



RESEARCH ARTICLE - ANTS

Dear Enemy Phenomenon in the Ant *Ectatomma brunneum* (Formicidae: Ectatomminae): Chemical Signals Mediate Intraspecific Aggressive Interactions

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Abstract

The integrity of ant colonies depends exclusively on social relationships between their individuals, especially the ability of communication between group members, which is mainly mediated through chemical signals. Another important feature of ant behavior is territory defense, since they need to gather large amounts of food to feed their larvae, males and breeding females. Thereby, ants might display behavioral strategies to defend their territories from intruders. Here we investigated whether *Ectatomma brunneum* displays the Dear Enemy Phenomenon, what is the relationship between Cuticular Hydrocarbon composition and levels of aggression during their intraspecific interactions and which compounds and/or classes of compounds might be the most important to modulate the level of aggression. To test our hypothesis, we evaluated the levels of aggression through behavioral observations during interactions between 23 pairs of colonies nested in two distinct sites at varied distances. Then, we analyzed the cuticular chemical profile of the individuals involved in the interactions, and compared these results with the levels of aggression displayed between colonies tested. The results allow us to confirm our hypothesis that the DEP occurs in *E. brunneum*. The higher tolerance between closer colonies can be explained due to their kinship level in addition to sharing the same microhabitats. The results also showed there are significant differences in CHCs profiles, especially between colonies nested at relatively greater distances, and it is likely that differences in content of some branched alkanes are the most important to establish these differences and, therefore, the levels of aggression during the interactions.

Introduction

As in other insects' societies, ants are characterized by a high degree of cooperation between different individuals in the colony. Colony members display social behaviors that complement each other, resulting in an overall development of the colony (Zinck et al., 2008). Workers, for example, actively work on building the nests, protecting the colony against predators, foraging and nursing (Ratnieks et al., 2006). Therefore, the integrity of the colony depends exclusively on the social relationships between its individuals and thus their

ability of communication between group members (Crozier & Pamilo, 1996). Social insects use mainly chemical signals for communication, especially the Cuticular Hydrocarbons (CHCs) which might vary between colonies or even between castes (e.g. Blomquist et al., 1998; Lenoir et al., 1999; Antonialli-Junior et al., 2007). CHCs have profiles both genetically and environmentally determined (Sorvari et al., 2008), which are used by workers as "signatures" for intra and interspecific recognition (Arnold et al., 2000). The existence of these recognition mechanisms is of great value, especially in intraspecific interactions. Considering that social insects are



not really clones, but groups of individuals with high degree of kinship that live in colonies and might have different interests (Cassill & Tschinkel, 1999; Ratnieks et al., 2006), conflicts between colonies are expected to be frequent.

Ants generally control and defend their territories, from where they extract resources to feed the large number of larvae, males and breeding females of their colonies (Newey et al., 2010). Thus, in most species, encounters between foragers from different colonies might trigger conflicts that can turn into clashes with physical contacts resulting in death of individuals (Matthews & Matthews, 2010). These conflicts can happen between individuals of several colonies or species for access to the food source, territory, or reproductive partners. Such disputes, indeed, generally involve aggressive behaviors (Huntingford & Turner, 1987). On the other hand, some species respond less aggressively to the entrance of neighbors in their territories than the entrance of a non-neighbor (stranger). This difference in intensity of aggressive response is known as “Dear Enemy Phenomenon” (DEP) and has been described in several species of vertebrates and invertebrates (e.g. Ydenberg et al., 1988; Temeles, 1994; Dimarco et al., 2010). In ants, the higher tolerance between neighbors has been described in species such as *Acromyrmex octospinosus* Reich (Formicidae: Myrmicinae) (Jutsum et al., 1979), *Temnothorax nylanderii* Foerster (Formicidae: Myrmicinae) (Heinze et al., 1996), *Pheidole gilvescens* Creighton and Gregg (Formicidae: Myrmicinae) and *Pheidole xerophila* Wheeler (Formicidae: Myrmicinae) (Langen et al., 2000), *Formica pratensis* Retzius (Formicidae: Formicinae) (Pirk et al., 2001), *Cataglyphis fortis* Forel (Formicidae: Formicinae) (Knaden & Wehner, 2003), *Acromyrmex lobicornis* Emery (Formicidae: Myrmicinae) (Dimarco et al., 2010) and ants of the *Neoponera apicalis* complex Latreille (Formicidae: Ponerinae) (Yagound et al., 2017). One explanation for this phenomenon is based on familiarity between neighbors, and once the territory boundaries are established, these neighbors show little or no threat, while an individual from a distant site corresponds to a stranger who may be looking for a new territory (Temeles, 1994). Another hypothesis is based on the reduction of energy costs of an aggressive interaction and preventing fights with neighbors, since there are frequent encounters during foraging (Ydenberg et al., 1988).

Investigations in several species such as *Camponotus fellah* Dalla Torre (Formicidae: Formicinae) (Boulay et al., 2004), *Cataglyphis niger* André (Formicidae: Formicinae) (Soroker et al., 1995), *Aphaenogaster senilis* Mayr (Formicidae: Myrmicinae) (Lenoir et al., 2001) and *Neoponera apicalis* Latreille (Formicidae: Ponerinae) (Soroker et al., 1998), have shown that CHCs undergo a homogenization between colony members through social interaction. Thus, many authors have evaluated the levels of aggression during intraspecific interactions in order to investigate the origin of individual recognition cues in different species of ants, since members of the same colony generally ignore each other during encounters,

but show different levels of aggression towards individuals from different colonies or species (Stuart & Herbers, 2000; Menzel et al., 2009).

Ectatomma brunneum Smith (Formicidae: Ectatomminae) is an ant with widespread distribution in Latin America, occurring from Panama to Argentina and can be found throughout the Brazilian territory, usually in areas with open vegetation such as forest borders or clearings, but also in crops, pasture and secondary vegetation (Brown, 1958). It is a generalist predator of terrestrial arthropods, alive or recently dead (Giannotti & Machado, 1992; Maques et al., 1995), and the food is generally collected on the ground, rarely on vegetation (Del-Claro et al., 1992). Despite being a species of widespread occurrence, little is known about many aspects of its biology. Here we investigated whether *E. brunneum* displays the DEP, what is the relationship between the CHC and levels of aggression during their intraspecific interactions and which compounds and/or classes of compounds might be the most important to modulate the level of aggression. We tested whether worker ants display different levels of aggression towards neighbors and non-neighbors. Our hypothesis was that if the DEP occurs in this species, ants should be more aggressive towards non-neighbors, since these ants could represent a threat to their already established territorial organization. Secondly, we analyzed the cuticular chemical composition, establishing the relationship between the CHC and levels of aggression during conspecific interactions and identifying which compounds and/or classes of compounds might be the most involved in modulating the level of aggression between these ants.

Material and Methods

Behavioral study

We collected nine colonies of *E. brunneum* (Table 1), five of them nested in the surroundings of the Universidade Estadual de Mato Grosso do Sul Campus in Dourados, Mato Grosso do Sul, Brazil (22°11'53.32"S, 54°55'50.90"W), in an area of pasture, with predominance of grass and shrub species (area 01). Other four colonies were nested in a forest board of Fazenda Coqueiro (22°12'45.78"S, 54°54'43.15"W) (area 02), classified as a Semideciduous forest, according to the Instituto Brasileiro de Geografia e Estatística - IBGE classification system (Veloso et al., 1991). The two areas were around 3 km apart from each other. Colonies were ranked according to collection site. We collected colonies nested at different distances and environments because the cuticular chemical compounds may be affected by both genetic and environmental factors (Sorvari et al., 2008), and this type of variation could influence on our results.

Colonies were collected according to the method described by Lapola et al. (2003). In order to standardize the time during which the colonies would be under controlled conditions and to control habituation, the period between collections was never greater than twenty days. Then, all

individuals were placed in artificial nests in Laboratório de Ecologia Comportamental of Universidade Estadual de Mato Grosso do Sul. All adults of each colony were marked with non-toxic paint in the pronotum region for identification. The mean colonial population in area 01 was of 124.5 ± 4.9 individuals and 142.5 ± 19 in area 02, all colonies had immature (larvae and pupae) and, except for colony 6, all of the others had a queen. We only used colonies for the experiment after two days of acclimatization in the artificial nests.

The experimental design consisted of two colonies at a time connected to a single foraging arena, consisting of a wooden box of 51cm x 42.5cm x 6 cm connected to both nests, where it was offered water in a Petri dish, larvae of *Tenebrio molitor* Linnaeus (Coleoptera: Tenebrionidae), and molasses. In this arena, we could observe the interactions between foragers of the two colonies during their activities outside the nest. For every new experiment, we used a new and neutral arena. We kept the connection between colonies and performed observations for three days maximum, in order to avoid familiarity between them. Before connecting different colonies, we induced encounters in the foraging arena between workers from the same colony as control tests. When the first worker left the colony, we captured it and waited until the next worker entered the foraging arena. Then, we placed the workers close to each other in order to induce the encounter, and recorded their behaviors. We observed each pair of colonies every day, 5 hours per day, totaling 15 hours of observation for every pair tested. Five induced encounters were performed between workers from the same colonies as a control test.

We evaluated the interactions between 23 pairs of colonies: nine encounters between colonies nested in the pasture (PP); six encounters between colonies nested in the forest (FF); and eight between colonies nested in forest and pasture (FP). We observed the colonies in pairs because it would be difficult to perform the experiment with all colonies at the same time, since the maintenance period under laboratory conditions, which implies standardization of diet, can lead to similarity in patterns of CHCs, interfere in habituation and generate different behavioral responses.

Table 1. Distance (m) between the nine colonies of *Ectatomma brunneum*. *P* means colony nested in pasture and *F* nested in forest.

	#1 F	#2 F	#3 F	#4 F	#5 P	#6 P	#7 P	#8 P	#9 P
#1 F	0								
#2 F	173	0							
#3 F	711	720	0						
#4 F	707	712	235	0					
#5 P	2087	2086	2480	2482	0				
#6 P	2083	2073	2475	2501	4	0			
#7 P	2006	2205	2386	2387	192	151	0		
#8 P	2100	2015	2397	2390	203	168	135	0	
#9 P	2077	2078	2485	3342	172	160	187	196	0

During the interactions, we observed and recorded all behaviors through the *ad libitum* method (Altman, 1974). We considered only the encounters one by one. The behaviors observed were: ignore, touch, escape, attempted seizure, seizure, antennal boxing, body lift, display of abdomen and fight. In order to evaluate the intensity of aggression between foragers, the behaviors exhibited received a rating scale, modified from Suarez et al. (1999), from 0 to 2, as follows: 0 for touch, escape and ignore; 1 for attempted seizure, seizure, antennal boxing, body lift and display of abdomen; and 2 for fight. At the end of 15 hours of observation, we compiled an arithmetic mean between the intensities of aggression displayed during each encounter between foragers of each pair of colonies.

Chemical analyses

In order to assess whether the mean level of aggression displayed during interactions correlates with the similarity or dissimilarity of each colony's CHCs composition, the cuticular chemical profile of the body of at least nine foragers from each colony that interacted in the arena were assessed by Gas Chromatography coupled to Mass Spectrometry (GC-MS).

For GC-MS analyses, the cuticular compounds were extracted with 5 ml of hexane (HPLC-VETEC) in ultrasonic bath for 3 min. After filtration, the solvent was dried under fume hood, and the dry extract was solubilized in 100 μ L of hexane prior to analysis. Analyses were performed employing a gas chromatograph (GC- 2010 Shimadzu, Kyoto, Japan) coupled to a mass spectrometer detector (QP 2010), using fused silica capillary column DB -5 (J & W, Folsom, CA, USA) 5% phenyl- dimethylpolysiloxane (30 m length \times 0.25 mm diameter \times 0.25 mm of film thickness). The analysis conditions were: helium as carrier gas (99.999%) at flow rate of 1.0 ml⁻¹, injection volume 1 μ l in splitless mode. The temperature ramp was programmed as follows: initial temperature of 50 °C, reaching 85 °C at a rate of 5 °C min⁻¹, followed by a ramp of 8 °C min⁻¹ up to 280 °C, and a second ramp of 10 °C min⁻¹ up to 300 °C remaining at the final temperature for 35 minutes. Temperature of injector, detector and transfer line was kept at 280 °C. The parameters of mass spectrometer included scanning MS voltage electron impact ionization of 70 eV, ranging from 45 to 600 m/z, with 0.5 s of scan interval.

Data processing was performed using a signal to noise ratio of three. The criteria for accepting a detected compound was a minimum of 900 of similarity match and manual inspection of the quality of the mass spectrum of each compound. Identification of compounds was performed employing the calculated retention index (Van den Dool & Kratz, 1963), using a standard mixture of linear alkanes (C₇-C₃₃), and comparing the calculated value with the retention index of literature associated with interpretation of mass spectra obtained from the samples and compared with the data bases NIST21 and WILEY229. The relative area for each chromatographic peak was employed as abundance approach to evaluate the contribution of each compound area to the total

area and for comparison between the samples. The sum of all peak areas was considered 100% of the sample and to each peak was assigned a percentage corresponding to its area.

Statistical analyses

In order to assess whether differences in levels of aggression between colonies of the two areas is significant, the normality of data was checked and then a t-test was performed with the mean levels of aggression between colonies from the same area and between different areas using the R software. In order to evaluate the relationship between the level of aggression and the distances between colonies we used the Pearson's Correlation Coefficient with the data of aggressiveness and distances between colonies nested in the two different areas. These analyses were performed in the R (R Development Core Team 2010) software. The GC-MS data were analyzed by Stepwise Discriminant Function Analysis, using the Systat 10.2 software, which can reveal the set of variables that best explains the difference between the groups, indicated by Wilk's Lambda (Quinn & Keough, 2002). With these results, which show the compounds that were most

significant for groups' separation, we performed a Cluster Analysis, in order to assess the relationship between colonies based on their cuticular chemical profiles. This analysis was also performed using the R software. Finally, in order to assess the relationship between differences in CHCs composition, distance and aggressiveness between colonies, we performed the Mantel test in the NTSYS-pc 2.1 software.

Results

We did not observe any aggressive behavior in control tests. We observed an average of 236 ± 144 encounters between foragers from distinct colonies. The t-test performed with mean values of levels of aggression between colonies nested in the same area, regardless of distance, did not indicate significant differences (FF x PP = $p > 0.05$). However, comparing the values obtained from encounters between colonies nested in the same area and in different areas, there were significant differences (FF + PP x FP $p < 0.05$). The correlation analyses showed that there is a certain level of intolerance between colonies regardless of nesting site and distance between them (Fig 1). The highest level of aggression between colonies was

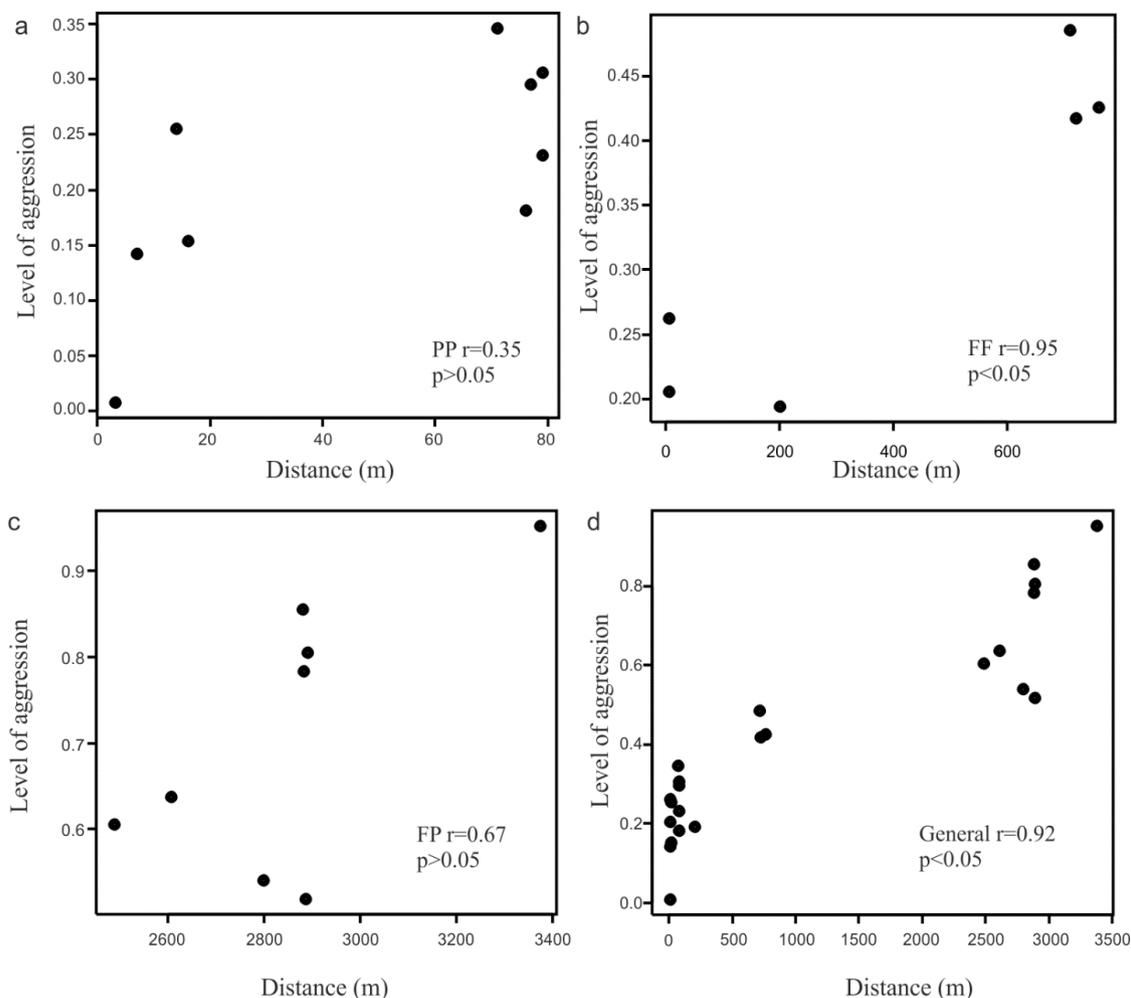


Fig 1. Scatter plot depicting the Pearson's Correlation tests results between the level of aggression and distances of colonies of *Ectatomma brunneum*. Markers (●) correspond to mean levels of aggression obtained from the interactions between colonies. (a) = Interactions between colonies nested in the pasture area; (b) = Interactions between colonies nested in the Forest area; (c) = Interactions between colonies nested in the pasture and colonies nested in the Forest area; (d) = General mean levels of aggression regardless of nesting site.

0.95 and they were 3.3 km apart from each other (Fig 1c); and the lowest was 0.008 and colonies were 3 m apart from each other (Fig 1a). The analyses showed that there was significant correlation between the level of aggression and distance in encounters between colonies nested in the forest area (Fig 1b), and when it was considered all encounters (Fig 1d). However, the correlation between the level of aggression and distance in encounters between colonies nested in the pasture area (PP) and between colonies nested in different areas (FP) was not significant (Fig 1a and c).

Forty-seven (47) CHCs, ranging from 20 to 31 carbon atoms. Linear alkanes represented 25% of compounds, branched alkanes 21%, alkenes 7% and non-identified compounds 47%

(Table 2). Linear alkanes, branched alkanes and alkenes represented more than 75% of the total area of compounds. The results of the Discriminant Function Analysis revealed that the CHCs profile of the nine colonies are significantly different (Wilk's Lambda = 0.000, F = 987.369, $p < 0.05$). Five compounds were the most significant for groups' separation (Table 2), four branched alkanes and one linear alkane. The Cluster Analysis displays the dissimilarities between the colonies based on their CHCs profiles (Fig 2; Cophenetic Correlation Coefficient = 0.818). The Mantel test ($t = 4.13$, $p < 0.05$) showed that colonies nested closer to each other, especially those from the same area, are the ones with the most similar cuticular chemical profiles and the lowest levels of aggression.

Table 2. Relative area (%) of CHCs compounds identified in workers from colonies of *Ectatomma brunneum*, obtained by GC-MS.

Compounds	Relative area (%) per colony								
	#1	#2	#3	#4	#5	#6	#7	#8	#9
C ₂₀ n-eicosane	0.516	0.759	0.754	0.782	1.526	1.604	1.666	1.731	1.764
C ₂₁ n-heneicosane	0.493	0.492	0.522	0.456	0.514	0.538	0.528	0.504	0.487
C ₂₂ n-docosane	0.21	0.211	0.27	0.256	0.306	0.29	0.293	0.29	0.308
C ₂₃ n-tricosane	7.697	7.839	7.953	7.944	7.066	7.078	6.993	6.928	7.06
C ₂₄ n-tetracosane	0.197	0.203	0.29	0.269	0.298	0.298	0.29	0.294	0.298
C ₂₅ n-pentacosane	13.74	13.82	12.96	12.93	11.81	11.73	12.02	12.01	11.06
C ₂₆ n-hexacosane	2.013	1.991	1.713	1.665	2.49	2.596	2.666	2.731	2.523
C ₂₇ n-heptacosane	12.70	12.86	12.77	12.93	15.12	15.38	15.17	15.19	15.18
C ₂₈ n-octacosane	0.206	0.209	0.27	0.254	0.294	0.28	0.293	0.29	0.306
C ₂₉ n-nonacosane	0.206	0.216	0.271	0.252	0.284	0.292	0.293	0.29	0.302
C ₃₀ n-triacontane	0.209	0.213	0.27	0.257	0.292	0.29	0.293	0.29	0.307
C ₃₁ n-hentriacontane	2.263	2.254	1.815	1.807	1.898	1.738	1.764	1.813	2.255
13-MeC ₂₅ 13-methylpentacosane	2.347	2.219	2.327	1.95	2.008	2.008	1.887	2.41	2.32
3-MeC ₂₅ 3-methylpentacosane	2.27	2.446	2.487	3.013	2.4	2.402	2.666	2.087	2.526
13-MeC ₂₇ 13-methylheptacosane	3.013	2.92	2.576	2.974	3.028	3.024	2.866	3.014	2.903
5-MeC ₂₇ 5-methylheptacosane	1.419	1.286	1.514	1.119	1.538	1.538	1.431	1.143	1.313
3-MeC ₂₇ 3-methylheptacosane	3.975	3.871	4.028	4.014	4.02	4.018	4.017	3.867	3.871
15-MeC ₂₉ 15-methylnonacosane	2.664	2.727	2.986	2.559	3.244	3.252	2.776	2.665	2.727
14-MeC ₂₈ 14-methyloctacosane	1.12	0.985	1.225	0.918	1.304	1.302	1.035	0.886	0.986
15-MeC ₂₉ 15-methylnonacosane	3.777	3.839	3.517	4.018	3.666	3.668	3.987	4.038	3.808
13-MeC ₃₁ 13-methylhentriacontane	1.976	1.929	2.088	1.948	2.112	2.108	1.853	1.78	1.987
13-MeC ₃₃ 13-methyltritriacontane	1.558	1.488	1.521	1.508	1.246	1.248	1.519	1.386	1.488
C _{27:1} n-heptacosene	1.288	1.166	1.33	1.018	1.24	1.234	1.086	1.115	1.168
C _{29:1} n-nonacosene:1	1.128	1.178	1.02	1.089	0.888	0.886	1.107	0.987	1.178
C _{29:2} n-nonacosene:2	0.487	0.546	0.6	0.519	0.558	0.558	0.726	0.849	0.548
C _{31:1} n-hentriacontene	0.756	0.815	0.51	0.836	0.556	0.558	0.675	0.907	0.802

Most important CHCs for groups' separation.

Discussion

Our results demonstrate that among the groups of encounters between colonies nested in the same area (FF and PP), the levels of aggression were not significantly different, but these changes when compared to the level of aggression observed in encounters between colonies from different areas (FP). Considering the correlation analyses performed with encounters between colonies from the same area (Fig 1a and b), the significant correlation observed in FF encounters is probably related to the kinship level between colonies, which tends to be lower between more distant colonies. Meanwhile, colonies nested in the pasture area were, in general, nested closer therefore the level of kinship and consequently the level of aggression should be lower than the ones of colonies from the forest, which explains the correlation result (Fig 1a).

This fact becomes clearer when we evaluate the level of aggression of encounters between colonies from different areas, whose correlation was not significant (Fig 1c). In this case, colonies were at least 2 Km apart from each other, so the level of kinship and probability of encounter during foraging activity under natural conditions should be so low that ants displayed high levels of aggression in all encounters regardless of the distance. Finally, considering the levels of aggression from all encounters, the correlation is highly significant confirming that indeed the greater the distance the higher the level of intolerance between colonies (Fig 1d).

These results agree with other studies with the ant species *Acromyrmex octospinosus* Reich (Formicidae: Myrmicinae) (Jutsum et al., 1979), *Temnothorax nylanderi* Foerster (Formicidae: Myrmicinae) (Heinze et al., 1996), *Pheidole gilvescens* Creighton and Gregg (Formicidae: Myrmicinae) and *Pheidole xerophila* Wheeler (Formicidae: Myrmicinae) (Langen et al., 2000), *Formica pratensis* Retzius (Formicidae: Formicinae) (Pirk et al., 2001), *Cataglyphis fortis* Forel (Formicidae: Formicinae) (Knaden & Wehner, 2003), which also present the DEP, since ants are more tolerant towards conspecifics from neighboring colonies. Temeles (1994) argues that this feature can be explained due to familiarity between neighbors, because once the territory boundaries are established, these neighbors show little or no threat, while an individual from a distant site corresponds to a stranger who may be looking for a new territory. In addition, there are frequent encounters between neighbors during foraging, so preventing fights would be an effective way of reducing energy costs due to aggressive interactions (Ydenberg et al., 1988).

The Discriminant Function Analysis showed that four of the five most important compounds for groups' separation, were branched alkanes and one linear alkane. These results corroborates the discussions of LeConte and Hefetz (2008), Blomquist and Bagnères (2010), Richard and Hunt (2013) and Lorenzi et al. (2014) regarding the role of branched alkanes as important signals for intraspecific recognition. However,

some studies also discuss the role of linear alkanes for this function (Lorenzi et al., 2004; Tannure-Nascimento et al., 2007; Ferreira et al., 2012).

Colonies nested at closer sites have more similarity in CHCs composition, and can use this information to mediate interactions during encounters (Fig 2). Groups' separation observed in the Similarity dendrogram shows, therefore, that there is a direct relationship between CHCs composition and colonies' nesting site (Fig 2). Although each colony had its own chemical signature as suggested by studies on CHCs in other ant species, such as *A. senilis* (Lenoir et al., 2001) and *Ectatomma vizottoi* Almeida (Formicidae: Ectatomminae) (Antoniali-Junior et al., 2007), there is greater similarity between profiles of colonies nested in nearby sites. One possible explanation is the higher level of kinship between these colonies due to a restricted dispersal strategy and/or by the phenomenon of colony fission already documented for *E. tuberculatum* Olivier (Formicidae: Ectatomminae) (Zinck et al., 2008). On the other hand, it is well known that CHCs composition is determined by two main components: genetic and environmental (Sorvari et al., 2008), thus this similarity in CHCs profile, as well as the low level of aggression, might be partially explained by environmental similarity, especially microhabitats shared by neighboring colonies.

In the particular case of the interaction between colonies #5 and #6, collected 3m apart from each other, in the same area, the level of aggression was the lowest (Fig 1a). The analysis based on these colonies' CHCs profiles showed that they are indeed relatively similar (Fig 2). Ten hours after being connected by the single foraging arena, they merged to form a single colony with adults and immature accepted by other colony members. This suggests that these two colonies could be one that had been divided into distinct nesting sites, thus consisting of a polydomic nest, supporting the hypothesis suggested by Lapola et al. (2003) and Vieira and Antoniali-Junior (2006) for the same species. On the other hand, the low level of aggression may be due to microhabitat similarity, which could lead to similarity in CHCs profiles because of both the higher level of kinship and the same types of food resource (Pirk et al., 2001). In this context, some authors have shown that colonies kept under the same diet during a large period can have their CHCs profile modified leading to similarity in composition (e.g. Zweden et al., 2009; Bernardi et al., 2014; Valadares et al., 2015).

The importance of the environmental component especially microhabitats on CHCs profiles is enhanced by the greater similarity between colonies #8 and #9 presented in the Cluster Analyses (Fig 2). Although these colonies were nested in pasture area, there were small trees and shrubs in their nesting sites, unlike the others, which were surrounded by grasses. This is likely the reason why these colonies separated from the others nested in the same area (Fig 2).

Therefore, these results demonstrate that the environmental component is also important to determine the

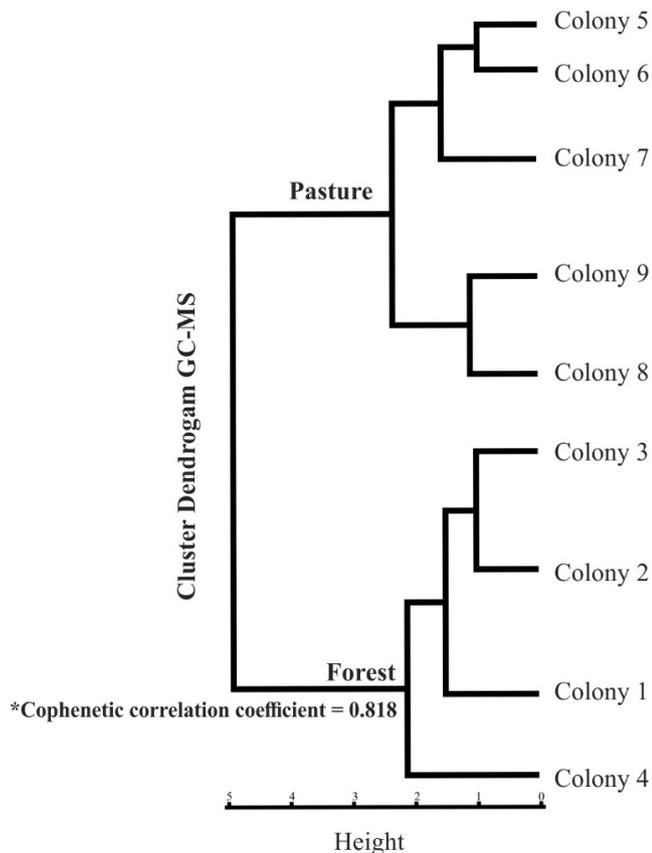


Fig 2. Similarity dendrogram of *Ectatomma brunneum* colonies based on cuticular hydrocarbons compounds data obtained by GC-MS. Cophenetic Correlation Coefficient = 0.818.

composition of CHCs. Indeed, Heinze et al. (1996) describe that when colonies share the same microhabitat there might occur similarity in odor produced by them, and as a result, workers reduce the aggressive response towards neighbors. Some authors suggest, for example, that diet is one of the factors that influence odor formation, and colonies that use similar resources should present similar colonial odor, therefore, have higher levels of tolerance when in situations of interaction (Jutsum et al., 1979; Liang & Silverman, 2000).

Conclusion

The results confirmed the hypothesis that *E. brunneum* presents the DEP, since ants were indeed more aggressive towards non-neighbors than towards neighbors. The lower level of aggression displayed between workers from closer colonies is likely explained due to their higher kinship levels, in addition to environmental factors such as sharing the same microhabitats. Furthermore, the results showed that there are significant differences in CHCs profiles, especially between colonies nested at relatively greater distances, and according to the analyses it is likely that the differences in content of some branched alkanes are the most important to establish these differences and, therefore, the levels of aggression during the interactions.

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Authors' contribution

MC Pereira and WF Antonialli-Junior conceived and design the experiments. MC Pereira and ELB Firmino performed the behavioral study. RC Bernardi and CAL Cardoso performed the chemical analyses. MC Pereira performed the data analyses. MC Pereira, LD Lima, IC Guimarães, CAL Cardoso and WF Antonialli-Junior wrote and reviewed the manuscript.

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