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Nest architecture development of grass-cutting ants

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ABSTRACT

Atta bisphaerica is a species of grass-cutting ants commonly found in the Cerrado biome. The Brazilian Cerrado (savanna) biome covers 2 million km 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 *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 *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 *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, *Atta sexdens*, *Atta laevigata*, *Atta bisphaerica* and *Atta cephalotes* nests present a soil mound only. On the other hand, *Atta capiguara* 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 *A. sexdens* and *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 *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), *A. bisphaerica*, *A. sexdens*, *A. laevigata* and *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 *A. capiguara* 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. vollenweideri* carried out by Bonetto (1959), Carvalho (1976) and Jonkman (1980b) indicated that living chambers had an oval shape with a flat bottom and that garbage 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. capiguara*, 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 microclimatic 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. vollenweideri*. In studies on *A. capiguara*, 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 Geijskes (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, $F_{2;13} = 16.96$, $p < 0.001$), that is, the colony growing as a function of time. However, *A. bisphaerica* nests of different ages did not present major changes in depth (ANOVA, $F_{2;13} = 1.35$, $p = 0.2973$).

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. vollenweideri* (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. capiguara*, fungus chambers are located outside the apparent core, distanced and

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