaining together 100% of the results. Therefore, it seems that every female within these three groups had a different physiological status within the colony, which leads to a difference in the cuticular chemical profile and probably in the recognition by other females of their position in the colony hierarchy.

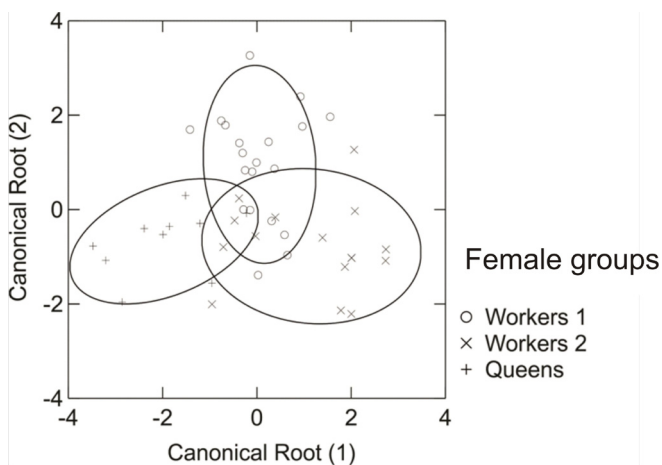


Fig 3. Dispersion diagram of the results of the stepwise discriminant analysis, showing the two canonical roots of differentiation of cuticular chemical profile in 3 different groups of females of *Polistes versicolor*.

According to Monnin (2006), there are correlations between reproductive status and the CHC profile in social insects, and that differentiation is important for the establishment of a hierarchy in independent-founding species, as it is the case for many Neotropical members of Polistinae. In colonies of *P. dominula* founded by a single female, the CHC profiles of the queen and workers were different (Dapporto et al., 2005). Bonckaert et al. (2012) investigated colonies of *Vespula vulgaris* (Linnaeus) and found that laying queens, queens in aggregate, virgin queens, and workers had different degrees of ovarian development, and this was correlated with their respective CHCs profiles.

The discriminant analysis of the 3 groups of females with different cuticular chemical profiles, however, showed no significant morphological differences (Wilks' Lambda = 0.851, $F = 3.182$, $P < 0.05$), indicating an absence of morphological differences among these groups. The absence of morphological differences among castes was also reported by Giannotti & Machado (1999) for *P. lanio* and by Murakami et al. (2009) for *M. cassununga*, all of them independent-founding species. However, Gobbi et al. (2006) observed that of *P. versicolor* female aggregate are significantly larger than first emerged females, foundress association and workers. Tannure-Nascimento et al. (2005) suggested that the morphological differences between reproductive and non-reproductive females of *Polistes satan* (Bequaert) during the colony cycle is due to seasonal nutritional differences.

In fact, most studies describe the absence of morphological differences among castes in *Polistes* and *Mischocyttarus* (Cumber, 1951; Giannotti & Machado, 1999; Tannure-Nascimento et al., 2005; Murakami et al., 2009), supporting the hypothesis of post-imaginal caste determination. However, studies such as those of Gadagkar et al. (1991), Keeping (2002), Dapporto et al. (2008) and Hunt et al. (2011) performed with *Ropalidia marginata* (Fabricius), *Belonogaster petiolata* (DeGeer), *Polistes metricus* (Say) e *P. dominula*, respectively, show that, at least in part, the distinction of castes may be pre-imaginal.

By analyzing the relative age of the females we found a higher frequency of young females during the post-emergence (pre-male) stage and, as the colony cycle advances, old females become more frequent during the post-emergence

Table 1. Wave numbers, coefficients of the two canonical roots, functional groups, and vibrations of the peaks identified in the infrared absorption spectra of the thorax of the wasps, for analysis of the cuticular chemical profile.

Peak	Wave number (cm^{-1})	Canonical root 1	Canonical root 2	Functional group	Vibration model
(1)	667	1.453	3.036	Out-of-plane C-H (benzene)	Bending
(2)	1030	3.185	-5.432	In plane C-H (benzene)	Bending
(3)	1238	5.766	-5.481	-C-N	Stretching
(4)	1377	-15.713	7.655	C-CH ₃	Symmetric bending
(5)	1450	2.659	1.669	C-CH ₂ and C-CH ₃	Asymmetric bending scissors
(6)	1523	3.216	-1.956	N-H	Bending
(7)	2634	0.173	1.456	C-N and N-H	Overtone bending

(post-male) and decline stages (Fig. 4). The chi-square test ($\chi^2 = 65.594$, $df = 6$, $P < 0.05$) indicated a relationship between the female age and the degree of ovarian development, in which the queens are always among the older females of the colony. The workers have a range of ages, which is probably related to the stage of the colony cycle.

Corroborating these results, Baio et al. (2003), Murakami et al. (2009) and Felippotti et al. (2010), investigating colonies of *Metapolybia docilis* (Richards), *M. cassununga* and three species of *Clypearia* respectively, reported that queens are among the older females in the colony, and that the presence of young and old females varies according to the stage of the colony cycle. Murakami et al. (2009) observed that females with the most advanced ovarian development are older and are also more aggressive in the hierarchical ranking. All of these results agree with the system of gerontocracy (Strassmann & Meyer, 1983), common in independent-founding species, which means that, as the workers grow older, they are subject to more aggressive acts of from the dominant female.

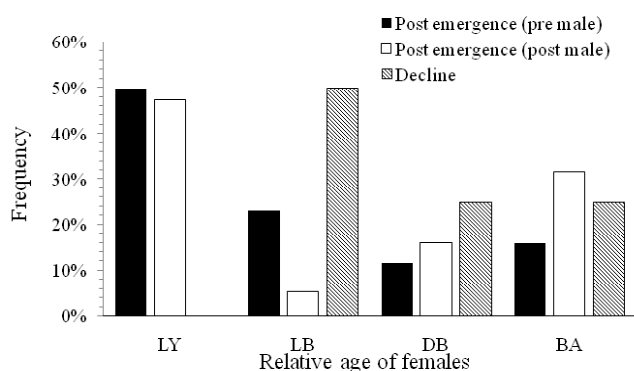


Fig 4. Frequency of the relative ages for females of *Polistes versicolor* from the ten colonies. Separation made according to the color patterns of the transverse apodeme in different colony cycle stages. LY: light yellow; LB: light brown, DB: dark brown; BA: black.

We conclude that there are three groups of females showing different cuticular chemical profiles in *P. versicolor* colonies. The first group is females with filamentous ovarioles, typical of workers; the second one is that of females with intermediate ovarioles; and the third one is the group of the queens, which are older, inseminated females with the greatest degree of ovarian development found among all females. On the other hand, no significant morphological differences were found among these female groups. Therefore, although there are no significant morphological differences among females, there are other factors such as the cuticular chemical composition, which are indicative of the reproductive physiological condition of each female in the colony.

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