1 Title: Longitudinal study of foraging networks in the grass-cutting ant *Atta capiguara* Gonçalves,
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15 Running title: Foraging networks of the ant *Atta capiguara*

Abstract

Colonies of leaf-cutting ants of the genus *Atta* need to collect large quantities of vegetal substrate in their environment to ensure their growth. They do so by building and extending over time a foraging network that consists of several underground tunnels extending above ground by physical trails. This paper presents a longitudinal study of the foraging network of two mature colonies of the grass-cutting ant *Atta capiguara* (Gonçalves) located in a pasture in central Brazil. Specifically, we investigated whether the extension of the foraging area of the colonies required to reach new resources occurs by building new and longer underground tunnels or by building new and longer physical trails. Each nest was surveyed at intervals of approximately 15 days during one year. At each survey we mapped the position of the tunnel entrances and foraging trails at which activity was observed. In addition, we assessed the excavation effort of the colonies since the last survey by the number and distance to the nest of new tunnel entrances, and the physical trail construction effort by the number and length of newly built physical trails. Our study reveals that in *A. capiguara* the collection of new resources around the nest required to ensure the continuous growth of the colonies is achieved mainly through the excavation of new underground tunnels, opening at greater distance from the nest, not through the building of longer aboveground physical trails.

Keywords: formicidae, pasture, tropical, Brazil

Introduction

Ant foraging trails are a notable example of transportation networks (Perna & Latty 2014). In some species (*Formica polystena* (Förster): Rosengren 1971, *Iridomyrmex purpureus* (Smith):

Cabanes *et al* 2015, *Messor barbarus* (L.): Lopez *et al* 1994, Plowes *et al* 2013, *Atta spp.*: Vasconcelos 1990, Wirth *et al* 2003, Kost *et al* 2005, Lopes *et al* 2016, Silva *et al* 2013) foraging workers build long-lasting conspicuous trails, called physical trails, that lead them from their nest directly to the location of the resources they exploit (Anderson & McShea 2001, Silva *et al* 2013). Ants act as true ecosystem engineers (Cuddington *et al* 2007) by modifying the environment through the cutting of the vegetation along these trails and the removal of the small obstacles that impede their locomotion (Howard 2001, Cevallos Dupuis & Harrison 2016, Bochynek *et al* 2016, 2019, Middleton *et al* 2019). These trails can be followed on the ground even in absence of ants on them and they can be maintained for periods of time that can extend to several years in some ant species (Rosengren 1971, Bochynek *et al* 2016).

Physical trails can have several functions for ant colonies. First, they offer a smooth substrate and thus allow ants to move faster from the food locations to their nest, to have a higher transport efficiency and to increase their food delivery rate (Sales *et al* 2015, Bouchebti *et al* 2018). Second, they allow colonies to share and gather information rapidly on the resources available in the environment (Shepherd 1982, Farji-Brener & Sierra 1998, Dussutour *et al* 2007, Farji-Brener *et al* 2010, Bouchebti *et al* 2015a). Third, physical trails can be considered as a “physical memory” of resource locations (Fowler & Stiles 1980, Rockwood & Hubell 1987, Wirth *et al* 2003, Kost *et al* 2005) that facilitates resource monitoring. And fourth, physical trails partition space between neighbouring colonies and thus reduce the effect of competition (Hölldobler & Lumsden 1980, Vilela & Howse 1986, Wirth *et al* 2003).

Physical trail networks typically are formed by the successive branching of foraging trails in most species of ants (Hölldobler & Möglich 1980, Buhl *et al* 2009, Silva *et al* 2013). However, the geometry of these networks and the persistence of the trails vary within and

between species according to the characteristics of the environment and the type of food collected (Carroll & Janzen 1973). For example, the seed-harvesting ant *Messor barbarus* adopts a “phalanx” strategy in areas of high resource density in which it builds networks with a high rate of trail bifurcations whereas in areas of low resource density it adopts a “guerilla” strategy with longer and less branching trails (Lopez *et al* 1993, 1994). The geometry of the trail networks also depends on the density of the vegetation, with branching angles at bifurcations being more acute in open areas with low vegetation density than in close areas with high vegetation density (Acosta *et al* 1993, Farji-Brener *et al* 2015). As for the persistence of the physical trails, it can vary according to the type of resource collected. For example, in the grass-cutting ant *Atta bisphaerica* (Forel) which exploits small and ephemeral patches of grass, most physical trails last only a few days (Lopes *et al* 2016). On the other hand, when the resources are stable or regularly renewed, e.g. colonies of Homoptera producing honeydew exploited by red wood ants or plants that are regularly defoliated by leaf-cutting ants, physical trails are generally highly persistent and the geometry of the trail networks show little change for long periods of time (Chauvin 1962, Rockwood & Hubell 1987, Kost *et al* 2005).

Longitudinal studies of foraging trail networks are relatively scarce in the ant literature (*Formica rufa* (L.): Skinner 1980, *Iridomyrmex purpureus*: Cabanes *et al* 2015; *Atta* spp.: Vasconcelos 1990, Kost *et al* 2005, Silva *et al* 2013, Lopes *et al* 2016). Yet, these studies allow for a better understanding of the interactions between resource availability, the growth of the colonies, the changes in meteorological conditions or in the environment surrounding the nest and the geometry of the foraging networks. Here, we present a longitudinal study of the geometry of the physical trail networks of the grass-cutting ant *Atta capiguara* which is frequently found in the pastures of the southern part of Brazil (Forti 1985, Fowler *et al* 1986,

Delabie *et al* 2011). As other species of ants of the genus *Atta* (*A. sexdens* (L.): Vasconcelos, 1990; *A. bisphaerica*: Moreira *et al.*, 2004, Lopes *et al.*, 2016; *A. laevigata* (Smith): Moreira *et al.*, 2004), *A. capiguara* builds underground tunnels that depart from their nest chambers, open to the outdoor environment at some distance from their nest and extend above ground to reach distant foraging grounds.

During a 12-month period we mapped the foraging network of two mature nests at intervals of approximately two weeks and monitored ant activity on the trails and around the tunnel entrances. First, we investigated the spatiotemporal dynamics of the trail networks and the way ants distribute their foraging effort around their nests and tunnel entrances during the monitoring period. Second, we investigated whether the extension of the foraging area of the colonies we observed occurred through the excavation of more underground tunnels, opening at greater distance from the nest, or through the building of more and longer physical trails, starting from existing tunnel entrances.

Material and Methods

Data collection was carried out during one year from November 2011 to October 2012 in a pasture area at Santana Farm, located in the city of Botucatu– SP (22°53'09" S; 48°26'42" W). The pasture consisted mainly of *Brachiaria decumbens* with spots of *Paspalum notatum*.

Two nests of *A. capiguara* were selected for our observation. Both nests had already produced alates. They were thus at least 3 years old (Autuori 1941) and were considered as mature. The size of the nests were estimated by measuring the area covered by loose soil on top of the nests. At the beginning of the monitoring period this measured area (estimated by the

product of its largest length by its largest width, according to the method used by Forti *et al* 2017) was 34.31 and 8.4 m² while at the end it was 228.00 and 113.70m², for Nest 1 and Nest 2 respectively, representing an increase by a factor of 6.6 and 13.5. Nest 1 was located in the middle of the pasture while Nest 2 was at approximately 20m from one of its edges and at a distance of about 20m from Nest 1.

For the mapping of foraging activities two 40x40m grids centered on each nest with stakes placed at 10 meter intervals over the grid were used. Each nest was surveyed at intervals of 15 days, always in the late afternoon, corresponding to the peak of foraging activity in *A. capiguara*, whatever the season (Caldato *et al* 2016). At the beginning of each survey, the area around each nest was inspected to find out whether new tunnel entrances had been excavated and new foraging trails constructed since the last survey.

To check whether the new trails really belonged to the studied nests, we used a variation of the method developed by Fowler *et al* (1993). Small acrylic particles of various colors, measuring approximately 0.7cm in length, were immersed into a water and sugarcane molasses (3:1) solution and then impregnated with sugarcane leaf powder. These particles were then distributed near the edges of the trails, with different colors used for each trail. After a period of 24 hours, we checked for the presence of the particles on the top of loose soil over the studied nests, confirming the trails as belonging to the nest.

The tunnel entrances were categorized as open with dispersed foraging activity around by isolated workers but with no visible foraging trails departing from them, open with one or several foraging trails departing from them with ant traffic, open but inactive (without ant foraging activity) or closed (when the entrance hole's opening was no longer visible). Finally, the positions of new entrance holes and of new trail ends were measured from the distance of the

two nearest stakes in the grid and then mapped at scale on a graph using the software CorelDraw X3.

Since we did not have any expectation as to what could be the trend followed by the change over time in the distance of the tunnel entrances to the nest and in the length of the foraging trails, we used a General Additive Model (GAM) (Zuur *et al*, 2009) with a gaussian error distribution and a cubic regression spline. The nest identity was entered as a fixed variable and the time series corresponding to each nest were allowed to have a different residual spread so that we could investigate whether there are differences between the two nests in the temporal trend of the distance of the tunnel entrances to the nest and in the length of the foraging trails. For each variable studied, to take into account the correlation between successive values in the time series, we added to the GAMs a correction term implementing a correlation structure in the residuals corresponding to an auto-regression of order 1, i.e. the simplest form of temporal correlation in which a value at time t in a time series depends only on the value at time $t-1$ (Zuur *et al* 2009). The nest identity was nested in the time variable so that the auto-correlation was applied at the level of each nest. The models were fitted with the *mgcv* R package (Wood 2017). Model validation was carried out by plotting the model residuals vs fitted values and vs time and by checking the normality of the residual distribution with a *qqplot*. All analyses and figures were done with R 3.4.3 software run under RStudio version 1.0.153.

Results

We divide the result section into two parts. The first part deals with the distribution of the foraging effort around the nests and around the tunnel entrances over the monitoring

period while the second part deals with the evolution of both the excavation effort of the colonies and their effort in building new physical trails over the monitoring period.

Distribution of foraging efforts

The foraging activity of nest 1 was homogeneously distributed around the nest during the first half of the monitoring period (Fig 1a) while that of nest 2 was concentrated in the eastern sector (Fig 1b). During the second half of the monitoring period the foraging activity of nest 1 was concentrated in the northwestern sector (Fig 1a) while that of nest 2 shifted to the southwest sector (Fig 1b).

The majority of foraging trails were built from already opened tunnel entrances so that several foraging trails were used successively over time at most tunnel entrances (Fig 1). In both nests most foraging trails extended away from the nest, more or less in the continuation of a straight line linking the nest location to the tunnel entrance.

More than half of the trails were active during one survey only and thus had a lifetime of less than 15 days (Fig 2) and among these trails 86% were created during the first half of the monitoring period, i.e. during the months with the highest rainfall. Two trails had a lifetime of more than 10 months. There was no correlation between the lifetime of the trails and their length (Spearman's rank correlation: $\rho = -0.06$, $P = 0.58$).

To investigate whether the foraging effort of ants was distributed randomly around the tunnel entrances or whether it was oriented in specific directions we analysed the geometry of the trail network of the two colonies.

First, we computed the distribution of the angles between the direction of the line joining the location of the nest and each tunnel entrance and the direction of the line joining the location

of the tunnel entrance and the end of each trail departing from this entrance (angle α in the inset of Fig 1b). We found that this distribution was approximately centered on 0° (mean $\pm$ SD: $17 \pm 91^\circ$, comparison of the mean of the angle distribution with 0° : t test= 1.712, $P= 0.09$, $N= 87$) and therefore that most trails extended in the continuation of the tunnel from which they originated. Second, we investigated whether ants build their trails in a random direction from the tunnel entrance or whether they build them so as to avoid reaching locations that are at a closer distance from a tunnel entrance already open. We found that for only 24% of the new foraging trails built by the two colonies studied, the end point of the trails were closer to a tunnel entrance already open than to the tunnel entrance from which they originated.

To investigate whether the same proportion would be found if ants were building their trails in a random direction, we ran a simulation in which the direction of the trails (with the same length as observed trails) from each tunnel entrance was picked randomly from a normal distribution centered on the direction of the line joining the tunnel entrance to the nest location and with a standard deviation corresponding to that calculated for the observed networks, i.e. 91° (see above). We ran the simulation 200 times and calculated for all runs of the simulations the average percentage of trails whose end point was closer to a tunnel entrance already open. The value we found was 46%, thus almost the double than the value calculated for the observed networks. This means therefore that ants were not building their trails in random directions and that in most cases the patches of grass they exploited could not be reached by a shorter trail built from a tunnel entrance already open. This resulted in a reduced overlap of the space exploited around each tunnel entrance and in a better partition of the foraging space at the level of the colonies, with the flow of ants from each tunnel entrance directed towards different locations.

Excavation and physical trail construction effort

Over the 12 month monitoring period a total of 36 tunnel entrances (24 for Nest 1 and 12 for Nest 2) were opened by the two studied colonies. The number of newly opened tunnel entrances increased continuously for both nests during the monitoring period, with phase of growth alternating with phase of stasis (Fig 3). The increase in the number of new tunnel entrances in nest 1 was steeper from November to April, when resources are plentiful, than from May to October, corresponding to dry season. However, there was no significant correlation in the monthly number of new tunnel entrances created and the cumulated monthly rainfall (Fig 4) (Spearman's rank correlation: $\rho = 0.47$, $P = 0.13$ and $\rho = 0.29$, $P = 0.36$ for nest 1 and nest 2, respectively). The overall increase in the number of new tunnel entrances was much greater for nest 1 than for nest 2, suggesting that the growth of the underground tunnel network occurs differently in the two nests, probably because of their difference in size. The mean distance of new tunnel entrances to the location of the nest increased over time in the same manner in both nests over the monitoring period (Fig 5; GAM, $df = 1$, $F = 15.22$, $P < 0.001$, $R^2 = 0.29$), suggesting an extension of the network of underground tunnels.

In both nests most tunnel entrances closed or became inactive after one to two months of activity (Fig 6). At some of the entrances that remained open, workers were observed removing soil particles, suggesting the excavation of new chambers within the nest. Note that some tunnel entrances closed and then reopened and became active again a few weeks later (e.g. entrance 4, 9, 12 of nest 1 in Fig 6).

A total of 87 physical foraging trails were built from the tunnel entrances in both nests during the monitoring period, 58 for nest 1, and 29 for nest 2. The mean length of newly built physical trails was longer for nest 1 compared to nest 2 (Fig. 7; GAM: $df = 1$, $F = 14.16$, $P < 0.001$,

$R^2= 0.17$) but in both nests it did not vary much over the monitoring period (GAM: $df= 3.42$, $F= 1.27$, $P=0.315$).

Discussion

Our study shows that the extension of the foraging area in *A. capiguara* and the shift to new locations at which vegetation is collected occurs mainly through the construction of new underground tunnels, opening at greater distance from the nest, not through the continuation of existing physical trails or the building of new and longer physical trails from existing tunnel entrances. In fact, while the distance from the nest of newly excavated tunnel entrances increased over time in both nests at each survey (Fig 5), the length of newly built physical trails remain approximately the same (Fig 7). Although more costly to build for the colonies, underground tunnels offer a better protection to the ants against adverse abiotic conditions (Bouchebti *et al*, 2015) than aboveground physical foraging trails. In addition they can also be used for longer periods of time.

Leaf-cutting ant colonies generally have an accelerated growth rate during the first three years after their foundation (Hernandez *et al* 1999, Grandeza *et al* 1999). After producing alates in their third year they then grow at a lower and steady rate. There are two ways ants can extend the foraging area of their colony. They can either stop excavating underground tunnels and build more and longer foraging trails from already existing tunnel entrances, or they can build more and longer tunnels and increase (or not) the number and the length of the foraging trails departing from these tunnels. Our observations show that in *A. capiguara*, contrary to what has been described in the species *A. sexdens* and *A. cephalotes* (L.) (Vasconcelos 1990), the extension of the foraging area is mainly achieved through the excavation of new underground tunnels, opening at greater distance from the nest, not through the building of longer foraging

trails. Although the cost of excavating longer tunnels is likely to be much higher than that of building physical foraging trails, the tunnels considerably reduce exposure to high temperatures and solar radiation which occurs both during the construction process of the physical trails (which can take minimally 5 to 6 days for physical foraging trails: Bouchebti *et al* 2018) and when travelling on these trails to exploit resources. They can also act as a thermal refuge in which the workers can find temporary protection against high outdoor temperatures (Bouchebti *et al* 2015b). Moreover, as noted by Vasconcelos (1990), their cost of maintenance is lower than that of foraging trails which is not negligible (Howard 2001, Bochynek *et al* 2016). While most foraging trails are used for short periods of time and then abandoned altogether, underground tunnels can be left unused for long periods of time and then rapidly reactivated to shift the location of the foraging activity of the colonies in order to exploit new patches of vegetation. Although the two *A. capiguara* nests we studied were about the same age and were located in the same pasture and thus submitted to the same meteorological conditions, the growth of their foraging network was different. This could be due to a variety of reasons, e.g. a heterogeneity in the availability of the resources offered by the pasture and/or differences in the productivity of the queens.

Similar to what has been observed in mature colonies of *A. bisphaerica* (Moreira *et al* 2004, Lopes *et al* 2016), *A. sexdens* (Vasconcelos 1990) and *A. laevigata* (Reed & Cherrett 1990) and contrary to what has been observed in mature colonies of *A. colombica* (Guérin-Méneville) (Wirth *et al* 2003) and *A. cephalotes* (Vasconcelos 1990, Silva *et al* 2013), none of the foraging trails of the two nests of *A. capiguara* studied departed directly from the heap of loose soil over the nests. All trails departed from underground tunnels whose entrance was at a distance of several meters from the nest. Therefore, in *A. capiguara*, as in most leaf-cutting ant

species (Shepherd 1982, Vasconcelos 1990, Reed & Cherrett 1990, Farji-Brener & Sierra 1998, Bouchebti *et al* 2018), foraging is centered on the trail system: the scouts depart directly from the tunnel entrances or from the foraging trails and the search for new resources is thus concentrated in the area close to these structures. Consequently, a large part of the foraging area in-between foraging trails remains unexploited (Vasconcelos 1990, Wirth *et al* 2003, Kost *et al* 2005, Lopes *et al* 2016).

The foraging trails of the two nests of *A. capiguara* studied were relatively short and almost never bifurcated (see also Forti 1985). This is at variance with what has been found in most leaf-cutting ants of the genus *Atta* (*A. cephalotes*, *A. colombica*, *A. sexdens*: Shepherd 1982, Vasconcelos 1990, Kost *et al* 2005) but similar to what has been observed in the grass-cutting ant *A. bisphaerica* (Lopes *et al* 2016). These differences in the organization and geometry of the foraging networks may be linked to differences in the type of environment in which ants of the genus *Atta* are found (e.g. close vs. open environment), in the spatio-temporal distribution of the resources collected (grasses or leaves), and/or in the resistance of the workers to high outdoor temperatures (Bouchebti *et al* 2015b). In *A. capiguara* a trail is built to exploit only one single patch of grass while in other species of *Atta*, a single trail can be used to exploit various plant units (Fowler *et al* 1986). Moreover, our analysis of the trail network shows that the trails were built so as to reduce the overlap in the space exploited around each tunnel entrance. The mechanism by which this process emerges remains to be investigated.

Throughout the 12-month monitoring period we observed a continuous increase of the number of newly opened tunnel entrances in both nests studied, suggesting an extension of the networks of underground tunnels. Excavation effort then slowed down from April on. Since there was no correlation between the monthly number of new tunnel entrances and the cumulated

monthly rainfall, this was probably not due to a hardening of the soil. Rather, this could be linked to a decrease in the amount of biomass collected (Caldato *et al* 2016) due to a reduction of the resource available to the colonies in the dry months of the year. Nevertheless, the number of physical trails with foraging activity did not vary much throughout the year. This is explained by the fact that ants kept using or reactivating the entrance holes of the tunnels that were already built. Ants also took advantage of the existing tunnels by building several physical trails in different directions from the same entrances. Finally, since the growth of the vegetation is linked to rainfall and thus slows down in the dry season, physical foraging trails should require less maintenance in the dry season and thus can be used for longer periods of time. Similar conclusions have been reached by Lopes *et al* (2016) in the leaf-cutting ant *A. bisphaerica*.

During the dry months of the year, characterized in Botucatu by higher air temperature and lower relative humidity (Caldato *et al* 2016), ants concentrated their foraging activity in a particular angular sector around their nests, whereas during the wet months their foraging activity was more homogeneously distributed, particularly for nest 1. This is concordant with the observations of Kost *et al* (2005) who found that the fractal dimension of the foraging trail networks, i.e. the area covered by the network, was higher in the wet season than in the dry season in mature colonies of the leaf-cutting ant *A. colombica*. Ants may take more time to exploit the same patch of grass during the dry months than during the wet months of the year because of the scarcity of palatable grass blades. This is indeed suggested by the lower proportion of ants carrying vegetation in nestbound traffic at high temperatures (Caldato *et al* 2016).

Overall, our study highlights the fact that, in the same way as in the grass-cutting ant *A. bisphaerica* (Lopes *et al* 2016), the extension of the foraging network required to ensure the

continuous provisioning of the colonies in the grass-cutting ant *A. capiguara* is achieved mainly through the excavation of new and longer underground tunnels. Once they exit these tunnels, ants only have to travel a mean distance of about 5 meters in order to reach the patches of grass. This could contribute to minimizing their exposure to predators, parasites or adverse abiotic conditions.

Authors' contributions: all authors contributed fully or partly to the conception and design of the work presented, to the acquisition and analysis of the data and to the writing of the manuscript.

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Fig 1: Map of the physical foraging trails built by (a) nest 1 and (b) nest 2. The black solid lines are the trails active in the first half of the monitoring period, i.e. from November 2011 to April 2012, the black dashed lines are those active in the second half and the grey lines are those active in both periods. The points represent the tunnel entrances. The nest lies at the intersection of the dashed lines. The black thick arrow shows the north direction. The inset in (b) shows the angle α which was calculated to investigate the distribution of foraging effort around the tunnel entrances.

Fig 2: Frequency histogram of the duration of usage of the physical trails for the two *A. capiguara* nests studied. The duration of usage of each trail is calculated by the interval elapsed between the first and last survey in which activity was observed on the trail. Since the surveys occurred on average every fifteen days the actual duration of usage of a trail could be one to fourteen days longer. $N= 84$ trails.

Fig 3: Cumulative number of newly opened tunnel entrances at each survey over the monitoring period for the two *A. capiguara* nests studied.

Fig 4: Cumulated monthly rainfall (in mm) recorded at Bauru (22.355°S –49.0°W - Altitude: 620m), at about 100Km distance from the study site, from November 2011 to November 2012. Data provided by the Centro de Meteorologia de Bauru - FC/Unesp. Superimposed is the monthly number of newly opened tunnel entrances for the two nests studied over the monitoring period.

Fig 5: Distance from the nest of newly opened tunnel entrances, at each survey over the monitoring period for the two *A. capiguara* nests studied. Each point corresponds to a single tunnel entrance. The solid line shows the prediction of a GAM model and the dashed lines the 95% confidence interval of the prediction.

Fig 6: Evolution of tunnel entrance status over the monitoring period for (a) nest 1 and (b) nest 2. The tunnel entrances were categorized as closed (when the entrance hole's opening was no longer visible), open but inactive (without ant foraging activity), open with dispersed foraging activity around when isolated workers could be spotted but no foraging trails departing from them were visible, and open with one or several foraging trails departing from them with ant traffic.

Fig 7: Length of the newly created foraging trails at each survey over the monitoring period for the two *A. capiguara* nests studied. Each point corresponds to a single trail. The solid lines show the predictions of a GAM model and the dashed lines the 95% confidence interval of the predictions.