Effect of the presence of brood and fungus on the nest architecture and digging activity of *Acromyrmex subterraneus* Forel (Hymenoptera, Formicidae)

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**A R T I C L E   I N F O**

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**A B S T R A C T**

This study investigated the stimuli that trigger digging behavior in *Acromyrmex subterraneus* during nest building. The hypothesis was that the presence of the fungus garden and/or brood triggers the excavation of tunnels and chambers. For the experiment, the excavation rate of individually marked workers kept in plastic cylinders filled with soil was recorded. Four treatments were applied: (1) 30 medium-sized workers, 5 g fungus garden and 30 brood items (larvae and pupae); (2) 30 medium-sized workers and 5 g fungus garden; (3) 30 medium-sized workers and 30 brood items; (4) 30 medium-sized workers without fungus and brood. After 24 h, morphological parameters of nest structure (length and width of the chambers and tunnels in cm) and the volume of excavated soil were recorded. In contrast to the expected findings, no change in morphological structure, rate of excavation by workers, or volume of excavated soil was observed between treatments, except for tunnel width, which was greater, when no brood or fungus garden was present. Thus, the results do not support the hypothesis that the fungus garden and/or brood are local stimuli for nest excavation or that they mold the internal architecture of the nest. Although this hypothesis was confirmed for *Acromyrmex lundii* and *Atta sexdens rubropilosa*, the same does not apply to *A. subterraneus*. The digging behavior of workers is probably the result of adaptation during nest building in different habitats.

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**Introduction**

The ability of workers of leaf-cutting ants to build nests of complex architecture is an important ecological aspect to be considered (Kleinedam and Roces, 2000; Moreira et al., 2004). The adult nests of leaf-cutting ants of the genus *Acromyrmex*, instead of those of the genus *Atta*, which consist of thousands of underground chambers (Camargo et al., 2013), are often smaller with highly variable number of fungus chamber. In *A. molestans* Santschi, 1925 for example it has been found rural nests with just 1 chamber whereas in the urban area they have up to four chambers (Lopes et al., 2011). In *A. rugosus* (Smith, 1858) the number of chambers ranges from 1 to 26 (Verza et al., 2007) and in *A. landolti* (Forel, 1885) from 1 to 11 (Silva Junior et al., 2013). For all *Acromyrmex* and *Atta* species chambers are connected to each other and to surface by tunnels (Camargo et al., 2013; Silva Junior et al., 2013; Lopes et al., 2011; Verza et al., 2007).

The complete structure of the underground nest from both genera is obtained by the simple digging behavior of workers, which remove soil particles with their mandibles (Cassill et al., 2002). Thus nests of leaf-cutting ants are the result of the coordinated and self-organized activity of specialized workers. Nest building represents a behavioral evolution in an attempt to optimize the protection against predators and vital maintenance of the colony, regulating the entry and exit of gases, temperature and humidity inside the nest and thus ensuring good development of the brood and symbiotic fungus (Bollazzi et al., 2008; Bollazzi and Roces, 2010a, b, c).

During excavation, workers should be involved in the activity in a coordinated manner and should respond actively to stimuli. After commencement of the excavation process, nest mates should be recruited to the digging site. This recruitment is probably
controlled by a positive feedback mechanism, which is activated by pheromones (Pielström and Roces, 2012; Camargo and Forti, 2014). The physical contact between nestmates also increases at digging sites because of the higher density of ants at these sites, a factor that seems to influence and trigger the excavation process with a high density of ants (Römer and Roces, 2015). The social organization of leaf-cutting ants itself produces a context in which workers should respond not only to their individual necessities, but also to the needs of the whole colony. In this respect, workers adjust the size of their nests according to the population size of the colony (Rassé and Deneubourg, 2001), as well as to the volume of the symbiotic fungus (Fröhle and Roces, 2009; Camargo et al., 2013). Also climatic variables, as soil temperature and internal nest humidity, act as short-term regulatory building stimuli for Acromyrmex (Bollazzi and Roces, 2010c).

The investigation of the excavation process provides an understanding of the behavioral patterns that are fundamental for the social organization of leaf-cutting ants. These patterns form an important basis for ecological studies on the behavioral dynamics that regulates the organization of collective activities in eusocial insects.

Camargo and Forti (2014) determined the stimuli for excavation in Atta sexdens rubripilosa (Linnaeus, 1758) and concluded that the presence of brood and symbiotic fungus is an important stimulus for workers to implement tasks involved in nest building, i.e., tasks associated with the protection of the brood and symbiotic fungus. Furthermore, Camargo et al. (2013) concluded that the symbiotic fungus is a blueprint for the construction of tunnels and chambers. However, it remains unknown whether and how much these factors act as stimulus for other leaf-cutting ant species.

The internal complexity and depth of nests vary widely in leaf-cutting ants. Some nests are shallow and others are deeper, and the number of chambers containing fungus varies (Gonçalves, 1961; Lopes et al., 2011). The three subspecies of A. subterraneus (Forel, 1893) build nests that are underground or partially underground in most cases and located near the root system of plants (Bondar, 1923 and Della Lucia, 2011; Gonçalves, 1961; Andrade, 2002). Furthermore, there are descriptions of young nests of Acromyrmex subterraneus brunneus (Forel, 1912), which consist externally of loose soil and internally of a single chamber containing the fungus (Camargo et al., 2004).

To investigate the stimuli that determine nest architecture and the digging activity of workers, the present study evaluated the physical parameters of A. subterraneus nests built under laboratory conditions in which the presence of the fungus and/or brood was manipulated. The premise was that the fungus garden and/or brood are stimuli for excavation and directly influence nest morphometry and worker activity.

**Material and methods**

**Studied species**

Sixteen A. subterraneus colonies maintained since 2012 in the Laboratory of Myrmecology, Universidade Federal de Juiz de Fora (UFJF), Juiz de Fora, Minas Gerais, Brazil, were used. Ant species identification was made according to Forti et al. (2006). The colonies are maintained in a closed system consisting of three compartments interconnected by clear plastic tubing, which correspond to the fungus chamber, foraging arena, and waste chamber. The colonies are maintained under controlled conditions of temperature (26 °C) and humidity (70%) and received Acalypha wilkesiana (Euphorbiaceae) leaves daily.

For the experiments, 30 workers were randomly selected from each colony. All workers selected for the experiment belonged to the medium size class, with a head width ranging from 1.2 to 1.6 mm.

All individuals were marked with a unique color combination on the pronotum to identify each worker. Edging™ markers were used for this purpose because of their excellent adhesion, rapid drying, and good visibility (Camargo et al., 2007). Workers were placed in plastic containers whose borders were covered with neutral talc to prevent escape until drying of the ink, and after 24 h transferred to another container put over the excavation cylinder where they remained until the time of filming.

Portions of the symbiotic fungus were also removed from the same colonies using a fungal mass of 5 g per excavation cylinder. Unmarked workers, which may have been removed together with the fungal portion, were returned to the colony. The larvae and pupae used in the experiment were removed from the same colony. Hence, the excavation cylinders contained elements (workers, symbiotic fungus, and brood) from the same colony.

**Observation cylinders**

Excavation cylinders, each measuring 25 cm in height and 10 cm in diameter, were filled with latosol collected at the campus of UFJF. The soil was removed from a depth of 60 cm and previously sieved. Considering the volume of the excavation cylinder, the soil was weighed to obtain a density of 1.6/cm³ according to Camargo et al. (2011).

A 250-ml plastic container was placed above each cylinder for observation. The container had a hole at the bottom to permit direct contact with the soil to be excavated. A monitoring camera was positioned perpendicular to the container to record the activities of workers for 24 h.

**Stimuli for excavation by workers**

Four treatments, each consisting of four repetitions applied to different colonies, which varied in terms of the stimuli for excavation, were applied:

- Treatment FB: 30 workers, 5 g fungus garden and 30 brood items (larvae or pupae);
- Treatment FG: 30 workers and 5 g fungus garden;
- Treatment LP: 30 workers and 30 brood items (larvae or pupae);
- Treatment WK: 30 workers without fungus garden and brood.

After filming, the excavated soil found above the excavation cylinder was weighed for the determination of wet weight and then dried in an oven (70 °C) for 72 h for the determination of dry weight (g).

**Digging rate by workers**

The first 10 min of each hour were evaluated per repetitions, recording the individual rate of digging activity (transport of the soil pellet).

**Tunnel architecture**

The method of modeling ant nests with cement used by some authors facilitates visualization of the shape of chambers and tunnels and permits to obtain an overall idea of nest architecture (Moreira, 2001). Thus, at the end of the excavation period and filming, the tunnels and chambers were filled with liquid plaster at a plaster–water proportion of 3:2 (Fig. 1). After the plaster had dried, the excavation cylinders were cut with a stylus and the soil was removed, thus obtaining plaster molds of the structure of the excavated nest. These molds were used to measure the length and width...
of the tunnels and chambers and to quantify the number of entrance holes.

Statistical analysis

The effects of the treatments on the architecture of emerging structures and digging activity were evaluated using generalized linear models (GLM). The response variables were wet and dry weight of excavated soil, length and width of the tunnels and chamber, number of tunnels, chambers and entrance holes, and excavation rate. Treatment was considered the explanatory variable. The analyses were performed using the R i386 3.1.1 program (R Core Team, 2015).

Results

Architecture of emerging structures

Nests composed of up to four tunnels were observed, but the number of tunnels did not differ significantly between treatments (GLM: $F = 1.41$; d.f. = 3; $p = 0.19$). The nests that possessed four tunnels were observed in treatment WK, which contained only workers. There was also no significant difference in the number of entrance holes (GLM: $F = 35.04$; d.f. = 3; $p = 0.69$). Only one of the repetitions submitted to treatment LP had seven entrance holes. The length of the tunnels did not differ significantly between treatments (GLM: $F = 28.30$; d.f. = 3; $p = 0.44$). However, the treatments exerted a significant effect on tunnel width (GLM: $F = 539.02$; d.f. = 3; $p = 0.04$), with the observation of wider tunnels in treatment WK (Table 1).

No significant difference in the number of chambers was observed between treatments (GLM: $F = 68.17$; GL = 3; $p = 0.22$), highlighting the occurrence of seven chambers in one repetition of treatment LP. There was also no significant difference in the length (GLM: $F = 52.65$; d.f. = 3; $p = 0.42$) or width of the chambers (GLM: $F = 117.73$; d.f. = 3; $p = 0.43$).

Digging activity

The dry weight of excavated soil did not differ between treatments (GLM: $F = 31.86$; d.f. = 3; $p = 0.35$). The same was observed for the wet weight of excavated soil (GLM: $F = 74.14$; d.f. = 3; $p = 0.26$) (Fig. 2A and B).

Digging activity also did not vary significantly as a function of treatment (GLM: $F = 0.40$; d.f. = 3; $p = 0.74$) or time (GLM: $F = 1.07$; d.f. = 3; $p = 0.37$). This activity appeared to be higher in the first 12 h. Peak activity was observed between the 8th and 12th hour in treatment LP (Fig. 3).

Fig. 1. Plaster mold of a nest excavated by Acromyrmex subterraneus workers. (A) Plaster mold of tunnels; (B) a molded and dried nest ready to be removed; (C) labeled and measured structure.
Table 1
Range of the number of entrance holes, number and size of tunnels and chambers excavated by Acromyrmex subterraneus workers.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Entrance hole (n)</th>
<th>Tunnels (n)</th>
<th>Chambers (n)</th>
<th>Tunnel length (cm)</th>
<th>Tunnel width (cm)</th>
<th>Chamber length (cm)</th>
<th>Chamber width (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FP</td>
<td>1–3</td>
<td>1–3</td>
<td>1–5</td>
<td>6.0–23.0</td>
<td>0.8–1.0</td>
<td>1.0–2.0</td>
<td>1.0–5.0</td>
</tr>
<tr>
<td>FG</td>
<td>1–3</td>
<td>1–3</td>
<td>1–3</td>
<td>2.5–6.0</td>
<td>0.7–2.0</td>
<td>0.7–3.0</td>
<td>0.7–3.0</td>
</tr>
<tr>
<td>LP</td>
<td>1–7</td>
<td>1–2</td>
<td>1–3</td>
<td>3.0–20.0</td>
<td>0.5–1.0</td>
<td>0.5–3.0</td>
<td>0.5–4.5</td>
</tr>
<tr>
<td>WK</td>
<td>1–4</td>
<td>1–4</td>
<td>1–3</td>
<td>1.0–1.5</td>
<td>1.0–1.5</td>
<td>1.5–2.0</td>
<td>1.5–3.3</td>
</tr>
</tbody>
</table>

Discussion

The results showed that the fungus garden or brood did not act as a stimulus for excavation in A. subterraneus. This finding is in contrast to the study of Camargo and Forti (2014) on Atta sexdens rubropilosa, in which the fungus garden and brood served as stimuli for workers to excavate chambers and tunnels.

Although Römer and Roces (2014, 2015) had reported an effect of these stimuli in Acromyrmex lundii, they additionally observed that the workers of this species are able to use available space in the nests instead of excavating new galleries. The same seems to apply to A. subterraneus, considering the similar measurements of nest structure and similar digging activity, regardless of the presence of the fungus or brood. This opportunistic behavior in nest building, in which preexisting empty spaces are used, has been reported for Acromyrmex subterraneus molestans (Lopes et al., 2011).

In a first instance, one may speculate that the fact of keeping the number of individuals constant in all treatments led to a similar rate of excavation in all treatments. The same number of workers permits the same rate of contacts between individuals, with the contact rate acting as a stimulus for workers (Römer and Roces, 2015). In the present study, this factor served as a similar stimulus in all treatments. According to Buhl et al. (2004), both the increase and the decrease in excavation rate are related to the perception of signals by the individuals. This fact has also been analyzed by Pielström and Roces (2012) who suggested stridulation as a trigger to recruit nestmates for digging activity.

The group of 30 individuals used in the experiment was considered sufficient to excavate the nest, with workers digging throughout the 24-h period (Fig. 3). Despite the lack of a significant difference over time, the excavation rate exhibited a more
linear distribution in treatments FB and FG, while the distribution was more exponential in treatment LP, with the observation of a marked increase followed by a decline. The same pattern has been observed by Fröhle and Roces (2009) for Acromyrmex lundii and by Camargo et al. (2013) for Atta sexdens rubropilosa. In contrast, this variation was less pronounced in treatment WK.

A difference could also be observed in the first 12 h (Fig. 2). If the excavation period were shorter, i.e., only 12 h, differences in structure and excavation rate could have been detected since a higher activity is observed in the first 12 h. It is therefore assumed that, during a period of 24 h, the differences became similar for all treatments, i.e., associating both issues, 30 workers were efficient in digging during the observation period.

The architecture of emerging structures did not show significant variations between treatments, with the observation of a similarity of these structures. An exception was observed in treatment WK, which exhibited wider tunnels and could be considered the control of the experiment. The absence of brood and fungus seems to have altered the digging behavior of workers, which built wider tunnels in the absence of other tasks to be performed. This fact is clearly supported by the “foraging-for-work” theory (Tofts, 1993), which proposes that workers of leaf-cutting ants tend to actively search for work. In the absence of stimuli, the ants may have searched for work, intensifying the enlargement of tunnels. From this point of view, it would then become necessary to decide to implement digging as a task due to the absence of other tasks to be performed. In contrast to the proposal of this study, in the situation observed specifically in the control treatment, it appears that the stimulus for excavation was exactly the absence of brood and fungus, in agreement with the theory of Tofts (1993).

With respect to the assumption that brood and fungus trigger excavation in A. subterraneus, no significant differences in the rate of excavation were observed between treatments. However, an increase in the excavation rate was seen in treatment LP, suggesting a role of the brood as a stimulus for excavation. The presence of brood may have exerted a positive effect on worker density at the site, stimulating excavation, since the aggregation of workers at a certain location triggers the excavation process (Römer and Roces, 2015). Workers exert their function to maintain stability and to ensure the success of colony growth. Larvae and pupae represent future workers and an intimate relationship exists between adult workers and their immature siblings in such a way that the former are responsible for the care of the latter, constantly seeking conditions that provide protection and ensure their integrity and full development. The priority would thus be the execution of actions that ensure the success of the future generation. Bollazzi and Roces (2002) add that the brood is always relocated first. Following this line of reasoning, Römer and Roces (2015) identified in a behavioral study that digging activity was more intense at sites containing brood compared to those where the brood was absent. Analyzing the issue from a priority point of view in nest building, the priority would be the execution of actions that ensure the success of the future generation. Although the symbiotic fungus is the only food source of the colony, in this case it does not represent an immediate priority that would justify the investment of energy in nest building, at least during the 24 h evaluated in the present experiment. After brood relocation, the possible next priority step could be the transport of the fungus to guarantee food resources.

In conclusion, the presence of brood or fungus did not serve as a stimulus for excavation, although this behavioral pattern has been observed in other leaf-cutting ants such as Acromyrmex lundii (Römer and Roces, 2015) and Atta sexdens rubropilosa (Camargo and Forti, 2014). This observation highlights the fact that A. subterraneus workers do not adhere to this behavioral pattern, probably because digging behavior is the result of adaptation during nest building in different habitats. However, further studies are needed to answer this question.

Conflicts of interest

The authors declare no conflicts of interest.

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