Dealing with water deficit in *Atta* ant colonies: large ants scout for water while small ants transport it

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Summary
Leafcutter ants (*Atta sexdens rubropilosa*) (Forel 1908) have an elaborate social organization, complete with caste divisions. Activities carried out by specialist groups contribute to the overall success and survival of the colony when it is confronted with environmental challenges such as dehydration. Ants detect variations in humidity inside the nest and react by activating several types of behavior that enhance water uptake and decrease water loss, but it is not clear whether or not a single caste collects water regardless of the cost of bringing this resource back to the colony. Accordingly, we investigated water collection activities in three colonies of *Atta sexdens rubropilosa* experimentally exposed to water stress. Specifically, we analyzed whether or not the same ant caste foraged for water, regardless of the absolute energetic cost (distance) of transporting this resource back to the colony. Our experimental design offered water sources at 0 m, 1 m and 10 m from the nest. We studied the body size of ants near the water sources from the initial offer of water (time = 0) to 120 min, and tested for specialization. We observed a reduction in the average size and variance of ants that corroborated the specialization hypothesis. Although the temporal course of specialization changed with distance, the final outcome was similar among distances. Thus, we conclude that, for this species, a specialist (our use of the word “specialist” does not mean exclusive) task force is responsible for collecting water, regardless of the cost of transporting water back to the colony.

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Key words: *Atta sexdens rubropilosa*, Water stress, Task division, Task specialization

Introduction
Eusocial animals engage in a number of different tasks such as foraging, brood care, or the removal of detritus from their nests (Wilson, 1971). In some species, task distribution among the different members of a colony is often associated with morphological and behavioral differentiation, such that groups of individuals similar in external morphology and body size perform similar tasks (Anderson et al., 2002). These groups, called “castes”, are particularly evident in highly eusocial ant species such as *Atta*, which exhibit noteworthy inter-individual morphological diversity (Wilson, 1971). In some cases, morphological diversity is easily interpretable by means of an optimization scenario, in which the relationship between body size, shape (form) and task (function) is evident. One classic example is the caste known as “soldiers”, whose main task is the defense of the colony, and they are therefore usually larger and physically more adept at fighting than the other castes. However, certain tasks are carried out by castes that do not exhibit morphological attributes that could intuitively be interpreted as specializations (see later). This observation suggests that castes may engage in specific tasks for reasons other than their superior physical ability to perform such tasks (Wilson, 1980a; Wilson, 1980b). For example, an important evolutionary factor that determines waste disposal among *Atta sexdens rubropilosa* may be to prevent contamination of the fungus garden (Hart and Ratnieks, 2001a; Hart and Ratnieks, 2002). A flexible pool of castes participates in this task, such that ants of different shapes and sizes may be recruited, as necessary, whenever there is more waste than usual to deal with (Waddington and Hughes, 2010).

The study of the relationship between task divisions, ant size and shape becomes even more complex when considering tasks that are split into sub-tasks (Anderson and Ratnieks, 1999; Hart and Ratnieks, 2001a; Hart and Ratnieks, 2001b; Burd and Howard, 2008). In leafcutter ants, for example, the task of “foraging”, understood as the process of bringing energy resources back to the colony, requires finding, cutting, transporting and inserting leaves into the fungus garden, as well as maintaining the garden and distributing food to individuals (Hölldobler and Wilson, 1990). Moreover, a number of parallel tasks may enhance foraging, such as the accumulation of leaf piles near the nest (Hart and Ratnieks, 2001b) and trail maintenance. The latter task is performed by small individuals, whose numbers on trails increase after a disturbance (Evison et al., 2008). Task partitioning is also important to improve the efficiency of foraging, as observed in *Acromyrmex*. Along the long trails produced by this genus, individuals may transfer pieces of leaves from one to another (Lopes et al., 2003) and also from one place to another (Bollazzi and Roces, 2010). In addition, some tasks that are essential for colony survival only need to be performed occasionally in the lifetime of a colony, and therefore they are unlikely to result in the creation of a specialist task force that would otherwise remain idle.
One task that is carried out only occasionally, and yet is essential for *Atta* colonies, is the humidification of the nest for proper fungus growth (Stahel and Geijskes, 1940; Roces and Kleineidam, 2000). Effective water transport to the colony requires collecting and transporting water to the nest, and this task is performed mainly by small ants when water sources are very close to the nest (Ribeiro and Navas, 2008). However, larger ants could transport more water and would maximize water uptake per unit of time, an even more important consideration when water sources are far from the nest. If efficiency of water transportation is a dominant driver for task distribution, increasingly larger ants would be expected to collect water as distances to the source increase. On the other hand, occasional tasks may rely on the task-force that is less disruptive to the colony. In this case, a fixed size distribution for water-collecting ants would be evident, regardless of the distance to the source. In order to unravel this problem, we ascertained which caste was responsible for collecting water in dehydrating colonies of *A. s. rubropilosa* (Forel 1908) that had water sources located at various distances. We also studied the temporal pattern of variance in the size of water-collecting ants from the onset of detection. We did this because, according to ant foraging theory (Gordon, 2010), scouts would have a role in discovering, informing and recruiting (Franks et al., 2002; Gan et al., 2010; Bollazzi and Roces, 2011), but not necessarily in gathering the resources themselves.

## Materials and Methods

### Set-up

We used 3 colonies maintained at the same lab facility at temperatures between 22°C and 27°C. These colonies lived in plastic pots in which a healthy fungus garden grew before the onset of the experiment. A pot containing 4 l liters of fungus was placed in a plastic tray (32 cm long, 22 cm wide and 7.5 cm high), whose edges were dusted with talcum powder. This tray was connected to other trays by a one-inch-diameter plastic tube. The trays were connected to one another as required by the experiment, which began by inducing dehydration in the colony while providing a viable and dry substrate for the fungus garden, which was made possible by replacing *Acalypha sp.* with corn grits. Subsequently, the colonies were monitored in order to ascertain the beginning of water collection behavior. After water collection behavior was detected, dehydration was continued for another 10 days (leaves replaced by corn grits with no water source available for the ants). At this point, a water source (15 ml in a Petri dish) was introduced into the most distant tray from the nest. The distances from the nests to the water source were 0 m, 1 m, and 10 m (Fig. 1).

### Overall design

We began gathering size data on the ants when vigorous water collection behavior was evident. In order to evaluate the size of ants collecting water, we sampled ants for two hours, and we always sampled between 1:00 p.m. and 6:00 p.m. to control for possible daily rhythm effect. Then, we separated individuals collected over time using a specific protocol (see ‘Ant collection and measurement of head capsule’ in Materials and Methods) and we collected size data. In order to separate individuals over time, we grouped ants collected between the onset of experiment and minute 20, between minutes 20 and 40, and so on until minute 120. This sampling protocol generated 6 samples that could be ranked across a temporal scale. Next, we calculated the average head size of each one of these samples and used them as an indicator of ant size as a function of time. This procedure was repeated for each colony tested. We are aware that removing ants from their colonies could have caused a small shift in the size distribution of ants, and in theory this could have affected subsequent results. However, this effect is likely negligible, as experimental sample sizes (all repetitions) were about 360 ants per colony, and the colonies had 10,000+ ants. In addition, ants of different sizes were removed, from scouting ants at the beginning of experiments to small ants that turned out to be the main collectors of water, so no biased sampling occurred. Finally, when (in separate experiments) water-collecting ants were removed only once after 12 h of water being available, the size distribution of those water-collecting ants was similar to that reported here (Ribeiro and Navas, 2008).

### Experimental treatment of distance to water source

Our aim was to expose each colony to three experimental conditions by varying the distance between the nest and the water source used by the ants for nest hydration. The distances selected for each individual experiment were 0 m, 1 m and 10 m. Due to technical problems, colony B could not be tested at 10 m. The numbers of ants tested appear in Table 1.

### Ant collection and measurement of head capsule

In all the experimental colonies, a Petri dish was introduced as a water source, and the dish had four equidistant markings on its edge. We first collected the individual taking water closest to the first mark. Then, we collected a second individual, in this case the one closest to mark 2. This procedure continued until the fifth ant (again, closest to mark 1) and the cycle was restarted. We measured cephalic capsules (width) with digital calipers (Mitutoyo 552-304, 0.05 mm accuracy, USA), and we kept collected ants in a container separate from the colony until the end of the experiment, when they were returned to the colony.

### Null distribution of size

To obtain a null distribution of ant size, we used colony A and assumed that the data would be equivalent for the other two colonies. This assumption is sound because the three colonies were comparable in origin, size, age and maintenance protocols. In order to collect a comprehensive and representative sample of all ants in the colony, we first placed an empty 300 ml plastic pot in the tray used to feed the colony. After 10 days a mini-colony had been established and the pot was full of fungus. We waited 10 days more to ensure stability, and thus after 20 days we moved the pot to a separate tray connected to the mother colony by a small wooden bridge and fed it for 5 more days under these new conditions. Then, we froze both pot-colony and tray and measured the head size of all ants (*n* =1686). From this sample we obtained what we considered a null size distribution for the colonies representing a central tendency and variance in the size of all ant castes. Water collection is a phenomenon described in detail only recently (Ribeiro and Navas, 2008), and we do not know whether individual ants that normally stay inside the nest may leave it to collect water. So, our null size distribution included all ant types in the nest and not only ants found outside under normal foraging. We used this size distribution for comparison with the distribution of water collecting ants, under the premise that comparative lower variation coefficients in collecting ants would suggest specialization.

## Results

The size of ants engaged in water collection progressively decreased from the onset of water collection (Kruskal-Wallis test comparing ant size at times 1 to 6: *H* (5, 3297) = 878.51, *P* < 0.001; Nemenyi test *Q*0.05 = *q* = 6.03) (Fig. 2A). This pattern had different temporal courses according to the distance to the water source, as deduced from the time*distance interaction term (GLM: *F*10, 90 = 1.9693, *P* = 0.045) (Fig. 3). The size coefficient of variation in water-capturing ants decreased progressively with time until time 3 (Kruskal-Wallis test: *H* (5, 141) = 29.95, *P* < 0.001; Table 1. Total number of ants for each time interval and distance for all experimental tests.

| Distance (m) | 0 m | 261 | 255 | 224 | 197 | 208 | 231 |
|-------------|-----|-----|-----|-----|-----|-----|-----|
| (time)      | 0–20| 40–60| 60–80| 80–100| 100–120|
|             |     |     |     |     |     |     |     |
| 1 m         | 251 | 223 | 202 | 180 | 188 | 199 |
| 10 m        | 139 | 124 | 122 | 102 | 91  | 106 |
The variation (CV) of size was smaller in water-collecting ants than in the null distribution colony, and this was true from the beginning to the end of water collection (Kruskal-Wallis test: $H (6, 144) = 53.74, P < 0.001$; Nemenyi test $Q_{0.05} = q = 5.91, P < 0.001$ for all comparisons). Moreover, the null distribution of ant size had a lower mean than that for ants collecting water at the beginning of the activity (Mann-Whitney $U$ Test: $n = 670$, $U = 137,566.5$, $Z = 28.68$ $P < 0.05$), and the end of the activity (Mann-Whitney $U$ Test: $n = 540$, $U = 294,808.0$, $Z = 12.34$ $P < 0.05$). The overall pattern of shift in the size distribution of ants collecting water appears as a frequency distribution in Fig. 4.

Discussion

Ants that collect water belong to a certain size class (Figs 3, 4). It takes time for this size class specialization to occur (our use of the word “specialization” does not mean an exclusive behavior). In the early stages of water collection, large workers are more common around water sources. Sometime after, water collecting ants show both lower than average and reduced variability in body size. The shifts in size distribution favors the hypothesis that the size class primarily engaged in water collection results from both a reduction in the number of large ants involved in this task and an increase in the corresponding number of small ants (Fig. 4). Our results support the specialization hypotheses for water harvesting in the genus *Atta* (Ribeiro and Navas, 2008). Bigger ants are better at scouting and finding new sources of water due to their ability to move out of the nest. However, soon after the water source is found, scout ants probably collect water as well, because they display the same behavior at the water source as other ants (Da-Silva et al., 2009). The population of both scout and workers who engage in water collection display a lower coefficient of variation than the null distribution, suggesting caste specialization for both finding and transporting water. This also indicates that the task of water collection changes from one caste to another.

Our results are not compatible with the hypothesis that a mere distance-time interaction explains the distribution of ant sizes along the experiment. If the dominant driver of our results had been the greater absolute speed of larger ants, we should have observed, at the beginning of the experiment, larger ants at the most distant water sources. However, this was not the case. So, our data corroborate the hypothesis of specialization for water collection, and thus a remaining question is why specialization for water collection occurs. One possibility is specialization associated with optimization. However, if optimization exists, it does not involve maximization of water transportation to the colony. Perhaps other factors such as efficiency of water deposition in the fungus garden are dominant as an evolutionary driver for this behavior. Our data do not say about this, but likely the fungus is more sensitive to dehydration than individual ants. If so, the water search and collection behavior would be elicited by the hydric condition of the fungus garden, and not by that of individual ants. Smaller ants would be better at hydrating the fungus garden, given their scale (Ribeiro and Navas, 2008) and their task location, usually related to the maintenance of the fungus (Hölldobler and Wilson, 1990). For example, smaller ants are better than larger ones at fungus-care tasks such as leaf implantation (Hölldobler and Wilson, 1990; Burd and Howard, 2008).
Although our current view of the problem favors a fungus-care hypothesis more than an individual thirst approach (as proposed in Ribeiro and Navas, 2008), we cannot discard a possible role of water transfer among individuals. However, the transfer of water is not as simple as the transfer of leaf fragments, because it requires trophallaxis. Under experimental conditions trophallaxis between two ants may require hours (Da-Silva et al., 2009), and therefore this behavior may not be proper as a mechanism for collective hydration. In addition, ants may gather water indirectly from eating hydrated fungus.

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Competing Interests
The authors have no competing interests to declare. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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