Sociobiology 62(3): 356-363 (September, 2015) DOI: 10.13102/sociobiology.v62i3.746

Sociobiology
An international journal on social insects

RESEARCH ARTICLE - ANTS

Nestmate Recognition in the Amazonian Myrmecophyte Ant *Pseudomyrmex concolor* Smith (Hymenoptera: Formicidae)

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Article History

Edited by
Gilberto M. M. Santos, UEFS, Brazil
Received 07 January 2015
Initial acceptance 27 March 2015
Final acceptance 02 April 2015

Keywords
Amazonia, social insects, chemical, cues, aggressiveness, kin selection.

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Abstract

Nestmate recognition is fundamental to colonial cohesion in social insects, since it allows altruistic behavior towards relatives, recognition of intruders, territorial monopoly and resources defense. In ants, olfactory cues is a key factor in this process. *Pseudomyrmex concolor* is a highly aggressive ant that defends their host plant *Tachigali myrmecophila* against herbivores. However, this defense depends on the ant ability to discriminate in order to treat differentially between members of their own colony and intruders . In this study we investigated "whether" and "how" *P. concolor* recognizes nestmates from non-nestmates. We hypothesized that *P. concolor* is skillful in recognizing nestmates and tested it in field with experiments using nestmates and non-nestmates. Additionally, to test the efficiency of resident ants against intraspecific competition during colony foundation, we simulate the plant occupation by a competitor queen, introducing non-nestmates queens in plants previously occupied by *P. concolor*. For the issue of the "how", we hypothesized that the main cue used by this ant in nestmate recognition is olfactory signal. Thus, we tested adaptive threshold model, which predicts that, if the individual odor and colony's internal template are discrepant enough, the resident nestmate will behave aggressively towards incoming individuals. In this case, we confined nestmates with non-nestmates odors, and then, we reintroduced them in its host plants. In each experiment the frequency of aggressive behaviors were recorded and compared. Results showed that *P. concolor* recognize and discriminate nestmates from non-nestmates workers (biting and stinging them) and exclude potential competitors queens. Workers reintroduced in their own colony after impregnated with non-familiar odor were treated as non-nestmates. The adaptive threshold hypothesis was confirmed, the main cue used by this ant species in nestmate recognition is olfactory signals.

Introduction

In social insects, altruistic behavior towards relatives is a direct result of nestmate recognition, which is the ability to discriminate members of their own colony from non-members (Breed & Bennett, 1987; Breed, 2014). The same pattern is repeated in other social arthropods (Tizo-Pedroso & Del-Claro, 2007; Del-Claro & Tizo-Pedroso, 2009). Nestmate recognition behavior is a central feature for maintaining the colonial cohesion in eusocial insects, since it allows not only altruistic acts towards relatives but also territorial-environmental resources defense, parasites avoidance and intruders recognition (Wilson, 1971; Crozier & Pamilo, 1996; Bos & d'Ettorre, 2012). Thus, the studies about nestmate recognition are fundamental to understand the adaptive success in eusocial insects (Hamilton, 1964a, 1964b; Hamilton, 1972; Sturgis & Gordon, 2012). This mechanism consists in the expression and detection of recognition signals, as well as the assessment of these cues and the behavioral responses which arise during the process (Beecher, 1982; Sherman & Holmes, 1985; Starks, 2004).

During the evolutionary process, the cuticular hydrocarbons, which are waterproof waxes that cover the external surface of

Open access journal: http://periodicos.uefs.br/ojs/index.php/sociobiology
ISSN: 0361-6525

insects, have an important role in the communication system of social insects (Wilson, 1971; Lockey, 1988; Nunes et al., 2009). These substances play a central role in ants such as recognition of species, sexual pheromones, marking of territories and parental recognition (Hölldobler & Wilson, 1990; Van Zweden & d'Ettorre, 2010). There are several evidences that olfactory cues (cuticular hydrocarbons) are responsible for recognition and discrimination between nestmates and non-nestmates in ants (Hölldobler, 1995; Sudd & Franks, 1987; Carlin, 1988; Errard & Hefetz, 1997; Vander Meer & Alonso, 1998; Astruc et al., 2001; Katzav-Gozansky, 2008; Van Zweden & d'Ettorre, 2010; Newey, 2011; Bos & d'Ettorre, 2012; Sturgis & Gordon, 2012; Nascimento et al., 2013). The individuals are recognized as nestmates when their recognition cues are congruent with internal patterns, template, of their own colony (Vander Meer & Alonso, 1998; Sturgis & Gordon, 2012).

In the adaptive threshold model, if the difference between odor of individual and internal template of colony is sufficiently discrepant, the resident nest member will aggress the intruder (Sherman et al., 1997). Nestmate recognition in social insects is mainly based on olfactory cues and, thereby, as predicts adaptive threshold model, ants should accept nestmates and reject non-nestmates. The rejection behavioral response, generally agonistic, is a consequence of this process and is used as a conspicuous sign for nestmate recognition in eusocial insects (Carlin & Hölldobler, 1983; Bos & d'Ettorre, 2012).

Ants are highly aggressive to intra- and inter-specific intruders, displaying a series of agonistics behaviors towards non-members of the colony (Carlin, 1988; Crosland, 1990; Johnson et al., 2012). This phenomenon is called discrimination mechanism, which is the display of different behaviors to nestmate and non-nestmates according to their recognition (Barnard & Aldhous, 1991). Thereby, nestmate recognition plays a central role during the discrimination.

In the north of the Amazon region, the stinging ant *Pseudomyrmex concolor* (Smith, 1860) nests inside hollow rachis (domatia) of the leaves of *Tachigali myrmecophila* Ducke (Fabaceae: Caesalpinioideae) (Wheeler, 1921). *Pseudomyrmex concolor* is a highly aggressive ant, attacking incoming individuals that cause any disturbance and thus, defending their host plant against herbivores. Plants from which *P. concolor* colony was experimentally removed are more susceptible to herbivory with the leaf longevity and apical growth almost twice smaller than plants without ants (Fonseca, 1994). Additionally, seedlings of *T. myrmecophila* in the early developmental stages are exclusively nested by *P. concolor*, revealing great competitive ability of this ant. These results suggest that *P. concolor* is skillful to defend its host plant, thus, it avoids the invasion and the competition of intruders. However, that defense depends upon the ability of *P. concolor* to recognize nestmates and non-nestmates, since natural selection favored kin recognition systems in order to detect effectively relatives, discriminating and avoiding potential competitors (Sherman et al., 1997).

Although abundant, ant-plant mutualistic relationships have been poorly studied in the Amazon region regarding the behavioral ecology, especially from behavior recognition perspective and aggression towards aliens mediated by olfactory cues. Studies about nestmate recognition system (Jaffé et al., 1986; Mintzer, 1982; Starks et al., 1998) and kinship (Mintzer & Vinson, 1985) in *Pseudomyrmex* genus are scarce and little is known about how the neotropical *Pseudomyrmex* ants recognize nestmates. Here, we investigated the nestmate recognition system in the stinging amazon ant *P. concolor*. At the first time we tested the hypothesis that *P. concolor* is skillful in recognizing and discriminating nestmates from non-nestmates workers. In order to test the hypothesis that colonies of ants are effective in preventing intraspecific competition, we simulated the plant occupation by a competitor queen, introducing intraspecific non-nestmates queens in host plants previously occupied by *P. concolor*. Lastly we also hypothesized that the main cue used by this ant species in kin recognition is olfactory signal and tested whether and how olfactory signals can influence the nestmate recognition ability in *P. concolor*. Based in adaptive threshold model, we predicted that *P. concolor* workers will behave aggressively towards nestmates impregnated with olfactory cues distinct of its own colony.

Material and methods

Study area

Field experiments were carried out during June and July 2013 and May to July 2014 in the Terra Firme Amazonian Forest environments of River Curiaú Reserve about 20 km north of Macapá, Amapá, Brazil, located between 00º 15'N and 51º 00' W. The vegetation is mainly evergreen and has a variable canopy about 30-50m in height and presents short frequency deciduousness in the driest season with flowering and fruiting throughout the wet season. The regional climate is classified, according to Köppen, as Wet Tropical, characterized mainly for high annual rainfall rate and the average annual temperature is 27,6º C, with 31ºC maximum and 23ºC minimum. The average annual rainfall is 2.850mm, being one of the rainiest places in Brazil (Alvares et al., 2013).

Nestmate recognition experiments and behavioral observations

Twenty plants of *Tachigali myrmecophila* (between 0.70 m and 2.17m) colonized by *Pseudomyrmex concolor* were paired according to height, number of leaves and leaflets. We tagged each plant pair with letters (A to J) and each plant received a numerical designation (e.g A1 and A2 composing A pair). Plants separated by a minimum distance of 7 meters were selected to compose the pair, but most plants distanced more than 15 meters from each other.

After pairing, we performed behavioral experiments based on two experimental groups: a) Control Group - removing and reintroducing an ant of the same colony (e.g. A1 x A1; A2 x A2;...) and b) Treatment Group - introducing ant of another colony (e.g. A1 x A2; A2 x A1; ...). We performed twenty introductions for each behavioral test.

Nestmate and non-nestmates were randomly collected with entomological feather-weight forceps, marked with acrylic white ink Docrafts Artistepara® and kept confined for ten minutes in glass tubes (7,5cm x1,0cm x0,8cm; new and clean) in order to reduce the handling of the collection. Then, a single related worker of *P. concolor* (control group) or unrelated (treatment group) was introduced on the distal leaflet of one *T. myrmecophila* leaf randomly chosen.

Furthermore, in order to simulate interspecific competition and the presence of a potential herbivore we performed twenty introductions of *Azteca* sp. (Formicidae) and termites (Termitidae) in each tested plant respectively. Ants of *Azteca* genus is a reasonable model for interspecific competition test, since it participates of ant colony replacements during ontogenetic succession of *Tachigali* genus in Amazon forest (Fonseca & Benson, 2003) and termites were used in order to simulate the presence of herbivores in host plant (Oliveira et al*.*, 1987).

Additionally and similarly, aiming to simulate the plant occupation by a competitor queen, we introduced fifteen intraspecific non-nestmates queens in fifteen different plants. Behavioral interactions were observed for five minutes, after the first contact resident-intruder, and we recorded absence (no aggression) or presence of bites and/or stings (aggression) since these behaviors are very conspicuous and easy to record and indicate discrimination between individuals. The data were subsequently analyzed according to the all occurrence sampling method (Altmann, 1974). All introductions were independent, in other words, no individual introduced was used twice and each plant was tested only once for each experimental group.

Finally, to test the influence of olfactory cues in the nestmate recognition ability of *P. concolor*, five nestmate or non-nestmate ants and other ant species (*Azteca* sp.) were accommodated in a clean new vial glass (42mm x 21mm x 7mm) for 60 minutes in order to impregnated the bottles with their smell. Then workers of *P. concolor* were collected, marked and confined for 60 minutes in the vials containing just odors of nestmates (control group), odors of intraspecific non-nestmates (treatment 1) and odors of other ant species; *Azteca* sp. (treatment 2). After confinement, nestmates were reintroduced in their host plants. We performed twenty introductions for each experimental group.

Statistical analyses

Behavioral experiments showed discreet and independent variables data, being categorized on the occurrence and absence of aggression and assessed through ranking testing within two positions. Thus, for data analyses we followed Breed model (Breed, 2003), where chi-square test was used to compare differences between treatment and control group in experiments about nestmate recognition.

Results

Pseudomyrmex concolor workers discriminate introduced non-nestmates from nestmates. During the experiments we recorded 80% of aggression towards intraspecific non-nestmates and just 10% of reintroduced nestmates were attacked by resident ants. Aggressive behaviors such as bites and stings were targeted significantly to workers of treatment group (χ^2=19.79, P<0.001, n=20) (Fig 1). Unsurprisingly, all *Azteca* workers and also termites introduced in the plant were beaten by *P. concolor*. Thus, the aggression frequency was similar between interspecific ants and termites. On the other hand, 60% of nestmate workers were allowed to enter the colony (χ^2=13.78, P<0.001, n=20) (Table 1). Thereby, workers of *P. concolor* treat both intra- and interspecific non-nestmates as potential competitors or herbivores. As expected, workers of *P. concolor* were effective in preventing competing queens to establish in the host plant. All introduced intraspecific queens non-nestmates (n=15) were attacked, chased and/or removed from the plant by resident workers.

Table 1. Comparison on the frequency of nestmates and non-nestmates allowed entering the domatia of *Tachigali myrmecophila* (Fabaceae) colonized by *Pseudomyrmex concolor* (Pseudoyrmecinae) ants in the Amazon forest.

Enter Inside the Domatia					
		Yes	**No**	χ^2	**P**
Pseudomyrmex concolor	Nestmate	12 60%	8 40%	13.78	<0.001
	Non-nestmate	1 5%	4 95%		

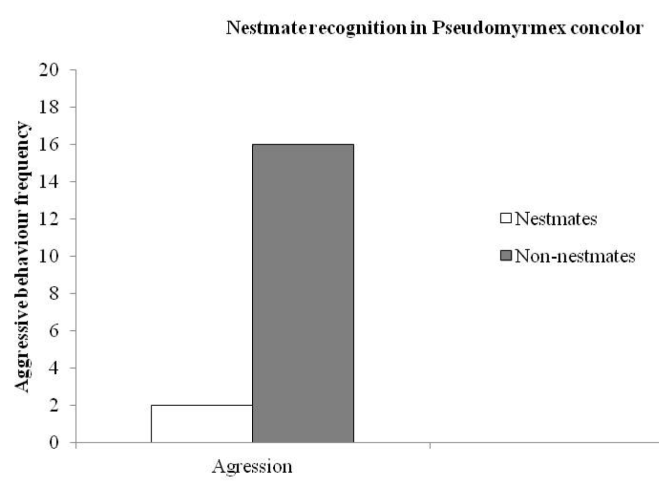

Fig 1. Occurrence of aggression between nestmate and non-nestmate (χ^2=19.79, P<0.001 n=20) in *Pseudomyrmex concolor* (Pseudoyrmecinae) ants observed during recognition experiments conducted in the Amazon forest host plant *Tachigali myrmecophila* (Fabaceae).

Finally, we confirmed the adaptive threshold model in *P. concolor*. After 1h of intraspecific non-nestmates odors exposition (treatment 1), ants reintroduced in their own host plant were attacked in 30% of introductions by its nestmates. On the other hand when ants were introduced into their colonies after contact with nestmates odors (control), aggression rate was just 5% (χ^2=4.32, P<0.05, n=20). Fifty-five percent of nestmates that came into contact with odors of *Azteca* workers (treatment 2) were bitten (χ^2=11.9, P<0.001, n=20). There was no significant difference of aggression frequency between ants confined in vial glass with intra or interspecific odors (30% vs 55%, χ^2=2.55, P>0.10) (Fig 2). Therefore, contact with disparate odors between internal template and individual odors provided interference in nestmate recognition ability in *P. concolor*. We recorded 21 behavioral acts, ranging from non-aggressive to aggressive ones recorded and described in Table 2 and Table 3. The analysis of recorded behaviors enable us to suggest a sequential behavioral pattern to the two occasions, when workers are and are not recognized by nestmates (Fig 3).

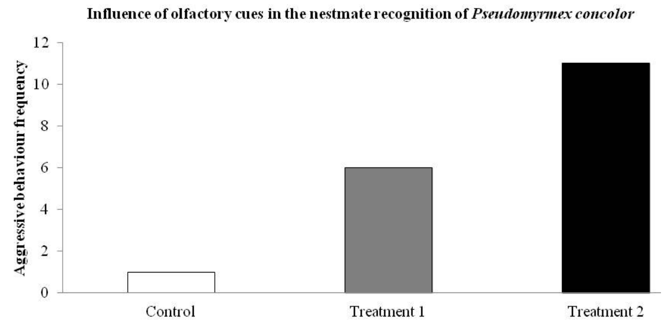

Fig 2. Frequency of aggression towards workers ants of *Pseudomyrmex concolor* (Pseudomyrmecinae) which remained in contact with odor of its nestmates (control, n=20), intraspecific non-nestmates odors (treatment 1, n=20) and interspecific ant odor (treatment 2, n=20). Control vs. treatment 1: χ^2=4.32, P<0.05; control vs. treatment 2: χ^2=11.9, P<0.001 and treatment 1 vs. treatment 2: χ^2=2.55, P>0.10.

Table 2. Behavioral acts exhibited by the ant *Pseudomyrmex concolor* (Pseudomyrmecinae) during nestmate recognition experiments conducted in the Amazon forest host plant *Tachigali myrmecophila* (Fabaceae).

To accept	Introduced ant is accepted and allowed to enter inside the foreign plant domatia
To lick	Resident ant touched with its palps the gaster of the introduced individual
To ignore	Introduced individual did not stimulate any behavior in the resident
Antennation	Reciprocal antennation between resident and introduced individual
To inspect	To touch with its antennas, the gaster, thorax and head of the introduced individual. This behavior can be performed by several resident ants, and the introduced remained still during the inspection
Self-grooming	Introduced ant rubbed parts of its body. This behavior can be performed between antennas and forelegs, as well as between hind legs and gaster
Gaster vibration	Semi-rotation movements of gaster shacking it
Avoidance	Introduced or resident ant changed in 90º or 180º the axis of its body before interaction
Assault	Resident ant changed its course towards the introduced individual, touching it with its antennas in the gaster or head of the intruder
To chase	Resident ant followed the intruder for more than three seconds touching it with its antennas in the gaster of the intruder
To scape	Introduced or resident ant changed in 90º or 180º the axis of its body after interaction
Nibbling	Biting without trapping the intruder's body with the jaws
Biting	Trapping and compressing the jaws in parts of the intruder's body, such as legs, antennae, gaster and jaws. The intruder was bitten by one or more residents simultaneously
Full attack	Resident moves towards the intruder and bites it without any prior interaction
To drag	Resident bites the introduced individual (antennae, legs and petiole) and moved them beyond its initial position
To carry	Resident ant bites and lifts the intruder above the level of its body
To expulse	Resident ant threw the intruder from its host plant or removed its corpse after death
Tug of war	Resident ants were biting and pulling the intruder in the opposite directions from its body axis. The intruder was immobilized because its bodily appendages were pulled in different directions, thus, it was unable to escape
Fighting	Residents and intruders were engaged in mutual bites. They were entwined and stuck with their mandibles, trapping and compressing their opponent. When fights were performed with interspecific ants, residents used the sting as pparatus attack. Sometimes, the intruder remained attached in the body of the resident even after death
To Sting	Resident ant ventrally doubled its gaster towards the intruder, leaned on the two pairs of hind legs and then, inserted the sting in the body of the intruder
To kill	After bites and/or stings the intruders stopped its body movements

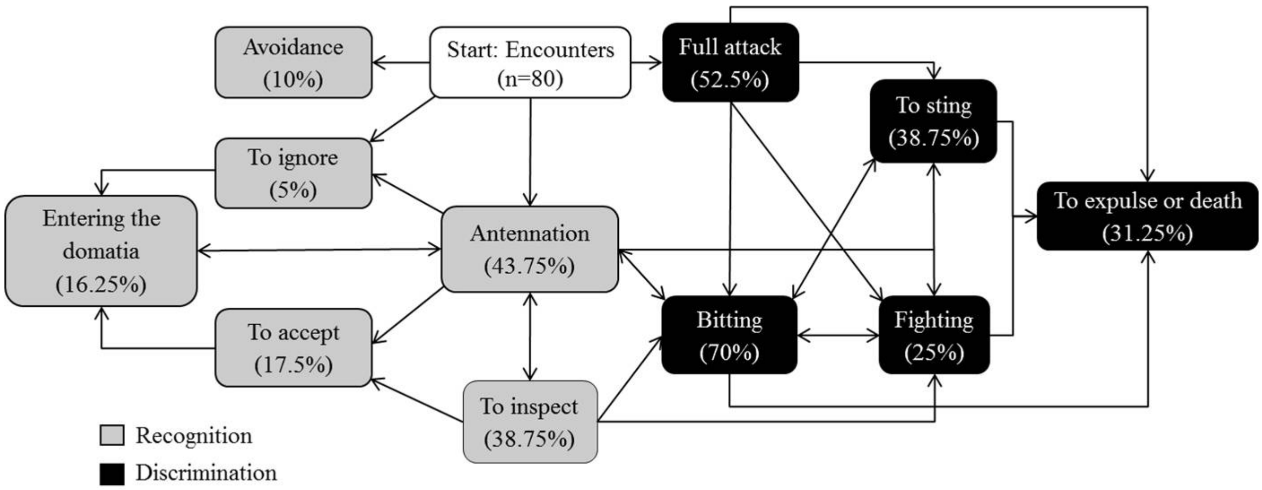

Fig 3. Behavioral pattern identified in *Pseudomyrmex concolor* (Pseudomyrmecinae) ants observed during nestmates recognition experiments conducted in the Amazon forest host plant *Tachigali myrmecophila* (Fabaceae). In brackets, the frequency of behaviors observed during the interactions (n=80) between nestmate (grey label) and non-nestmates (black label).

Table 3. Results of interactions between the resident ant *Pseudomyrmex concolor* (Pseudoyrmecinae) and introduced nestmates (control group), intraspecific non-nestmates (*Azteca* sp.) and termites, in the Amazon forest host plant *Tachigali myrmecophila* (Fabaceae). The line below "avoidance" separate between non- (above) and aggressive (below) behaviors.

Behaviors	**Control** (n = 20)	**Conspecific** (n = 20)	**Interspecific ant** (n = 20)	**Termites** (n = 20)
To accept	13	1	0	0
To lick	11	0	0	0
To ignore	0	0	1	3
Antennation	19	9	4	3
To inspect	15	8	3	5
Self-grooming	18	8	0	0
Gaster vibration	0	1	0	0
Avoidance	0	1	3	4
Assault	3	0	0	0
To chase	0	10	0	0
To scape	0	6	1	0
Nibbling	1	1	0	0
Biting	0	16	20	20
Full attack	0	11	15	16
To drag	0	7	11	5
To carry	0	2	6	1
To expulse	0	1	4	1
Tug of war	0	1	0	0
Fighting	0	5	13	2
To Sting	0	0	18	13
To kill	0	0	16	3

Discussion

The manipulation experiments confirmed the hypothesis that *P. concolor* is skillful in recognizing nestmates and discriminating non-nestmates according to its host plant. The adaptive threshold hypothesis was also confirmed; results demonstrated that the main cue used by this ant species in nestmate recognition is olfactory signal. Workers of *P. concolor* were significantly more aggressive towards non-nestmates, both intra and interspecific, than against nestmates. Similar results were observed to other ant species (Bos & d'Ettorre, 2012; Sturgis & Gordon, 2012) and social arthropods (Breed & Bennett, 1987; Jungnickel et al., 2004; Nunes et al., 2008; Tizo-Pedroso & Del-Claro, 2014).

Similar to other Formicidae, *P. concolor* workers also recognize and avoid, chasing or attacking all alien introduced queens. The second hypothesis that workers prevent a second nesting in previously colonized plants, reducing intraspecific competition, was also corroborated. Newly fertilized queens searching for nesting sites are often killed or removed by *Pseudomyrmex* ants that establish obligatory mutualistic relationships with myrmecophyte plants (Janzen, 1967; Janzen, 1973). The low frequency of *T. myrmecophila* trees unoccupied in similar sites in the Amazon forest (Fonseca & Benson, 2003), suggest that the competition for this plant species by *P. concolor* queens is strong. Thus, the defense of the host plant by resident ants against herbivores and any other incoming individual is imperative, not arising from the success of this mutualism, but as a result of the highly effective colonial defense due to nestmate recognition.

The behaviors recorded in *P. concolor* revealed a common behavioral pattern during the nestmate recognition mechanism in social insects (Wallis, 1970; Wilson, 1971; Carlin & Hölldobler, 1983; Carlin & Hölldobler, 1986; Carlin & Hölldobler, 1987; Hölldobler & Wilson, 1990; Breed &

Page, 1991;), systematized in Figure 3. During behavioral interactions, the first contact of the resident ant was a touch with its antennas on the introduced individual's body or mutual antennal touches between them. After this inspection the individual introduced could be tolerated, ignored or attacked by the resident ants. However, the physical contact has not occurred often before the aggressive interactions. In this case, the resident ant threw himself towards the incoming individual before there was any body-to-body contact, biting legs, antennas, mandibles and petiole. This aggression, which occurs even without investigation, reduces the time that the intruder could remain on the plant.

Recognition is the ability of the identify individuals, while discrimination is the differential treatment for another individual based on recognition (Hepper, 1986; Barnard & Aldous, 1991). Thus, the reduced time between recognition and discrimination demonstrated in *P. concolor* combined with its highly aggressive behavior and constant patrolling in the host plant provides an efficient defense against incoming individuals and competitors. The same is also observed in other arthropods (Del-Claro & Tizo-Pedroso, 2009).

Behavioral tests about the influence of odor during nestmate recognition demonstrated that workers of *P. concolor* introduced in their colonies, after contact with both interspecific and intraspecific non-familiar odors, were treated differentially by their nestmates. The one-hour exposure to non-familiar odors sets off aggressive behaviors such as biting and threats to those introduced ants. It's suggest that transfers of olfactory cues used in recognition through contact with the unfamiliar odor may have occurred. The contact with discrepant odors from the cuticular pattern interferes in the nestmate recognition in this ant species. Thus, workers of *P. concolor* utilize olfactory similarity cues during recognition and discrimination process.

As expected by the adaptive threshold model, differences between the individual odor and colony's internal template triggered aggressive behaviors for non-nestmates. Ants in contact with unfamiliar odors were often assaulted by their nestmates. Thus, it is clear that *P. concolor*, as well as *P. ferruginea* (Mintzer, 1982; Mintzer & Vinson, 1985), uses chemical similarity to discriminate between nestmates and non-nestmates. The difference of the frequency of aggression between nestmates confined in vials impregnated with interspecific and intraspecific odor was not significant. These ants recognize olfactory discrepancies derived both interspecific and intraspecific in a similar way, since workers from two treatments were attacked. Therefore, workers of *P. concolor* perceive individual variations of cuticular hydrocarbons with their internal template and thus behave appropriately for nestmates and non-nestmates.

During the evolutionary process, natural selection must have favored ants which were more and more efficient in recognizing nestmates and discriminating non-nestmates. This feature is critical for maintaining obligatory mutualism between ants and *myrmecophyte* plants. Thus preventing intruders is a benefit not only to the colony but also to the host plant, since an efficient colony in recognizing and discriminating between nestmates and intruders should also be efficient in expelling herbivores and defending their host plant. Therefore, individuals with discrepant olfactory cues of the internal colonial pattern should be avoided, battered or forced out by *P. concolor* workers that nest in *T. myrmecophila.*

In this study we clearly demonstrated that *P. concolor* ant is skilled in recognizing and discriminating nestmates from non-nestmates using highly aggressive behaviors, being effective in the defense of the colony against competitors and their plant against herbivores. During this process, workers of *P. concolor* perceive olfactory signals from other individuals and so, compare these cues with its odoriferous identity and the colony's internal template. According to chemical similarities assessed ants may accept or reject, through aggression, individuals present in their host plant. However, more studies (eg: chemical analysis) are required to confirm that nestmate recognition behavior is directly related to the chemical compounds present on the surface of the body of *P. concolor*. Furthermore, it is possible that *P. concolor* uses other information such as visual cues, common mechanism for members of the subfamily Pseudomyrmecinae (see Ward & Downie, 2005) and other social insects (see Sheehan & Tibbetts, 2011; Tibbetts & Sheehan, 2011), during nestmate recognition.

Acknowledgments

We thank Leiliana Rocha and Danilo Gonçalves for field assistance. We are also grateful to Everton Tizo-Pedroso (Federal University of Góias) for his valuable criticism and suggestions for the final version of the manuscript. This research was supported by CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico, PHd grant, process 142213/2012-8; and also PQ grant 301605/2013-0).

References

Altmann, J. (1974). Observational study of behavior: sampling methods. Behaviour, 49: 227-267.

Alvares, C.A., Stape, J.L., Sentelhas, P.C., De Moraes Gonçalves, J.L. & Sparovek, G. (2013). Köppen's climate classification map for Brazil. Meteorologische Zeitschrift, 22: 711-728.

Astruc, C., Malosse, C. & Errard, C. (2001). Lack of intraspecific aggression in the ant *Tetramorium bicarinatum*: A chemical hypothesis. Journal of Chemical Ecology, 27: 1229-1248.

Barnard, C.J., Aldhous, P. (1991). Kinship, kin discrimination and mate choice. In P. G. Hepper (Eds.), Kin recognition (pp.125-147). England: Cambridge University Press.

Beecher, M.D. (1982). Signature systems and kin recognition. Integrative and Comparative Biology, 22: 477-490.

Bos, N. & d'Ettorre, P. (2012). Recognition of social identity in ants. Frontiers in Psychology, 3: 1-6.

Breed, M.D. (2003). Nestmate Recognition Assays as a Tool for Population and Ecological Studies in Eusocial Insects. Journal of the Kansas Entomological Society, 76: 539-550.

Breed, M.D. (2014). Kin and nestmate recognition: The influence of W. D. Hamilton on 50years of research. Animal Behaviour, 92: 271-279.

Breed, M.D. & Bennett, B. (1987). Kin recognition in highly eusocial insects. In D.J.C. Fletcher, & C. D. Michener (Eds.), Kin recognition in animals (pp. 243-285). J. Wiley & Sons Ltd.

Breed, M.D., & Page, R.E. (1991). Intra- and interspecific nestmate recognition in Melipona workers (Hymenoptera: Apidae). Journal of Insect Behavior, 4: 463-469.

Carlin, N.F. (1988). Discrimination Between and Within Colonies of Social Insects: Two Null Hypotheses. Netherlands Journal of Zoology, 39: 86-100.

Carlin, N.F. & Hölldobler, B. (1983). Nestmate and kin recognition in interspecific mixed colonies of ants. Science, 222: 1027-1029.

Carlin, N.F. & Hölldobler, B. (1986). The kin recognition system of carpenter ants (*Camponotus* spp.): I Hierarchical cues in small colonies. Behavioral Ecology Sociobiology, 19: 123-124.

Carlin, N.F. & Hölldobler, B. (1987). The kin recognition system of carpenter ants (*Camponotus* spp.) - II. Larger colonies. Behavioral Ecology and Sociobiology, 20: 209-217.

Crosland, M.W.J. (1990). Variation in ant aggression and kin descrimination ability with and between colonies. Journal of Insect Behavior, 3: 359-379.

Crozier, R.H. & Pamilo, P. (1996). Evolution of Social Insects Colonies. Oxford: Oxford University Press.

Del-Claro, K. & Tizo-Pedroso, E. (2009). Ecological and evolutionary pathways of social behavior in Pseudoscorpions (Arachnida: Pseudoscorpiones). Acta Ethologica, 12: 13-22.

Errard, C. & Hefetz, A. (1997). Label familiarity and discriminatory ability of ants reared in mixed groups. Insectes Sociaux, 44: 189-198.

Fonseca, C.R. (1994). Herbivory and the Long-Lived Leaves of an Amazonian Ant-Tree. Journal of Ecology, 82: 833-842.

Fonseca, C.R., Benson, W.W. (2003). Ontogenetic succession in Amazonian ant trees. Oikos, 102: 407-412.

Hamilton, W.D. (1964a). The genetical evolution of social behavior I. Journal of Theoretical Biology*,* 7: 1-6.

Hamilton, W.D. (1964b). The genetical evolution of social behavior II. Journal of Theoretical Biology*,* 7: 17-52.

Hamilton, W.D. (1972). Altruism and related phenomena, mainly in social insects. Annual Review of Ecology and Systematics*,* 3: 193-232.

Hepper, P.G. (1986). Kin recognition: functions and mechanisms, a review. Biological Reviews, 61: 63-93.

Hölldobler, B. (1995). The chemistry of social regulation: multicomponent signals in ant societies. Proceedings of the National Academy of Sciences of the United States of America, 92: 19-22.

Hölldobler, B. & Wilson, E. O. (1990). The Ants. Cambridge: Harvard University Press.

Jaffé, K., Lopez, M.E. & Aragort, W. (1986). On the communication systems of the ants *Pseudomyrmex termitarius* and *P. triplarinus*. Insectes Sociaux, 33: 105-117.

Janzen, D.H. (1967). Interaction of the bull's horn acacia (Acacia cornigera L.) with an ant inhabitant *(Pseudomyrmex ferruginea* F. Smith) in eastern Mexico. Kansas, USA: University of Kansas Publications.

Janzen, D.H. (1973). Evolution of polygynous obligate acacia-ants in western Mexico. Journal of Animal Ecology, 42: 727-750.

Johnson, B.R., Van Wilgenburg, E. & Tsutsui, N.D. (2012). Nestmate recognition in social insects is sometimes more complex than an individual based decision to accept or reject. Behavioral Ecology and Sociobiology*,* 66: 343–346.

Jungnickel, H., Da Costa, A.J.S., Tentschert, J., Patricio, E.F.L.R.A., Imperatriz-Fonseca, V.L., Drijfhout, F. & Morgan, E.D. (2004). Chemical basis for inter-colonial aggression in the stingless bee *Scaptotrigona bipunctata* (Hymenoptera: Apidae). Journal of Insect Physiology, 50: 761-766.

Katzav-Gozansky, T., Boulay, R., Ionescu-Hirsh, A. & Hefetz, A. (2008). Nest volatiles as modulators of nestmate recognition in the ant *Camponotus fellah*. Journal of Insect Physiology, 54: 378-385.

Lockey, K. H. (1988). Lipids of the insect cuticle: origin, composition and function. Comparative biochemistry and physiology, 89: 595-645.

Mintzer, A. (1982). Nestmate recognition and incompatibility between colonies of the acacia-ant *Pseudomyrmex ferruginea*. Behavioral Ecology and Sociobiology, 10: 165-168.

Mintzer, A. & Vinson, S.B. (1985). Kinship and incompatibility between colonies of the acacia ant *Pseudomyrmex ferruginea*. Behavioral Ecology and Sociobiology, 17: 75-78.

Nascimento, F.S., Tannure-Nascimento, I.C., Dantas, J.O., Turatti, I.C. & Lopes, N.P. (2013). Task-Related Variation of Cuticular Hydrocarbon Profiles Affect Nestmate Recognition in the Giant ant *Dinoponera quadriceps*. Journal of Insect Behavior, 26: 212-222.

Newey, P. (2011). Not one odour but two: A new model for nestmate recognition. Journal of Theoretical Biology, 270: 7-12.

Nunes, T.M., Nascimento, F.S., Turatti, I.C., Lopes, N.P. & Zucchi, R. (2008). Nestmate recognition in a stingless bee: does the similarity of chemical cues determine guard acceptance? Animal Behaviour, *75*: 1165-1171.

Nunes, T. M., Turantti, I. C. C., & Mateus, S. (2009). Cuticular hydrocarbons in the stingless bee *Scharziana quandripunctata* (Hymenoptera, Apidae, Meliponini): differences between colonies, castes and age. Genetics and Molecular Research, 8: 589-595.

Oliveira, P.S., Oliveira-Filho, A.T., Cintra, R. (1987). Ant foraging on ant-inhabited Triplaris (Polygonaceae) in western Brazil: a field experiment using live termite-baits. Journal of Tropical Ecology, 3:193–200.

Sheehan, M.J. & Tibbetts, E. A. (2011). Specialized Face Learning Is Associated with Individual Recognition in Paper Wasps. Science, 334: 1272-1275.Sherman, P. W., & Holmes, W. G. (1985). Kin recognition: issues and evidence. Fortschritte der Zoologie, 31: 437-460

Sherman, P. W., Reeve, H. K. & Pfennig, D. W. (1997). Recognition systems. In J. R. Krebs, & N.B. Davis (Eds.), Behavioural ecology: An evolutionary approach (pp. 69-96). Oxford, UK.: Blackwell.

Smith, F. (1860). Descriptions of new genera and species of exotic Hymenoptera. Journal of Entomology, 2: 65-84.

Starks, P.T. (2004). Recognition systems (Special issue). Annales Zoologici Fennici. 41: 689-892.

Starks, P.T., Watson, R.E., Dipaola, M.J. & Dipaola, C.P. (1998). The effect of queen number on nestmate discrimination in the facultatively polygynous ant *Pseudomyrmex pallidus* (Hymenoptera : Formicidae). Ethology, 104: 573-584.

Sturgis, S.J. & Gordon, D.M. (2012). Nestmate recognition in ants (Hymenoptera : Formicidae): a review. Biological Invasions, 16: 101-110.

Sudd, J.H. & Franks, N.R. (1987). The behavioural ecology of ants (1st ed.). USA, Chapman & Hall.

Tibbetts, E.A. & Sheehan, M.J. (2011). Facial Patterns are a Conventional Signal of Agonistic Ability in *Polistes exclamans* Paper Wasps. Ethology, 117: 1138-1146.

Tizo-Pedroso, E. & Del-Claro, K. (2007). Cooperation in the neotropical pseudoscorpion, *Paratemnoides nidificator* (Balzan, 1888): Feeding and dispersal behavior. Insectes Sociaux, 54: 124-131.

Tizo-Pedroso, E. & Del-Claro, K. (2014). Social parasitism: emergence of the cuckoo strategy between pseudoscorpions. Behavioral Ecology, 25: 335–343.

Van Zweden, J. S. & D'ettorre, P. (2010). Nestmate recognition in social insects and role of hydrocarbons. In Blomquist, G.J (Eds.), Insect Hydrocarbons Biology, Biochemistry, and Chemical Ecology (pp. 222-243). England: Cambridge University Press.

Vander Meer, R.K.V. & Alonso, L.E. (1998). Pheromone directed behavior in ants. In R.K. Vander Meer, M.D. Breed, K.E. Espelie & M.L.P. Winston (Eds.), Pheromone communication in social insects (pp.159-191). USA: Westview Press.

Wallis, D.I. (1970). Aggression in social insects. In C.H. Southwick, (Eds), Animal aggression: selected readings (94-102). New York, USA: Van Nostrand Reinhold Company,

Ward, P.S. & Downie, D.A. (2005). The ant subfamily Pseudomyrmecinae (Hymenoptera: Formicidae): Phylogeny and evolution of big-eyed arboreal ants. Systematic Entomology, 30: 310-335.

Wheeler, W.M. (1921). The Tachigali ants. Zoologica, 3: 137-168.

Wilson, E.O. (1971). The Insect Societies. Cambridge: Belknap Press, Harvard University.