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Recognition and Aggression of conspecific and heterospecific worker in *Acromyrmex subterraneus subterraneus* (Forel) (Hymenoptera: Formicidae)

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Abstract

Aggressive behavior is important for social insects because it makes possible for the colony to defend itself and the offspring from the action of invasive species. We studied the recognition and aggressiveness of the leaf-cutting ant *Acromyrmex subterraneus subterraneus* (Forel) to co-specific workers from other nest and heterospecific workers of *Acromyrmex subterraneus molestans* Santschi, *Acromyrmex subterraneus brunneus* (Forel) and *Acromyrmex niger* (Smith); and queens of their social parasite *Acromyrmex ameliae* De Souza, Soares and Della Lucia. Workers of other species were placed in contact with those of *A. subterraneus subterraneus* for three minutes and during this period the behavioral interactions were quantified. The aggressiveness index (AI) for each agonistic encounter was obtained. *Acromyrmex subterraneus subterraneus* workers exhibited greater aggressiveness against heterospecific than against conspecific competitors. Aggressiveness is connected to differences in the chemical profiles, which are larger in heterospecifics colonies.

Introduction

An important feature of social species is the existence of elaborate recognition systems that facilitate cooperation among members of a group, because they help to maintain the integrity of the society, reducing the negative impact caused by predators, competitors and social parasites (Crosland, 1990; Fishwild & Gamboa, 1992; Crowley et al., 1996; Wiley, 2013). Ants maintain coherence within the colony through chemical recognition of nestmates, allowing workers to differentiate other individuals between friend and foe through specific chemical signatures of each colony (Lenoir et al., 2001; Akino, 2008; Guerrieri et al., 2009).

Leaf-cutting ants (Hymenoptera: Formicidae: Myrmicinae: Attini) are dominant herbivores in the Neotropics (Hölldobler & Wilson, 1990). *Acromyrmex* Mayr, with 62 species and nine subspecies (Forti et al., 2006; Souza et al., 2007; Brandão et al., 2011), causes economic losses in agriculture and is one of the most important pests of forest plantations (Boulogne et al., 2012).

These ants are models for behavioral studies because of their elaborate social organization and interactions between individuals of the colony and with other organisms (Camargo et al., 2006). Workers of different sizes exhibit different behaviors and division of labor (polyethism), which maximizes foraging (Hart et al., 2002). However, there are activities of the colony that are still poorly studied, such as some interactions between the workers of a nest.

Social insects exhibit aggressive behavior towards individuals from other nests and invading organisms (Hölldobler & Michener, 1980). This behavior allows the nests to store resources (Hamilton, 1972) and protect their workers and offspring from external threats (Pollock & Rissing, 1989; Sakagami, 1993; Mori et al., 2000; Allon et al., 2012).

The recognition of nestmates occurs through a specific "label" called "colony odor" (Crozier & Dix, 1979). This label is made of cuticular hydrocarbons in social insects (Lenoir et al., 2001a) and allows ants to recognize nestmates and distinguish them from intruders (Hölldober & Michener,

1980). Soil characteristics, food source and other nest materials participate in the formation of this label (Heinze et al., 1996). Diet is more important than the influence of the queen and their genetic origin in the formation of odor in nests of *Acromyrmex subterraneus subterraneus* (Forel) (Hymenoptera: Formicidae) and, consequently, in the recognition of the ants (Richard et al., 2004). The fungi recognition behavior (Viana et al., 2001) and trophallaxis (Moreira et al., 2006) were studied in this subspecies. However, several types of behavior of *A. subterraneus subterraneus* workers such as aggressiveness have not yet been evaluated. We hypothesized that workers of *A. subterraneus subterraneus* exhibit greater aggressiveness against heterospecifics than against conspecific ones.

The objective of this study was to describe and compare the behavior of recognition and aggression of *A. subterraneus subterraneus* workers in relation to workers of: (1) *A. subterraneus subterraneus* from different colonies; (2) *Acromyrmex subterraneus molestans* Santschi; (3) *Acromyrmex subterraneus brunneus* (Forel); (4) *Acromyrmex niger* (Smith); and their social parasite queens (5) *Acromyrmex ameliae* De Souza, Soares & Della Lucia.

Material and Methods

Study site and species

The study was conducted at the Laboratório de Formigas Cortadeiras (LFC) of the Universidade Federal de Viçosa (UFV) in Viçosa, Minas Gerais State, Brazil in November 2011. *Acromyrmex subterraneus subterraneus*, *A. subterraneus molestans*, and *A. niger* naturally co-occur in the region of Viçosa. The social parasite *A. ameliae* occurs only in nests of *A. subterraneus subterraneus* and *A. subterraneus brunneus* in another region of Minas Gerais State, at about 300 km from Viçosa. All individuals used in the experiment were obtained from a total of ten colonies maintained in the LFC at 24 ± 2ºC and ± 75% RH; and fed with *Ligustrum japonicum* Thunb. (Oleaceae) and *Acalipha wilkesiana* Müll. Arg. (Euphorbiaceae) leaves. One of the colonies of *A. subterraneus subterraneus* was collected on the UFV campus and kept under the same conditions as the others, in a separate room.

Recognition and agressiveness evaluation

The aggressiveness and recognition among workers were assessed in a neutral arena, a plastic container (250 mL) with the inner upper half covered with talc. Five workers of *A. subterraneus subterraneus* were randomly selected and introduced into the arena and, after 10 minutes, another individual was introduced as the following treatments: nestmate worker (Control), co-specific worker of nest collected in the field (ASS); worker of *A. subterraneus molestans* (ASM); worker of *A. subterraneus brunneus* (ASB); winged queens of *A. ameliae* (AA); and worker of *A. niger* (AN).

We avoided the use of *A. subterraneus subterraneus* workers that were weak, sick or injured in the experiment, because the use of non-healthy individuals may produce inconsistent results (Roulston et al., 2003). Agonistic encounters were observed for 3 minutes and the evaluated behavioral interactions were divided into six different levels of aggression (modified from Suarez et al., 1999 and Velasquez et al., 2006): 0 - ignore; 1 - quick or repeated antennation on another individual; 2 - retreat towards contrary direction after contact; 3 - intimidation by opening the mandibles; 4 - biting and gaster flexing; and 5 - hold/dominate the other individual and try to remove it from the arena. The latency, the time between the release of the stimulus and the first reaction of the workers of *A. subterraneus subterraneus*, was also recorded. Each arena was washed with distilled water and neutral detergent and then wiped with 70% alcohol after each evaluation to remove any substance that could influence the ants' behavior. Ten replicates were conducted for each treatment.

The frequency of each behavior was determined for each treatment and an aggressiveness index (AI) was calculated (Velasquez et al., 2006): $AI = \sum_{i=1}^{5} \frac{OB_i * F_i}{N}$ where OB_i is the observed behavior i, F_i is the frequency of each behavior during three minutes of observation and N is the total number of interactions observed during the period.

Statistical analysis

The difference in total frequencies in each agression level per treatment was determined using the total number of behaviors per treatment. The average proportion of each independent aggression level and the aggressiveness index were compared among treatments using analysis of variance (ANOVA) and the Fisher test (LSD) with the STATISTICA 7.0 software (Statsoft Inc., Tulsa, USA).
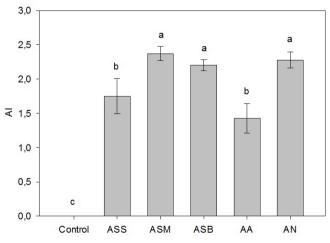

Fig 1. Aggressiveness index (AI) resulting from observed behaviors during interactions among workers of *A. subterraneus subterraneus* and conspecific nestmate workers (Control), conspecifics collected from a colony in the field (ASS), workers of *A. subterraneus molestans* (ASM), *A. subterraneus brunneus* (ASB), queens of *A. ameliae* (AA) or workers of *A. niger* (AN) with their standard errors. Different letters indicate significant differences by *post hoc* Fisher test (LSD).

Results

The aggressiveness index (AI) was higher in encounters between workers of *A. subterraneus subterraneus* and those of *A. subterraneus molestans*, *A. subterraneus brunneus* and *A. niger* (Fig 1) compared to the other treatments. *Acromyrmex subterraneus subterraneus* workers showed only antennation and ignored their nestmates (Table 1).

The workers in control treatment showed agression levels between 0 and 1, while all other treatments showed all aggression levels (Fig 2). Level 0 was more common in the control, followed by treatment with the parasite queen *A. ameliae* (Fig 2). Aggression levels 1, 2 and 5 did not differ among treatments (Fig 2). Levels 3 and 4 were more frequent in treatments with workers of *A. subterraneus brunneus* and *A. niger*, respectively.

Table 1. Observed behaviors during the interactions among workers of *A. subterraneus subterraneus* and conspecific nestmates (Control), conspecifics of colony collected in the field (ASS), workers of *A. subterraneus molestans* (ASM), *A. subterraneus brunneus* (ASB), queens of *A. ameliae* (AA) or workers of *A. niger* (AN).

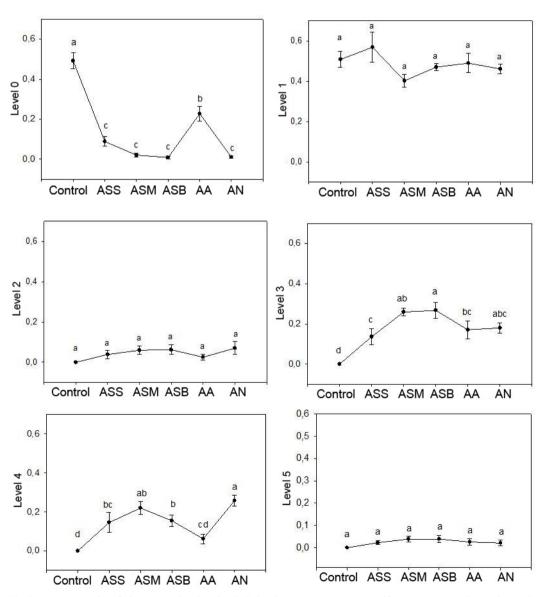| Behavior	Control	ASS	ASM	ASB	AA	AN
Ignore	74	14	6	2	49	2
Antennation	79	103	120	130	107	123
Retreat	0	6	19	16	6	10
Intimidation	0	37	80	79	41	56
Bite	0	28	49	24	13	47
Gaster flexing	0	20	23	20	2	35
Grab/Dominate	0	7	10	8	1	7
Remove	0	0	1	2	5	2
Total	153	215	308	281	224	282

Fig 2. Average ratio of six aggression levels (0-5) in six treatments: conspecific nestmate workers (Control); conspecific from colony collected in the field (ASS), workers of *A. subterraneus molestans* (ASM), *A. subterraneus brunneus* (ASB), queens of *A. ameliae* (AA) or workers of *A. niger* (AN) with their standard errors. Different letters indicate significant differences by *post hoc* Fisher test (LSD).

Discussion

The presence of workers of *A. subterraneus molestans*, *A. subterraneus brunneus* and *A. niger* induced more aggression than the other intruder workers. Workers of *A. subterraneus subterraneus* made distinction between conspecific nestmates and non-nestmates, being more aggressive when in contact with non-nestmates, unlike the observed for *A. subterraneus molestans* (Souza et al., 2006). This is probably related to the ants diet. Colonies of *A. subterraneus subterraneus* maintained in the laboratory and the field had different food sources; this was the condition reported by Souza et al. (2006). Although the diet of colonies of *A. subterraneus subterraneus*, *A. subterraneus molestans*, *A. subterraneus brunneus* and *A. niger* kept in the laboratory was the same, the high aggressiveness of workers on these heteroepecifics is a sign that other factors, such as genetic influence, interfere with the composition of the "colony odor" (Vanzweden et al., 2010; Krasnec & Breed, 2013).

The low aggressiveness of workers of *A. subterraneus subterraneus* when in contact with winged queens of *A. ameliae* differs from that observed for workers of the genus *Temnothorax* (Hymenoptera: Formicidae), which responded more aggressively to the presence of workers of the social parasite *Protomognathus americanus* (Emery) (Hymenoptera: Formicidae) than to conspecifics or heterospecifics (Pamminger et al., 2011; Scharf et al., 2011). The tolerance observed in this experiment may be related to the phenomenon called "chemical insignificance". Just after emergence, immature ants are devoid of cuticular chemicals, acquiring the odor of the colony and integrating to other workers later (Lenoir et al., 1999). The weak signal of young workers allows them to be more easily accepted in other colonies than older workers (Lenoir et al., 2001a), similar to what can have occurred with the queens of *A. ameliae*. Adaptations in the morphology of these insects and the production of similar chemicals are possibly linked to non-aggression towards these queens (Martin et al., 2010; Bauer et al., 2009). Chemical camouflage, which occurs when the social parasite acquires the odor by direct contact with the host when entering the host colony, may also be occurring (Lenoir et al., 2001b). Males of *Bombus vestalis vestalis* (Fourcroy) (Hymenoptera: Apidae) lack the morphological and chemical adaptations that females have to infiltrate the host colony, but they produce a repellent odor that prevents them from being attacked by workers of the host species (Lhomme et al., 2012). It should be investigated if a similar phenomenon occurs with winged queens of *A. ameliae*.

Leaf-cutting ants defend their territories against conspecific and heterospecific intruders, protecting food resources and their offspring. The species *A. subterraneus subterraneus*, A. *subterraneus molestans*, *A. subterraneus brunneus* and *A. niger* co-exist in their natural range, besides having habits that make them potential competitors for feeding resources. On the other hand, the possible similarity of the cuticular chemical profile of different colonies of *A. subterraneus subterraneus* tested and the strategy of the parasite *A. ameliae* to infiltrate in the host colony may explain the lower intensity of aggression suffered by the latter.

Workers of *A. subterraneus subterraneus* are more aggressive when in contact with heterospecifics competitors compared to the co-especifics and the social parasite *A. ameliae*. The mechanisms involved in the reduced aggression towards these treatments have to be evaluated to have a better understanding of the ecological process between competitors and social parasites in these ants.

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