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Polydomy in the ant *Ectatomma opaciventre*

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Abstract

Tropical ants commonly exhibit a hyper-dispersed pattern of spatial distribution of nests. In polydomous species, nests may be satellites, that is, secondary structures of the main nest, where the queen is found. In order to evaluate whether the ant *Ectatomma opaciventre* Roger (Formicidae: Ectatomminae) uses the strategy of building polydomous nests, the spatial distribution pattern of 33 nests in a 1,800 m² degraded area located in Rio Claro, SP, Brazil, were investigated using the nearest neighbor method. To complement the results of this investigation, the cuticular chemical profile of eight colonies was analyzed using Fourier transform infrared photoacoustic spectroscopy (FTIR-PAS). The nests of *E. opaciventre* presented a hyper-dispersed or regular distribution, which is the most common in ants. The analysis of the cuticular hydrocarbons apparently confirmed the hypothesis that this species is polydomous, since the chemical profiles of all studied colonies with nests at different sites were very similar to the chemical signature of the single found queen and were also different from those of colonies used as control.

Keywords: colony organization, cuticular hydrocarbons, nestmate recognition, satellite nests, spatial distribution

Abbreviations: FTIR-PAS, Fourier transform infrared photoacoustic spectroscopy

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Introduction

Ants of the genus *Ectatomma* belong to the family Ectatomminae (Bolton et al. 2007) and are endemic to the Neotropical Region (Brown Jr 1958; Lattke 1994, 2003). Among the 14 species of the genus, 10 can be found in Brazil: *E. brunneum*, *E. edentatum*, *E. lugens*, *E. muticum*, *E. opaciventre*, *E. permagnum*, *E. planidens*, *E. ruidum*, *E. suzanae*, and *E. tuberculatum* (Kugler and Brown 1982; Oliveira and Brandão 1991; Santos et al. 1999; Silvestre and Silva 2001; Marinho et al. 2002; Franz and Weislo 2003; Corrêa et al. 2006; Marques and Del-Claro 2006; Delabie et al. 2007; Vieira et al. 2007; Scott-Santos et al. 2008).

Studies done on this genus deal mainly with its interactions with plants that have extrafloral nectaries (Oliveira and Brandão 1991; Oliveira and Freitas 2004; Oliveira and Del-Claro 2005), fauna surveys (Marinho et al. 2002; Marques and Del-Claro 2006), ecology (Silvestre and Silva 2001; Pie 2004; Erdogmus 2010; Tofolo et al. 2006, 2010, 2011), population dynamics (Antonialli-Junior and Giannotti 1997, 2001; Tofolo and Giannotti 2005; Vieira et al. 2009, 2010), division of labor (Antonialli-Junior and Giannotti 2002; Vieira et al. 2010), nest architecture (Antonialli-Junior and Giannotti 1997; Lapola et al. 2003; Vieira et al. 2007), and chemical ecology (Antonialli-Junior et al. 2007, 2008).

For most social insects, colonies are organized with the presence of a queen, workers, and brood, and the physical architecture of nests varies according to the group (Hölldobler and Wilson 1990). In ants, due to the large number of living species (Agosti and Johnson 2005), this variation occurs at the species level (Hölldobler and Wilson 1990). In *Ectatomma*, for example, the architecture of nests and population dynamics meet the general stand-

ards of the group, such as simple architecture and a small number of adults, as well as the behaviors and habits of life (lack of recruitment between workers, predominantly carnivorous habits, and age polyethism). However, according to the species, small variations are found, such as the number of chambers (2 to 10), the nest maximum depth (68 cm to 360 cm), the average number of adults (22 to 94) and, more intriguingly, the presence or absence of fertilized queens in the nests excavated. In *E. brunneum*, *E. opaciventre*, *E. edentatum*, and *E. vizottoi*, varying amounts of virgin queens were found; however, fertilized queens were not present in all nests excavated, although all of them contained immature individuals (Paiva and Brandão 1989; Antonialli-Junior and Giannotti 1997, 2001; Lapola et al. 2003; Vieira and Antonialli-Junior 2006; Vieira et al. 2007; Tofolo et al. 2010). In cases where nests were kept in the laboratory after collection, workers emerged from the field pupae; over time, this caste developed ovaries and began oviposition, resulting only in males until the end of their life cycle (Lapola et al. 2003; Tofolo et al. 2010).

The difficulty in finding queens in this ant genus is supported by the theory of polydomy. According to Hölldobler and Wilson (1990), a polydomous nest is a central structure (which houses workers, queens, and brood) with interconnecting secondary units, called satellite nests, containing a portion of workers and sometimes brood. Seifert (2010) considers that polydomy implies the existence of specific features, such as polygyny, reduced size of males and gynes, intranidal mating or short-range nuptial flights, sharing of the food source, exchange of individuals and brood between nests, and the development of a system of recognition among nestmates. Anderson and McShea (2001) and Lanan et al.

(2011) also consider that the limits to which the nest belongs are not restricted only to the physical structure that houses the queen, workers, and immatures, but also involve adaptive changes in the environment explored by the colony, the so-called outspots (Hölldobler and Wilson 1990) and outstations (Anderson and McShea 2001). These structures include shelters dug into the ground that foragers use to rest for an extended period or, in the case of photophobic ants, to protect themselves temporarily from sunlight, as in *Formica integra* (Step 1924), *Lasius fuliginosus*, *L. niger*, *F. pratensis*, (Dobrzańska 1966), *Oecophylla* sp. (Hölldobler and Wilson 1977), *Eurhopalothrix heliscata* (Wilson and Brown 1984), and *Crematogaster torosa* (Lanan et al. 2011).

Polydomy, therefore, is not restricted to the number of queens present in the secondary units, as it occurs in both mono- and polygynous species (Laskis and Tschinkel 2009). It would be a strategy (temporary or permanent) adopted by some ant species to avoid space constraints that would occur if the entire colony remained only in the central nest (Jaffé 1993). It also allows for a more efficient exploitation of resources dispersed in the environment (Laskis and Tschinkel 2009), although it may hinder the distribution of resources, social regulation, and communication between the subunits created (Crozier and Pamilo 1996).

In *Ectatomma*, satellite nests are known to be an inherent feature of Ectatomminae (Hölldobler and Wilson 1990); however, until now, no experiment has in fact proven that it is associated with polydomy. During the excavation of the nests used in studies with *E. brunneum* (Lapola et al. 2003; Tofolo and Giannotti 2005; Locher et al. 2009; Tofolo et al. 2010), *E. opaciventre* (Antonialli-Junior

and Giannotti 1997; Tofolo et al. 2011), *E. planidens* (Antonialli-Junior and Giannotti 2000, 2001, 2002, 2003; Antonialli-Junior et al. 2007), and *E. vizottoi* (Vieira et al. 2009), it was noticed that not all of them had queens, as some had only workers and brood. Furthermore, no underground or surface structure delimiting trails and connecting nests nearby has been found so far, as occurs in species that build outstations.

Although *E. opaciventre* do not have sophisticated recruitment methods, communication between the subunits created is made possible with the use chemical cues (Hölldobler and Wilson 1990), maintaining colony cohesion and preventing the exploitation of its resources by invaders (Crozier and Pamilo 1996). This recognition occurs through a mixture of chemical compounds present on the cuticle of individuals called surface pheromones, which are both genetically and environmentally determined. They are largely composed of saturated and unsaturated hydrocarbons (Blomquist et al. 1998; Lenoir et al. 1999; Blomquist and Bagnères 2010). These cuticular hydrocarbons play a particularly important role in nestmate recognition because they are the most abundant compounds on the cuticle of ants and often contain clues used to distinguish between nestmates and non-nestmates (Provost 1989; Singer 1998; Sturgis and Gordon 2012). They tend to be species-specific, although they may vary depending on the species, colony, caste, diet, age, and environmental conditions (Howard 1993; Howard and Blomquist 2005; Blomquist and Bagnères 2010).

According to Crozier and Dix (1979), nestmates must keep contact on a regular basis to effectively maintain the ability to tolerate their nestmates. Nevertheless, this contact is hampered when satellite nests are concerned

because individuals do not keep continuous contact with one another, which may produce a unique chemical label within each nest, causing a mosaic of odors (Dahbi and Lenoir 1998). According to Bonavitta-Cougourdan et al. (1987) and Meunier et al. (2011), workers memorize specific proportions of these chemical substances, which enables them to detect and reject any intruder (workers or eggs) that presents quantitative differences in their chemical profiles. This behavior may be recorded by differences in the level of aggression between workers of different nests (Nowbahari et al. 1990).

The investigation of polydomy in *E. opaciventre* may reveal part of its natural history and determine what the spatial and colonial limits of the species are, that is, how individuals use space and how the colony is distributed and organized regarding the different structures that compose the nest. This type of information can be the basis for further studies on behavior, reproduction, and evolution. Proving the existence of subgroups within a social species reveals different complexities in the relationship between individuals of a colony.

In this work, a combination of physical data (location and structure of colonies) and chemical data (cuticular chemical profiles) was used to explore and understand how the spatial organization of colonies of *E. opaciventre* Roger (Formicidae: Ectatomminae) takes place. Recently this subfamily was classified into the Poneroid clade for presenting more basal features, and more recently Brady et al. (2006) and Moreau (2006) relocated this subfamily into the Formicoid clade, along with the most derived species. The existence of polydomous nests in this species is one of the main questions to be answered, given the difficulty of collecting queens between nests

nearby and the absence of trails connecting the subunits built.

Methods

Study species and location

Ectatomma opaciventre is an ant species endemic to the Neotropical Region (Brown Jr 1958), as in northern Argentina and southeastern Venezuela and also in areas of cerrado and caatinga in central Brazil (Kugler and Brown 1982). It presents carnivorous habits (Fernández 1991). The workers forage individually in the epigeal stratum in the daytime period (Pie 2004), collecting mainly living or dead individuals of Hymenoptera, Coleoptera, Lepidoptera, and Orthoptera (Tofolo et al. 2011). Unlike other *Ectatomma*, no liquid food, such as honeydew or nectar, was observed (Erdogmus 2010). They exhibit foraging area fidelity and employ individual foraging strategies, i.e., there is no recruitment of nestmates for food search or transport (Pie 2004).

Ectatomma opaciventre nests in the hypogeal stratum (Fernández 1991) and builds vertical nests at depths of up to 68 cm. The architecture of these nests is simple, with a single chimney-like entrance cavity of 0.9 ± 0.2 cm in diameter and 1.6 ± 0.6 cm high, which gives access to a maximum of five chambers. However, the inner walls of the chambers and the tunnels connecting them are coated with tightly-packed soil because of the composition of the salivary secretion of these ants (Antonialli-Junior and Giannotti 1997). Thus, the entire nest structure is clearly visible during excavation, allowing the exploration of all chambers. Their colonies can present from nine to just over 100 individuals in the dry and rainy seasons, respectively (Antonialli-Junior and Giannotti 1997; Tofolo 2011).

The spatial distribution of the ant nests may be considered regular or hyper-dispersed, as a result of the competition for food sources between colonies (Traniello 1989) or the predation of founding queens by the oldest colonies, which prevents new colonies from settling (Ryti and Case 1986; Hölldobler and Wilson 1990; Deslippe and Savolainen 1995). Random or clumped distributions may occur as a result of interspecific interactions (Levings and Traniello 1981; Ryti and Case 1984, 1986; Hölldobler and Wilson 1990). In nests of *E. opaciventre* in preserved sites, Pie (2004) found a hyper-dispersed pattern of spatial distribution, the most common in this kind of area.

The active nests of *E. opaciventre* were located in a degraded area of 1,800 m² (designated as area A) in the city of Rio Claro, SP, Brazil (22° 22' 49.73" S; 47° 33' 48.87" W). This area is located in the urban region and is therefore surrounded by homes. It is predominantly covered by grasses and constantly suffers the action of burning and brush cutters. The area presents no other shrubby or woody vegetation, so the landscape has little heterogeneity and the richness of species of ants and other arthropods is low.

Among the nests found in area A, eight were chosen for collection because their foraging workers showed higher activity. Because the nests were vertical, with one chamber built below the other, the collection started 30 cm away from the only entrance hole, where a round trench was excavated to a depth of 50 to 100 cm. This way, the resulting cylinder of soil contained the entire nest. With the aid of a spatula, the excavation was then carried out laterally, moving inwards, until each chamber was reached one by one and all the individuals in them were collected. The individuals of each colony were transferred to the laboratory

in artificial plaster nests containing three chambers of increasing sizes, as in Antonialli-Junior and Giannotti (1997). The ants were fed every day for three months with a source of protein (larvae of *Tenebrio molitor* L. (Coleoptera: Tenebrionidae)), carbohydrate (sugar-water solution 1:1), and water until they were used in the experiments for extraction of cuticular hydrocarbons.

Nest location spatial distribution pattern

In order to analyze the pattern of spatial distribution of nests of *E. opaciventre*, the spatial position of each nest was recorded using a tape measure and then plotted as X and Y coordinates on a chart. The average distance between them and the pattern of spatial distribution were calculated by means of the nearest-neighbor method by Clark and Evans (1954). According to this method, an aggregation index (R) equal to 1 means a random distribution pattern; if it approaches zero, an aggregated pattern; if it is higher than 1, with an upper limit of 2.12, it is a hyper-dispersed pattern.

Fourier transform infrared-photoacoustic spectroscopy (FTIR-PAS)

To identify the similarity between the cuticular chemical profiles of individuals of eight colonies in area A, 15 workers (n = 120), one gyne, and the single queen were stored in 70% alcohol. As a control, 15 workers and one queen collected in another similar area (designated as area B, 22° 22' 49.44" S; 47° 33' 02.08" W, approximately 1 km away from area A) were used.

The gaster was extracted from the ants and placed in a vacuum oven for 48 hr to minimize moisture absorption, which would be undesirable in this study. Infrared beams of light were directed at the gaster of the ants because this is a part of the body that presents

high concentrations of cuticular hydrocarbons and it is handled easily (Cuvillier-Hot et al. 2001). The equipment used was a Thermo-Nicolet Nexus 670 (Thermo Scientific, www.thermoscientific.com) spectrophotometer coupled to a MTEC-300 photoacoustic detector (MTEC, www.mtecpcas.com); the data were processed using Omnic software (Thermo Scientific) supplied by the equipment manufacturer, following the report by Antonialli-Junior et al. (2007, 2008).

The degree of similarity between individuals of different colonies was evaluated by the intensity of the peaks obtained via FTIR-PAS. To confirm the results, the data went through a principal component analysis (PCA). The Euclidean distances between the colonies sampled were correlated with spatial position using a Mantel test (999 permutations) to quantify the influence of spatial proximity in FTIR-PAS similarity (Valentin 2000).

Results

Nest spatial distribution

A total of 33 active colonies of *E. opaciventre* were located, and the nest density in the area (Figure 1) was 0.018 nests/m². According to Clark-Evans (1954) method, the pattern of spatial distribution of the nests is regular or hyper-dispersed ($R = 1.51$), with an average distance between nests of 5.6 ± 0.4 m (ranging from 1.93 m to 12.96 m).

The population data from the nesting colonies in area A show that only one nest had a queen and, in this nest, the number of larvae of different instars was relatively higher than in the other groups (Table 1). The average number of chambers found was 4 ± 1 ; the highest number was six and the smallest was three. None of the nests collected had eggs or pupae.

No trails, surfaces, or underground structures connecting these nests were found. There was no record of aggressive behavior between foragers at trail intersections.

FTIR-PAS

The analysis of the cuticular hydrocarbons of ants of the eight most active colonies indicated 17 mid-infrared peaks (Figure 2) with superior absorption; they were considered the most important to separate the groups. Table 2 shows the functional groups and the vibrational mode of each of the peaks previously described by Antonialli-Junior et al. (2007, 2008).

The PCA analysis showed that the profiles of cuticular hydrocarbons of queens, workers, and gynes were different from one other quantitatively. The data of the colonies evaluated in area A overlapped more, and therefore there was a greater similarity among them than among those in area B (Figure 3). Thus, it was possible to distinguish between at least two groups, one composed of the data of the profiles of cuticular hydrocarbons of queens, gynes, and workers of eight colonies of area A, and the other consisting of the control samples in area B (Figure 3).

Considering the loadings of the FTIR-PAS peaks in the first axis, the A group had higher intensity of peaks 895 and 2634 cm⁻¹, while the control group presented smaller intensity of these peaks and higher intensity of many other peaks, such as 1377, 2877, and 2962 cm⁻¹. In the second axis, the peaks 1523, 1651, and 3290 cm⁻¹ had higher intensity in the gyne and queen of the A group (Table 2). The Mantel test showed that there was a significant correlation between nest distances and the chemical profiles of their cuticular hydrocarbons ($r = 0.64$; $p = 0.01$). Thus, colonies near

each other presented more similar cuticular hydrocarbon signatures.

Discussion

The spatial distribution pattern of the nests of this species was regular or hyper-dispersed, the most common pattern in ants (Levings and Traniello 1981), such as *Aphaenogaster senilis*, *Messor barbarus*, *Solenopsis latro*, *Tetramorium semilaeve*, *Tapinoma nigerrima*, and *Plagiolepis pygmaea* (Redolfi et al. 2005). This probably occurs because all these species are territorial and, upon increasing nest density, the competition between them also increases (Greenslade 1975; Ryti and Case 1984, 1986; Cushman et al. 1988). In fact, when nests of *E. opaciventre* were studied in a preserved area, Pie (2004) reported the same hyper-dispersed pattern of spatial distribution, with a density smaller than that found in the current study. This was an expected result, considering that the diversity and resources available to species are higher in a preserved area, resulting in greater spacing between nests. This difference was clearly observed by Deslippe and Savolainen (1994) in nests of *Formica podzolica* located in forest edges with high resource availability and in meadows.

Other studies showed that nest density in ants of the genus *Ectatomma* varies widely, unlike the pattern of spatial distribution, which in most cases is also hyper-dispersed, as in *E. ruidum* (Breed et al. 1990; Pratt 1989), *E. permagnun* (Paiva and Brandão 1989), and *E. tuberculatum* (Wheeler 1986). Probably, *E. opaciventre* established unicoloniality in the study area, as there was a complete absence of hostility between occupants from different nests (Crozier and Pamilo 1996), even when foraging trails overlapped.

The results of our study demonstrate that there are strong indications that this species has polydomy, because among the colonies evaluated, only one had a queen and it contained a greater number of immatures (Table 1). Moreover, the analysis of the profiles of cuticular hydrocarbons shows that most of these colonies had, in fact, greater similarity between them than those considered as controls, and that many of the profiles of cuticular hydrocarbons of workers from different colonies were close to that of the queen of colony 11. The large distance between the data regarding colonies 5, 8, and 9 in area A and the data concerning the remaining colonies suggests that they may be part of another set of polydomous nests.

Mantel's test confirms that the smaller the distance between them, the greater the similarity of their profiles of cuticular hydrocarbons, indicating the possibility that the individuals evaluated might come from eggs laid by a single queen, i.e., that these individuals could belong to a single colony.

Although interaction tests between ants from different nesting sites were not performed, in numerous personal observations workers were caught entering and leaving nests near each other, carrying other workers and immatures. Such behavior was not uncommon during the excavations.

Further evidence should be taken into account. In previous work with this species, a relatively high proportion of colonies collected in the field had no queens (Antonialli-Junior and Giannotti 1997). In addition, other species such as *E. brunneum* present the same evidence as well. Lapola et al. (2003) and Vieira and Antonialli (2007) also suggest polydomy for this species. In *E. tuberculatum*, Zinck et al. (2008) proved polydomy based on the re-

sponses to the recognition mechanisms between workers of the same nest and of different nests.

Considering that all colonies analyzed were raised in a laboratory after collection, and that the larvae developed into new workers, it becomes more evident that these workers may be daughters of the same queen, since non-fecundated workers could not produce females, but only males. In fact, Antonialli-Junior and Giannotti (1997) observed workers of *E. opaciventre* laying trophic eggs and eggs that gave rise to males, but never to other females.

In fact, the hyper-dispersed pattern of spatial distribution and the building of structures secondary to the main nest (satellites and outstations) seem to be an alternative to the energy expenditure required during environment exploration. Colony division, for example, allows a decrease in the foraging costs of the central nest so that the secondary units increase the dispersion area of the species and resource collection (Debout et al. 2007). The concentration of workers in various subunits over space may also allow quick recruitment when necessary (Anderson and McShea 2011).

According to a definition by Forel (1974), polydomy is characterized by several nests with the absence of brood or queens in at least one of them; they grow following a strategy that minimizes problems with space (Hölldobler and Wilson 1990) and temperature (Pedersen and Boomsma 1999) and reproductive conflict with the queen (Banschbach and Herbers 1996). In the case of the colonies of *E. opaciventre* collected in our study, all of them had workers and brood and only one of them had a queen, since it is a monogynous species (Table 1). The nests

without a queen may be regarded as satellites of the central nest, forming a polydomous structure.

Therefore, the chemical identity of the workers from colony 11 was expected to approach that of the queen from same colony. The level of similarity between the workers belonging to the other nests in area A was not the same between all of them. This variability is also expected, considering that each nest has a specific microenvironment and that the formation of cuticular hydrocarbons depends not only on genetic factors (heritable cues), but may also be environmentally influenced (Howard and Blomquist 2005; van Zweden and D'ettorre 2010). It is important to acknowledge that these local differences identified them as a group different from that of area B (control area).

Nonetheless, it is worth considering that the genetic similarity between the colonies of area A may also be explained by the degree of relatedness between them, due to the limited dispersal of the colonies. In this case, they should all present queens, which was not the case. It is still possible that the absence of queens was due to some problem during excavation and collection. However, careful measures were taken so that these problems were minimized. Moreover, other studies report the absence of queens after collection of colonies of the same species (Antonialli-Junior and Giannotti 1997) and other species of the genus (Lapola et al. 2003; Vieira and Antonialli 2007).

On the other hand, it is known that the profile of cuticular hydrocarbons may be influenced by environmental factors, such as the incorporation of hydrocarbons from food sources and nesting material. Some studies have shown that the differences in the chemical profiles of

individuals from different nests remain for a short time (Jutsum et al. 1979; Liang and Silverman 2000), with a predominance of the genetically determined composition (Bagnères and Blomquist 2010). Once individuals or colonies are subjected to the same biotic and abiotic conditions, it becomes much more likely that the predominant chemical profile is more genetically than environmentally determined (Howard and Blomquist 2005). The results found for the composition of the cuticular chemical profile of the ants did not suffer or suffered little environmental and food influence, as the ants used were not subjected to the chemical analysis immediately after collection in the field but were taken to the laboratory and held for more than three months under the same biotic/abiotic conditions.

Conclusion

Ectatomma opaciventre presents hyper-dispersed spatial distribution of nests, as do most species of tropical regions. As already discussed in other works, both this form of spatial distribution of nests and polydomy may be strategies to reduce intra- and inter-specific competition. Polydomy could be a strategy to allow species dispersion, minimizing foraging costs and diluting the effects of predation.

In this study, evidence was found that this species may have polydomous nests, because most of the colonies showed no queens, only workers and immatures. The chemical analysis of these colonies showed that those closest to one another had similar profiles of cuticular hydrocarbons, especially compared to the only colony in which a queen was found. These findings thus reinforce the hypothesis formulated, based both on the populations presented by each colony and on laboratory and field observations.

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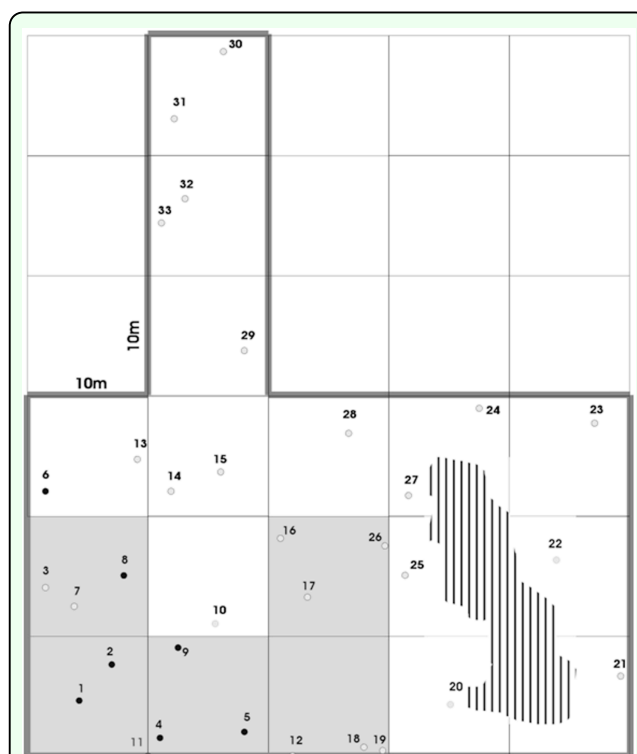


Figure 1. Map of the 33 nests of *Ectatomma opaciventre* found in a 1,800 m² area. The study area A is delimited by a gray line, and the hatched area corresponds to a waste dump. The black circles indicate the nests collected. The single queen was found in nest 11. High quality figures are available online.

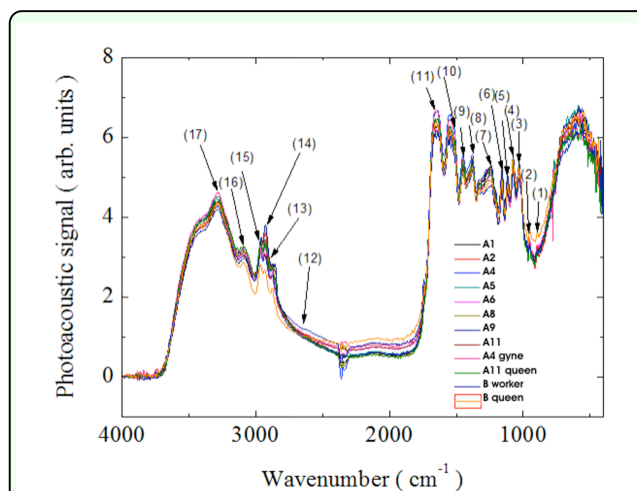


Figure 2. Spectra of the cuticular hydrocarbons extracted from adults of eight colonies of *Ectatomma opaciventre*. High quality figures are available online.

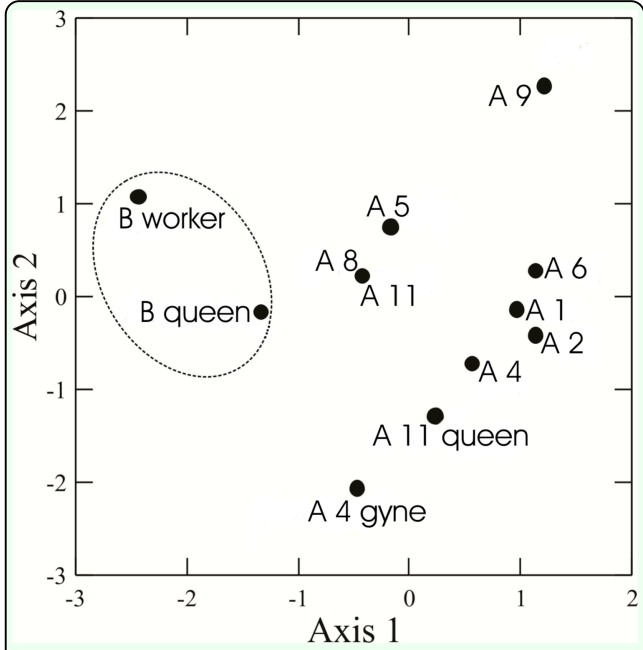


Figure 3. Scatterplot of principal component analysis based on the profiles of the cuticular hydrocarbons from different colonies of the ant *Ectatomma opaciventre*: A 1, A 2, A 4, A 4 gyne, A 5, A 6, A 8, A 9, A 11, and A 11 queen correspond to the samples of adults collected from nests of area A; B queen and B worker correspond to adults collected from area B (control area). High quality figures are available online.

Table 1. Populational and physical data of the eight nests of *Ectatomma opaciventre* collected in area A in the city of Rio Claro, SP, Brazil.

| Nest | Population | | | Chamber volume (cm ³) | | | | | | Distance (m) from the nest of the queen (no. 11) |
|------|------------|---------|--------|-----------------------------------|-------|-------|-----|-----|-------|--|
| | Queens | Workers | Larvae | 1 | 2 | 3 | 4 | 5 | 6 | |
| A1 | 0 | 112 | 2 | 112.5 | 112.5 | 72 | - | - | - | 5.42 |
| A2 | 0 | 54 | 2 | 80 | 78 | 114 | 306 | 700 | 522.5 | 7.92 |
| A4 | 0 | 67 | 11 | 105 | 112 | 270 | 567 | - | - | 1.67 |
| A5 | 0 | 32 | 9 | 96 | 72 | 112 | - | - | - | 8.33 |
| A6 | 0 | 58 | 10 | 84 | 144 | 780 | 360 | - | - | 23.33 |
| A8 | 0 | 57 | 21 | 60 | 160 | 360 | 80 | 336 | 768 | 15 |
| A9 | 0 | 62 | 19 | 126 | 387.5 | 127.5 | 80 | - | - | 9.17 |
| A11 | 1 | 29 | 35 | 204 | 840 | 240 | - | - | - | - |

Table 2. Representation of the photoacoustic peaks of higher vibration corresponding to the extraction of cuticular hydrocarbons from the abdomen of workers of eight colonies of *Ectatomma opaciventre* and loadings of each peak on the first two axes of the PCA results.

| Peaks | Wave number (cm ⁻¹) | Functional group | Vibrational mode | Axis 1 | Axis 2 |
|-------|---------------------------------|-----------------------------------|------------------------|--------|--------|
| 1 | 895 | C-H – Alkenes | Out-of-plane bending | -0.817 | 0.255 |
| 2 | 953 | C-H – Alkenes | Out-of-plane bending | -0.711 | 0.263 |
| 3 | 1030 | C-O-C – Ether | Asymmetric Stretching | 0.743 | 0.418 |
| 4 | 1076 | O-C-C – Saturated Ester | Stretching | 0.871 | 0.174 |
| 5 | 1115 | O-C-C – Aromatic Ester | Stretching | 0.927 | 0.328 |
| 6 | 1157 | C-N – Saturated Amine II | Stretching | 0.956 | 0.224 |
| 7 | 1242 | C-C-O – Saturated Ester (Acetate) | Stretching | 0.954 | -0.086 |
| 8 | 1377 | -C-CH3 – Alkanes | Symmetric bending mode | 0.973 | -0.135 |
| 9 | 1454 | -C-CH2 – Alkanes | Scissor | 0.909 | -0.302 |
| 10 | 1523 | -N-H – Amide II | In-plane bending | -0.311 | -0.875 |
| 11 | 1651 | C=O – Amide I, II e III | Stretching | -0.338 | -0.895 |
| 12 | 2634 | -C-N and -N-H | Overtone bending | -0.885 | 0.048 |
| 13 | 2877 | -C-H (CH ₃) | Symmetric Stretching | 0.967 | 0.04 |
| 14 | 2931 | -C-H (CH ₂) | Asymmetric Stretching | 0.938 | 0.18 |
| 15 | 2962 | -C-H (CH ₃) | Asymmetric Stretching | 0.966 | -0.029 |
| 16 | 3082 | =C-H | Stretching | 0.64 | -0.699 |
| 17 | 3291 | -N-H | Bending | 0.437 | -0.828 |