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Nest architecture development of grass-cutting ants

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\textbf{ABSTRACT}

\textit{Atta bisphaerica} is a species of grass-cutting ants commonly found in the Cerrado biome. The Brazilian Cerrado (savanna) biome covers 2 million km\textsuperscript{2} representing 23\% of the area of the country. It is an ancient biome with rich biodiversity, estimated at 160,000 species of plants, fungi and animals. However, little is known about their nest architecture development. This study investigated the architecture of fourteen \textit{A. bisphaerica} nests from Botucatu, São Paulo, Brazil. Molds were made of the nests by filling them with cement to allow better visualization of internal structures such as chambers and tunnels. After excavation, the depth and dimensions (length, width, and height) of the chambers were measured. As expected, there was a lateral development in the nests and increase in the number of chambers over time. Results showed that in nests with an estimated age of 14 months, the average depth was 1.6 ± 0.4 m; for those with 18 months it was 2.2 ± 0.7 m and at 28 months it was 2.5 ± 0.7 m. The number of chambers varied from 4 to 7 in 28-month nests, 2 to 4 in 18-month nests, and from 2 to 3 in 14-month nests. With respect to the dimensions of the internal tunnels, there were variations in their average width, increasing with time. The fungus chambers were located beneath the largest mound of loose soil. This study contributes to a better understanding of the so far unknown nest architecture development of \textit{A. bisphaerica} grass-cutting ants.

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Introduction

The nests of leaf-cutting ants consist of an externally visible part, a mound of loose soil, which is characterized by having a large number of holes that lead to the internal part, with the latter being made up of tunnels of various diameters and shapes, which allow the traffic of the ants and interconnect holes with chambers. The tunnels that reach the surface of the loose soil of the nest open through holes that are the exits of the landfill tunnels, along which the ants transport the soil removed from their excavations. The foraging tunnels, along which workers cut leaves, open to the outside of the nest in holes (Gonçalves, 1964) usually located outside the limits of the loose soil area at variable distances from the latter.

The mound of loose soil of \textit{Atta} nests is one of the most relevant aspects, since it is a characteristic initially observed in the field for identification of species, as small or large variations may occur depending on the species. According to Mariconi (1970), in a general way, \textit{Atta sexdens}, \textit{Atta laevigata}, \textit{Atta bisphaerica} and \textit{Atta cephalotes} nests present a soil mound only. On the other hand, \textit{Atta capigrua} also shows a certain number of secondary areas.

Although most species present a single mound, there are variations in the form of soil deposition by the ants and in the choice of the site to build these nests. The \textit{A. sexdens} and \textit{A. cephalotes} species build their nests preferably in shady places, and land deposition occurs irregularly, through the formation of small mounds of soil, with the shape of small volcanoes located around the holes above the mound of loose soil. In \textit{A. laevigata}, deposition occurs in a regular way, being concentrated and convex, and nests are constructed in both sunny and shady places (Pereira-da-Silva, 1975; Moreira et al., 2004a).

Normally, the loose soils are on the cluster of chambers. According to Eidmann (1932), Autuori (1942), Jacoby (1950), Mariconi and Paiva Castro (1960), Paiva Castro et al. (1961), Amante (1967), Gonçalves (1967), Pereira-da-Silva (1975), Jonkman (1980a,b), Pretto (1996) and Moreira et al. (2004a,b), \textit{A. bisphaerica}, \textit{A. sexdens}, \textit{A. laevigata} and \textit{Atta vollenweideri} ants build their chambers in the underground, in the projection of loose soil. Therefore, the loose soil is the ant colony's core, while, according to Amante (1967) and Forti (1985), the \textit{A. capigrua} species constructs its nest in a different way – the apparent core does not coincide with the real one, and huge garbage chambers can be found under the apparent core.

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Chambers vary in shape, location and dimensions, depending on the species and in some cases of the role. Studies on A. volleweideri carried out by Bonetto (1959; Carvalho (1976) and Jonkman (1980b) indicated that living chambers had an oval shape with a flat bottom and that active chambers had a conical shape. For A. sexdens, Jacoby (1950) concluded that the chamber structure is simple, varying from a spherical enclosure or an ellipsoid one with closed dome, while Gonçalves (1964) found spherical chambers with a flat bottom.

The chambers of A. sexdens nests studied by Gonçalves (1964) presented diameter between 20 and 40 cm, while Pretto (1996) found chambers with an average height of 18.31 cm, width of 24.25 cm and length of 24.24 cm. For A. capigurara, Mariconi et al. (1961) found small or medium chambers measuring approximately 21 cm in height by 27 cm, 15 cm and 17 cm in width, and 17 cm and 16 cm in length.

The chambers of A. laevigata, in Paiva Castro et al. (1961), were mostly located between 0.50 and 1.80 m deep, while Pereira-da-Silva (1975) found them on the surface (including in the loose soil area) up to 6 m deep, and Moreira et al. (2004a) up to 7 m deep. Thus, depending probably on loose soil area, soil type, species studied and the colony’s micromotic needs, depth may vary.

As for depth of location of the chambers, Bonetto (1959) found chambers from 0.70 to 3.0 m, concentrated in the center of the nest of A. volleweideri. In studies on A. capigurara, Mariconi et al. (1961) observed that the chambers were concentrated between 1.0 and 1.60 m deep. In A. sexdens, Gonçalves (1964) found a larger number of them in the center of the anthill and between 1 and 2 m deep, while Pretto (1996) found only 22% up to 1 m, with the others being below this.

In studies on nest architecture of A. cephalotes, Stahel and Geijsskes (1939) found a total of 373 chambers, of which 344 contained fungus. For A. sexdens, a total of 1027 chambers were found by Autuori (1942), which generated a density of 8.52 chambers m⁻³, while Pretto (1996) obtained densities of 1.75; 2.43, 2.66 and 4.17 chambers m⁻³ for the four nests studied.

The mentioned studies were carried out in adult nests, but little is known about the growth of the colony and, consequently, of the nest. The present study described nest architecture development in three periods: estimated ages of 14, 18 and 28 months of A. bisphaerica grass-cutting ants.

Material and methods

Studied area

The study was conducted at Santana Farm (20°50′46″S; 48°26′2″W), located near UNESP’s Lageado Experimental Farm, Botucatu, São Paulo, Brazil, in a Cerrado fragment.

Age of nests

Two thousand founder queens were observed immediately after the nuptial flight on 4 October 2008 at the Lageado Experimental Farm, FCA/UNESP, Botucatu, Brazil, after, they started to dig their nests, we marked individually all nests with wood white stake. After that, fourteen nests were excavated for architecture study.

Studied nests

Fourteen nests were used in this study – with approximately 14 months (4 nests), eighteen months (5 nests) and twenty-eight months (5 nests) after nest foundation.

The size of the nests was confirmed by mapping two or three active entrance holes near the nests. The holes were mapped using plastic straw baits of different colors and cut types (totaling 48 types of baits).

The straws were cut into 3–4 mm pieces and immersed in a solution of concentrated orange juice containing sugar (forming a syrup). The straw fragments were removed with sieves and transferred to trays where a mixture of citrus pulp and sugarcane leaf powder was added. The pieces were carefully moved inside these trays so the mixture impregnated well on the straws and was highly attractive to the ants. The straws were dried for 24 h at room temperature. After this period, the baits were stored in plastic bags identified with numbers by color and type of straw to facilitate handling in the field.

In the field, the plastic straw baits were placed in the active holes near the nests. The number corresponding to each type of bait was marked on the stake. After 24 h, the mound of loose soil to which each straw was returned by the ants was identified and the type and color found, which indicate which entrance holes belong to each nest, were recorded.

Internal architecture

The nests were molded using a mixture of 5 kg of cement in 10 L of water, which was poured into all open holes above the soil mound of the nest and into the holes spread on the soil surface (foraging holes) as described by Moreira et al. (2004a, b). The water-cement mixture was poured into the holes using aluminum funnels. Excavation started 7 days after application of the cement.

Prior to excavation, the external area of the nest was determined by the traditional method, which consists of measuring the greatest width and greatest length of the area comprising all mounds of loose soil. Two nylon strings were then stretched over the nest area, forming two orthogonal axes (x; y), whose center corresponded to the center of the nest area. These axes were used to identify the chambers and tunnels.

The areas of the nests filled with cement were excavated using small manual tools to avoid their destruction. A 0.70-m wide and 1-m deep ditch was opened around the nest area and excavation was performed from the outside to the inside. The ditch was deepened until complete appearance of the chambers, which were numbered for subsequent measurement. After excavation, the chambers and tunnels were measured and photographed. The following parameters were obtained: dimension (width, height, and length), depth from the soil surface, and position on the orthogonal axes (x, y). The mean, standard deviation and range of the measurements were calculated for statistical analysis. The variables studied were compared between nests at the 3 different ages, by means of ANOVA, at 5% of significance.

Results and discussion

As expected, there was a lateral development in the nests and increase in the number of chambers over time (ANOVA, F2,13 = 16.96, p < 0.001), that the colony growing as a function of time. However, A. bisphaerica nests of different ages did not present major changes in depth (ANOVA, F2,13 = 1.35, p = 0.2973).

Morphologically, the chambers of A. bisphaerica nests studied were located in the projection of the loose soil mound, as already reported by Amante (1967) and Moreira et al. (2004b). Other Atta species also construct their chambers in the projection of loose soil mound, including: A. sexdens (Eidmann, 1932; Autuori, 1942; Jacoby, 1950; Amante, 1967; Gonçalves, 1967; Pretto, 1996), A. volleweideri (Jonkman, 1980b) and A. laevigata (Mariconi and Paiva Castro, 1960; Paiva Castro et al., 1961; Pereira-da-Silva, 1975; Moreira et al., 2004a). However, in colonies of A. capigurara, fungus chambers are located outside the apparent core, distanced and
distributed laterally below ground level, and garbage chambers are located below this core (Amane, 1967; Forti et al., 2017).

The determination of the place of highest concentration of fungus chambers has been a relevant factor in the application of measures for Atta control, because when the limits of loose soil area coincides with the area of highest concentration of chambers in the nest, control is much easier (Moreira et al., 2004a).

About the internal structure of the nests, it was observed that among nests of different ages there was no great variation in depth (Fig. 1, Table 1); however, a lateral growth was observed (Fig. 1), as already reported by Moreira et al. (2004b) in mature nests.

In nests with an estimated age of 14 months, the average depth was 1.6 ± 0.4 m, ranging from 1.2 to 2.1 m, while for those with 18 months, it was 2.2 ± 0.7 m and ranged from 1.4 to 2.9 m. For 18-month nests, the average depth was 2.5 ± 0.7, ranging from 1.3 to 2.9 m (Table 1). As observed in A. bisphaerica, chambers containing fungus garden with freshly incorporated plant fragments are found near the soil surface. This fact suggests an adaptation of the species to withstand variations in temperature and soil humidity, since it usually builds its nests in open areas with plenty of sunlight; even deep soils chambers are not located at great depths. It therefore seems that nest depth is not limited by the ground water table (Moreira et al., 2004b). On the other hand, due to the complexity of the nests, ants choose the best location within the nest in terms of temperature and humidity gradients to rear eggs, larvae and pupae, as well as for the symbiotic fungus and all biota involved (Moreira et al., 2004b).

The nests of A. bisphaerica studied presented chambers of varied forms (Fig. 2), as already observed by Moreira et al. (2004b), Pereira-da-Silva (1975), and Moreira et al. (2004a) for A. laevigata. It was observed, however, that the spherical shape was predominant for the species studied, as concluded by Moreira et al. (2004b) for the same species, and Moreira et al. (2004a) for A. laevigata.

The number of chambers varied from 4 to 7 in 28-month nests, from 2 to 4 in 18-month ones and from 2 to 3 in 14-month nests (Table 1, Fig. 1).

The width, height, and length dimensions of the chambers (Table 2) were variable, showing some differences between nests, already observed by Jonkman (1980b) when analyzing the average height of A. vollenweideri chambers, attributing this difference to colony age, and by Moreira et al. (2004a,b), who observed variations in chamber dimensions between A. laevigata and A. bisphaerica nests. Variations in the dimensions of chambers of the same species may derive from the nest's need to increase fungus cultivation, due to colony growth.

When compared to the dimensions of A. bisphaerica nest chambers studied, variations were found as to average width, height and length. For nests from I to IV, with an approximate age of 14

<table>
<thead>
<tr>
<th>Nest</th>
<th>Estimated age (months)</th>
<th>Chambers (No)</th>
<th>Nest total depth (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>3</td>
<td>1.8</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>2</td>
<td>2.1</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>2</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>3</td>
<td>1.2</td>
<td></td>
</tr>
</tbody>
</table>

Table 1: Number of chambers and total depth of the nests and respective data on mean, mode and standard deviation (S*), maximum and minimum number of chambers by estimated age of Atta bisphaerica nests, Botucatu, SP, 2014.
Table 2
Mode, mean, standard deviation (*S*), and maximum and minimum values of dimensions (width, height and length) of Atta biphylica nest chambers, Botucatu, SP, 2014.

<table>
<thead>
<tr>
<th>Nests</th>
<th>Width (cm)</th>
<th>Height (cm)</th>
<th>Length (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mode</td>
<td>Mean</td>
<td><em>S</em></td>
</tr>
<tr>
<td>I</td>
<td>ne</td>
<td>7.3</td>
<td>1.5</td>
</tr>
<tr>
<td>II</td>
<td>ne</td>
<td>14.0</td>
<td>1.4</td>
</tr>
<tr>
<td>III</td>
<td>ne</td>
<td>10.5</td>
<td>2.1</td>
</tr>
<tr>
<td>IV</td>
<td>8</td>
<td>8.3</td>
<td>0.6</td>
</tr>
<tr>
<td>V</td>
<td>ne</td>
<td>9.0</td>
<td>2.6</td>
</tr>
<tr>
<td>VI</td>
<td>ne</td>
<td>12.7</td>
<td>2.1</td>
</tr>
<tr>
<td>VII</td>
<td>10</td>
<td>10.0</td>
<td>0.0</td>
</tr>
<tr>
<td>VIII</td>
<td>ne</td>
<td>11.0</td>
<td>2.6</td>
</tr>
<tr>
<td>IX</td>
<td>8</td>
<td>10.0</td>
<td>4.7</td>
</tr>
<tr>
<td>X</td>
<td>ne</td>
<td>7.5</td>
<td>3.1</td>
</tr>
<tr>
<td>XI</td>
<td>13</td>
<td>13.3</td>
<td>4.2</td>
</tr>
<tr>
<td>XII</td>
<td>ne</td>
<td>12.9</td>
<td>3.8</td>
</tr>
<tr>
<td>XIII</td>
<td>11</td>
<td>11.8</td>
<td>2.9</td>
</tr>
<tr>
<td>XIV</td>
<td>11</td>
<td>11.6</td>
<td>4.5</td>
</tr>
</tbody>
</table>

*S*, standard deviation; ne, non-existent repeated values.

months, the average width ranged from 7.3 ± 1.5 to 14 ± 1.4 cm; the average height was 8.3 ± 0.6 to 14 ± 1.4 cm, and the average length was 9.3 ± 1.5 to 14.5 ± 2.1 cm. For nests V to IX, with approximate age of 18 months, the average width ranged from 9.0 ± 2.6 to 12.7 ± 2.1 cm, the average height was 9.3 ± 2.1 to 11.5 ± 5.8 cm, and the average length was 11.7 ± 4 to 14.0 ± 1.7 cm. As for nests X to XIV, with an approximate age of 28 months, the average width ranged from 7.5 ± 3.1 to 13.3 ± 4.2 cm, with average height of 9.0 ± 3.9 to 15.4 ± 4.4 cm and average length of 9.5 ± 4.8 to 15.4 ± 4.8 cm (Table 2). However, there was no significant difference in chamber width (ANOVA, *F*₂,₁₃ = 0.24, *p* = 0.7870), height (ANOVA, *F*₂,₁₃ = 0.93, *p* = 0.5772) and length (ANOVA, *F*₂,₁₃ = 0.96, *p* = 0.5881) between the 3 ages studied.

The first chambers of *A. biphylica* were located at small depths (between 13 and 20 cm); possibly, this species does not need very stable conditions of temperature and humidity, since these nests occur in open areas with a lot of insolation and in soils that retain little water. In species that nest in shady areas, such as *A. sexdens*, temperature ranges from 21 to 29 °C and humidity is sometimes around 50% (Eidmann, 1932).

Concerning internal tunnel dimensions, variations were found in average width and height. For nests I to IV, with approximate age of 14 months, the average width ranged from 1.5 ± 0.2 to 1.9 ± 0.6 cm and the average height was 1.4 ± 0.2 to 1.6 ± 0.3 cm. For nests V to IX, with an approximate age of 18 months, the average width varied from 1.6 ± 0.8 to 2.8 ± 1.9 cm and the average height was 1.3 ± 0.2 to 2.6 ± 0.9 cm. As for nests X to XIV, with an approximate age of 28 months, the average width ranged from 3.4 ± 1.7 to 2 ± 0.6 cm and the average height was 1.8 ± 0.3 to 2.6 ± 1.6 cm (Table 3). Significant difference was found as to the width (ANOVA, *F*₂,₁₃ = 13.40, *p* < 0.05) of the tunnels between the different ages studied. The same was not observed for the height of the tunnels (ANOVA, *F*₂,₁₃ = 0.2143, *p* = 0.8117). Probably, this difference found in the width of the tunnels occurred with the growth of the colony and, consequently, of the flow of workers inside the nest.

The measures found by Paiva Castro et al. (1961) for internal tunnels in *A. laevigata* were 3.5 cm wide and 2.8 cm high. Perhaps, the difference in the width of the tunnels between the different species is due to the greater or lesser flow of workers inside them, which are widened with the growth of the nest. In studies on *Atta* nests by the process of cement molding, Jacoby (1938, 1950) detected the existence of several types of tunnels with different purposes. In addition to narrow tunnels that served as communication between larger tunnels, circular tunnels, more or less perpendicular, with 3–4 cm in diameter, were found, as well as elliptical tunnels, usually horizontal, with a diameter of 10–12 cm.

There are some reports on tunnels inside *A. sexdens* nests in Stahel and Geijskes (1939), which found the presence of tunnels descending toward the water table, having a role in the supply of water to the colony. The study also observed the movement of small puffs of smoke injected into holes above the nest, showing the existence of a primitive ventilation system in intact nests. Access to the chambers, as per observations by Bonetto (1959), for *A. volkenweideri*, is possible through tubular galleries of diameter varying between 5 and 8 cm.

Supply tunnels of *A. laevigata* studied by Moreira et al. (2004a), molded with cement in some portions, also had an elliptical section, with average width of 14.12 cm and average height of 2 cm. Maximum and minimum widths and heights were 24 and 3 cm and 9.5 and 1 cm, respectively, with the supply holes interconnected. They reached a single tunnel that went to the area with the highest concentration of chambers, with average depth of 55 cm from ground level, maximum of 73 cm and minimum of 27 cm. It was observed that, as the tunnel approached the royal core of the nest, the depth...
increased, and the nest sometimes reached a depth greater than 1.20 m, a fact observed by Pretto (1996) for tunnels of *A. sexdens*.

**Main remarks**

The nests of *A. bisphaerica* of different ages did not show great changes in depth, probably due to a stability in the temperature and humidity of the soil, propitious for the good development of the colony. A lateral development in the nests and an increase in the number of chambers over time were found, probably due to the growth of the colony (population and fungus garden) as a whole. The width of the tunnels increased over time, probably due to the higher flow of workers.

**Authors contributions**

Conceived and designed the experiments: Luiz Carlos Forti, Aldenise Alves Moreira. Performed the experiments: Aldenise Alves Moreira. Analyzed the data: Luiz Carlos Forti, Aldenise Alves Moreira. Contributed with reagents/materials/analysis tools: Luiz Carlos Forti. Wrote the paper: Luiz Carlos Forti, Roberto da Silva Camargo, Aldenise Alves Moreira, Maria Aparecida Castellani, Nadia Caldato.

**Conflicts of interest**

The authors declare no conflict of interest.

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