



## Nest Architecture Development of Grass-Cutting Ants, *Atta capiguara* (Hymenoptera: Formicidae)

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### ABSTRACT

*Atta capiguara* grass-cutting ants are commonly found in the Cerrado biome, in open fields. Although grass-cutting ants build giant nests, little has been elucidated about this building pattern and when chambers and tunnels emerge. The present study describes the nest architecture development of *A. capiguara* grass-cutting ants from data on 31 cement-molded nests. *A. capiguara* nests grow with increases in the number of fungus chambers and emergence and increase of waste chambers and foraging tunnels. The structural growth of *A. capiguara* nests in the first year and a half of age (18 months) is vertical, with the building of the first chambers in the soil profile. After 18 months, the nests grow sideways with the addition of chambers and tunnels, and the first waste chambers appear. Between 18 and 54 months, the number of fungus chambers increases from 1-3 to 21-32, and the chambers are concentrated at the soil surface, although they can be found more than 3 m deep. In addition, the total volume of the waste chambers increases with the increment in the fungus chambers volume. Thus, this study contributes to understanding the nest architecture development of *A. capiguara* grass-cutting ants and demonstrates that the total volume of waste chambers is proportional to the total volume of fungus chambers suitable for the colony.

Leaf-cutting ants (Formicidae: Myrmicinae: Attini), besides associating interdependently with the symbiotic fungus, are organized and invest in the rapid growth of the colony (Hölldobler and Wilson, 1990). However, the extraordinary ecological success of leaf-cutting ants is mostly attributed to their great capacity to change or explore the environment for them to build nests (Hölldobler and Wilson, 1990; Passera and Aron, 2005), which protect them from biotic and abiotic factors that affect the survival and development of the colony (Bollazzi et al., 2008).

Among the 17 known species of *Atta* (F.), *A. sexdens*, *A. laevigata*, *A. bisphaerica*, *A. capiguara* and *A. cephalotes* are described and cause economic damages to crops in Brazil (Bolton et al., 2006). *Atta capiguara* (G.), commonly found in the Cerrado biome, is a grass-cutting ant that causes damages to pastures, as well as to rice, maize and sugarcane crops (Mariconi et al., 1961; Forti et al., 2017). Although leaf-cutting ants are distributed in the Neotropical region, *A. capiguara* is present only in South America (Mariconi et al., 1961; Forti et al., 2017).

Few studies about the nest architecture development of *Atta* are available (Autuori, 1941; Forti et al., 2018). In the case of *A. capiguara*,

research on architecture has been conducted only in nests of colonies considered mature (> 5 years of age) (Mariconi et al., 1961; Forti et al., 2017). In the *A. capiguara* nest excavated by Forti et al. (2017), aged around 8 years, 72 fungus chambers and 10 waste chambers were found, most of them connected to the main tunnel, at an average depth of 1.2 meter. In addition to waste chambers, *A. capiguara* builds its nests differently from other species of *Atta*, *A. sexdens*, *A. laevigata*, *A. bisphaerica*, *A. cephalotes*, which fungus chambers grouped outside of the loose soil mound (nest center apparent) (Autuori, 1941; Moreira et al., 2004; Forti et al., 2017).

The nests of leaf-cutting ants are founded by the queen (Hölldobler and Wilson, 1990), which builds the first chamber at a depth of 15-20 cm (Camargo et al., 2011). After worker population growth, workers take on the task of digging new tunnels and chambers (Hölldobler and Wilson, 1990). Therefore, the workers adjust the size of the nest according to the size of the population in the colony (Rasse and Deneubourg, 2001), and the growth of the symbiotic fungus along with the presence of brood stimulates the expansion of the space (Camargo and Forti, 2014; Römer and Roces, 2014, 2015).

Nest architecture is an important behavioral feature and can clarify points of the ant's social organization level since there are few studies on nest building patterns, especially subterranean nests (Guimarães et al.,

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2018). The present study describes the nest architecture development of *A. capiguara* grass-cutting ants from data on 31 cement-molded nests.

This study was conducted at Santana Farm, Botucatu, São Paulo, Brazil (22°50'46" S; 48°26'2" W). The pasture of the farm consists of *Brachiaria* spp., with some spots of *Paspalum* spp., the preferred nesting places of grass-cutting ants. The soil is a Dark Red Latosol of medium texture as described by Moreira et al. (2004). *A. capiguara* nests were mapped after the nuptial flight of October 2008, according to Forti et al. (2018), and with 2, 14-18, 30-36 and 42-54 months of foundation, 8, 11, 9, 3 nests of each age, respectively, were molded for the study.

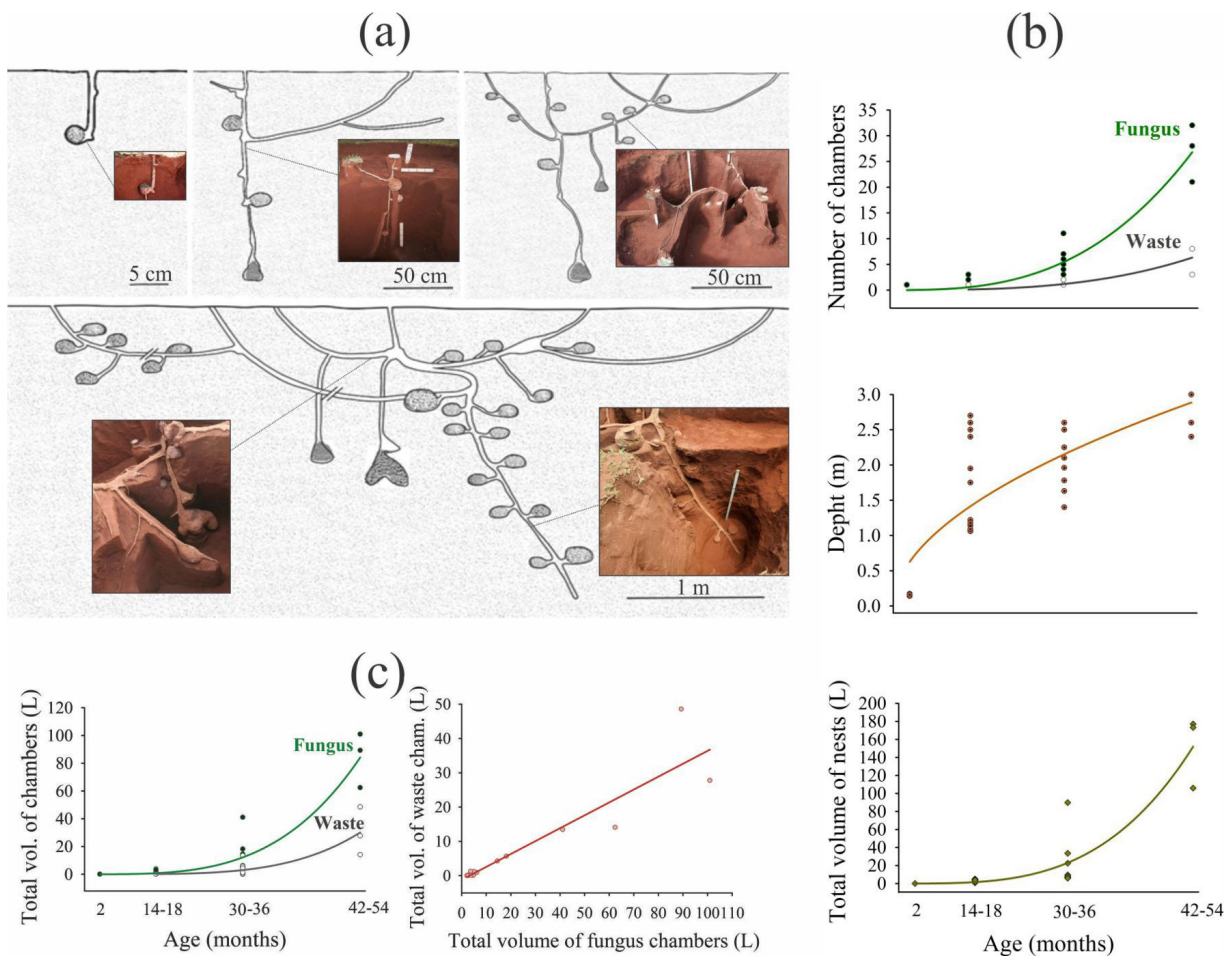
The *A. capiguara* nests were molded with cement, an efficient and economical technique (Moreira et al., 2004; Forti et al., 2017, 2018). The cement was mixed with water, at a proportion of 5 kg of cement to 10 liters of water. Nest excavation was manual, after the cement solidified, and consisted of opening a trench next to the nest, in the region with the smallest number of tunnels, with the said region being widened and dug deeper toward the holes, according to observations made of the chambers and tunnels. The fungus chambers were identified by cylindrical shape and grasses fragments fixed in the cement and waste chambers by conical shape and the presence of degraded and humid material. The fungus and waste chambers were measured for width, height and depth from the soil surface and foraging tunnels measured for height, length and depth.

The volume of the fungus and waste chambers was estimated from the volume of the geometric figures to which they were most similar,

according to Forti et al. (2017). The fungus chambers were compared to cylinders, while the waste chambers were compared to cones and the tunnels to the trunks of the cone. With the high complexity of the nests aged 42-54 months, data on the volume of foraging tunnels were obtained from one nest only.

The number of fungus chambers, number of waste chambers, nest depth, number of foraging tunnels, total fungus volume, waste and foraging tunnel volume were subjected to the Spearman's correlation analyses. Regression analyses using a power function category were performed between the number and total volume of chambers (fungus and waste), nest depth, total volume of nests and age of the nests 2, 14-18, 30-36 and 42-54 months. In addition, regression analysis using a polynomial function category was performed between total fungus chamber volume and total waste chamber volume of nests. Data on measures referring to the chambers and tunnels of nests of different ages were subjected to descriptive statistical analysis for mean and standard deviation values to be obtained. All analyses were performed with Statistica software version 7.0 (StatSoft Inc., 2004).

The molded structures revealed an increase in the number and total volume of chambers (fungus and waste), depth and total volume of nests as the *A. capiguara* nest grew (Fig. 1a, b, c). The increase of the number of fungus chambers with the growth of the nests was correlated significantly with the increment in the number of foraging tunnels, depth, total fungus and waste chamber volume, and total foraging tunnel volume (Table 1). The increase of the number of fungus



**Figure 1** Development of *Atta capiguara* nests (nests with 2, 14-18, 30-36 and 42-54 months) (a); increase in the number of chambers (fungus:  $y = 2.83X^{2.87}$ ,  $R^2 = 0.90$ ,  $P < 0.01$ ; waste:  $y = 3.51X^{3.03}$ ,  $R^2 = 0.74$ ,  $P < 0.01$ ), depth ( $y = 0.35X^{0.53}$ ,  $R^2 = 0.64$ ,  $P < 0.01$ ) and total volume of nests ( $y = 1.95X^{3.40}$ ,  $R^2 = 0.82$ ,  $P < 0.01$ ) as a function of age (b); total volume of chambers (fungus:  $y = 7.73X^{3.48}$ ,  $R^2 = 0.89$ ,  $P < 0.01$ ; waste:  $y = 1.99X^{4.14}$ ,  $R^2 = 0.68$ ,  $P = 0.02$ ) and increase in the volume of waste chambers with increment of the fungus chambers volume ( $y = -1.18 + 0.37X$ ,  $R^2 = 0.84$ ,  $P < 0.01$ ) (c).

chambers and waste chambers over time were not correlated, but the total volume of the waste chambers increases with the increment in volume of the fungus chambers (Table 1 and Fig. 1c).

The number of fungus chambers varied more in the older nests, from 21 to 32 in the nests aged 42-54 months, 2 to 11 in nests aged 30-36 months, while the younger ones, 14-18 months, had 1-3 chambers, and the nests with 2 months had only 1 chamber. Waste chambers were found in nests with 18 months, but only in 2 of 6 nests with this age (Fig. 1a). Foraging tunnels were found in nests with 30-36 and 42-54 months. The averages of height, width and volume of chambers and averages of height, length and volume of foraging tunnels are in Table 2.

The molded structures revealed that, as in other species of ants, *A. capiguara* nest size increases as the colonies grow, with effect mainly on the number of chambers and on nest depth as the number of workers increment (Fig. 1a) (Tschinkel, 2015). The nests grow with the addition of chambers (fungus and waste chambers, in the case of the grass-cutting ants of this study), as well as changes in the shape and volume of these structures (Fig. 1b, c) (Tschinkel, 2015). The building of *A. capiguara* fungus chambers at different depth in the soil is attributed to condition around 25 °C for symbiont fungus, eggs, larvae and pupae growth (Fig. 1a, b) (Camargo and Forti, 2014; Römer and Roces, 2014, 2015).

In the first year of *A. capiguara* nests, the structural growth is vertical, without lateral expansion of chambers and tunnels (Fig. 1a). After 18 months the nests grow laterally by the building of more chambers and tunnels (Fig. 1a). This growth pattern in young nests also was observed in *A. sexdens* (Camargo and Forti, 2013). Although the greatest number of fungus chambers for *A. capiguara* is concentrated at the soil surface (0-2 m), the chambers can be found more than 3 meters of depth (Fig. 1a, b). This information contributes to the establishment of the ideal method for grass-cutting ants controlling (e.g., the application of formicides in dry powder formulation in colonies aged more than one year reaches only a few chambers and ants on the soil surface and therefore the control is inefficient). Thus, the control by toxic baits is efficient because ants transport and uniformly distribute the baits inside in the colony (Moreira et al., 2003).

In this study, the variation in the dimensions of the *A. capiguara* fungus chambers is attributed to the symbiont fungus that acts as a mold and determines the final chamber size (Fröhle and Roces, 2009) (Table 2). Workers dig around the reallocated fungus, thus enlarging the initial size of the chamber to accommodate the total amount of fungus grown (Römer and Roces, 2014). However, there seems to be a maximum size for the chambers, so, at a certain point, new chambers need to be dug (Römer and Roces, 2014). The tunnel's dimensions vary with nest age, probably because of the greater flow of workers (Table 2) (Forti et al., 2018). In addition to interconnecting chambers and the environment with the nest, tunnels are of paramount importance in gas diffusion (Bollazzi et al., 2012).

Some species of leaf-cutting ants as *A. capiguara* build chambers specifically for waste disposal. Studies have established that the waste of *Atta* and *Acromyrmex* (M.) colonies concentrate species of the *Escovopsis* pathogenic fungus, which is harmful to the symbiont fungus garden and causes the mortality of ants (Currie et al., 1999; Bot et al., 2001). Thus, waste chambers are sectorized and isolated from the fungus chambers, showing the importance of separating residues to prevent contamination (Forti et al., 2017). Unlike most ants of the *Atta* genus, *Atta colombica* (G. Meneville) ants do not deposit waste inside the nest but need around 11% of all workers to execute this task outside the nest (Hart and Ratnieks, 2002; Farji-Brener et al., 2016). In this study, the formation of waste chambers was observed in nests aged 18 months (Fig. 1a). Most likely, due to the complexity of the structure, *A. capiguara* prefers to build waste chambers over recruiting a larger number of workers to deposit waste outside the nest. Another hypothesis is that, although the construction of waste chambers is influenced by phylogeny (close species, *A. sexdens*, *A. robusta*, *A. bisphaerica*, *A. capiguara*, *A. laevigata*, *A. saltensis* and *A. vollenweideri*, build waste chambers), the habitat of ants is the main fact that determines waste site (Farji-Brener et al., 2016). In environmental conditions that are harmful to pathogenic organisms, that is, desert habitats, leaf-cutting ants usually avoid excavation costs, with minimal sanitary risk when depositing waste outside the nest. On the other hand, humid habitats such as tropical and subtropical forests or Cerrado provide suitable conditions for pathogen proliferation (Farji-Brener et al., 2016).

**Table 1**

Matrix based on Spearman's correlation between the number of fungus chambers (FC), number of waste chambers (WC), number of foraging tunnels (FT), nest depth (ND), total fungus chamber volume (FCV), total waste chamber volume (WCV), foraging tunnel volume (FTV) and total nest volume (NV) along the development of *Atta capiguara* nests, in Botucatu, SP.

	FC	WC	FT	ND	FCV	WCV	FTV	NV
FC	-	0.541	0.722*	0.807**	0.846**	0.564	0.916**	0.860**
WC	-	-	0.502	0.530*	0.794**	0.674*	0.358	0.794*
FT	-	-	-	0.433	0.689*	0.688*	0.866**	0.688**
ND	-	-	-	-	0.832**	0.414	0.334	0.622*
FCV	-	-	-	-	-	0.845**	0.667*	0.995**
WCV	-	-	-	-	-	-	0.619	0.873**
FTV	-	-	-	-	-	-	-	0.667**

\* Show significant difference at  $P < 0.05$ ; \*\* indicate significant difference at  $P < 0.01$ .

**Table 2**

Dimensions of *Atta capiguara* chambers and tunnels along the nests architecture development.

Age (months)	Fungus chambers							Waste chambers							Foraging tunnels						
	Height (cm)		Width (cm)		Volume (L)		n*	Height (cm)		Width (cm)		Volume (L)		n*	Height (cm)		Length (m)		Volume (L)		n*
	Mean	SD**	Mean	SD	Mean	SD		Mean	SD	Mean	SD	Mean	SD		Mean	SD	Mean	SD	Mean	SD	
2	3.29	0.26	4.11	0.17	0.04	0.005	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14-18	9.81	2.70	10.02	2.05	0.85	0.56	26	11.50	0.71	11.50	4.95	0.07	0.05	2	-	-	-	-	-	-	-
30-36	12.32	4.66	13.20	5.02	2.24	2.89	44	21.60	15.73	18.84	11.19	0.63	2.72	10	1.60	0.28	0.95	1.04	0.28	0.17	5
42-54	15.23	3.40	16.00	3.88	3.10	2.64	57	27.11	12.37	25.11	16.60	4.74	3.80	9	1.69	0.29	1.93	1.46	2.18	1.86	14

\*n: number of chambers or tunnels found; \*\* SD: Standard Deviation.



The relationship between the total waste chambers volume increment with the increase in total fungus chambers volume in *A. capiguara* indicates an adjustment according to the need of the colony (Fig. 1c). The adjustment of the number or volume of waste chambers according to fungus required by colonies was suggested by Jonkman (1980). This author observed waste chambers empty in nests excavated without cement, suggesting that each chamber is built before he is essential.

The structural growth of *A. capiguara* nests in the first year and a half of age (18 months) is vertical, probably due to the microclimatic demands for the symbiont fungus and worker young/immature forms. After 18 months of age, nests grow laterally by the addition of fungus chambers and tunnels, and the first waste chambers are found, certainly because of the rapid growth of the colony in population and cultivated fungus. Between 18 and 54 months, the number of fungus chambers increases from 1-3 to 32, and the chambers are concentrated at the soil surface, although they can be found more than 3 m deep. This information also contributes to the establishment of the ideal method for grass-cutting ants controlling. Besides, the total volume of waste chambers is proportional to the total volume of fungus chambers suitable for the colony.

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### Conflicts of interest

The authors declare that they have no conflict of interest.

### Author contribution statement

APF, RSC, NC and LCF conceived/designed the research. APF, NC and LCF conducted the experiments. LCF contributed with new materials and/or analytical tools. APF and RSC analyzed the data. APF wrote the manuscript. All authors read, corrected and approved the manuscript.

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