

## Sequential load transport during foraging in *Acromyrmex* (Hymenoptera: Formicidae) leaf-cutting ants

Mariane A. NICKELE, Wilson REIS FILHO & Marcio R. PIE



### Abstract

The complex social organization of leaf cutter ants is closely linked with intricate patterns of division of labor in the worker force. For instance, foraging might involve division of labor between cutters and carriers, as well as task partitioning during leaf transport. However, little is known about division of labor during foraging in *Acromyrmex*, particularly under field conditions. The goal of the present study is to investigate the behavior of *Acromyrmex* leaf-cutting ants on trails of different lengths in order to elucidate the effect of foraging distance on the occurrence of transport chains under field and laboratory conditions, and to discuss the hypotheses to explain the occurrence of transport chains. In *Acromyrmex crassispinus* (FOREL, 1909) and *Acromyrmex subterraneus subterraneus* (FOREL, 1893), cutting and carrying of fragments were clearly separated activities performed by distinct worker groups differing in body size. In addition, the behavior of foragers of both species differed significantly according to variation in trail distances. On short trails (1 m), cutters frequently transported the fragments directly to the nest, whereas on long trails (more than 10 m), most cutters transferred the fragments to other workers. Transport chains happened more frequently when workers harvested plants far from the nest. These results demonstrate that *Acromyrmex* species display both division of labor between cutters and carriers, and task partitioning during leaf transport, with trail lengths showing marked effects on the likelihood of sequential transport. Furthermore, the results of this study provide support for the hypothesis that the behavioral response of transferring fragments in *Acromyrmex* species would have been selected for because of its positive effect on the information flow between workers.

**Key words:** Division of labor, task partitioning, foraging trail, leaf-cutting ants, leaf transfer, transport chains.

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### Introduction

Leaf-cutting ants (*Atta* and *Acromyrmex*) are commonly observed in many tropical and subtropical regions of the New World (HÖLLDOBLER & WILSON 1990). *Atta* and *Acromyrmex* have some of the largest colonies of the tribe Attini; however, the social complexity of *Atta* species is much greater than that in *Acromyrmex*. For instance, *Atta* workers show a more pronounced level of polymorphism, including the presence of soldiers, and their foraging trails are wider and reach greater distances than *Acromyrmex* (see WEBER 1972). Therefore, studying *Acromyrmex* can provide a window into the intermediate steps that were necessary to attain the higher levels of social organization found in *Atta*.

Insect societies in general, and leaf-cutting ants in particular, depend largely on the effective and efficient organization of the workforce through division of labor (HART 2013). Division of labor is the division of the workforce into different tasks, which can in turn be partitioned into sub-tasks connected into a single process by a transfer of

materials (JEANNE 1986, RATNIEKS & ANDERSON 1999). In leaf-cutting ants, this form of work organization occurs in all stages of leaf collection, leaf transportation, and leaf processing within the nest; in the deposition of refuse (leaves and dead fungal garden) to internal or external dumps; and in colony emigration (HART & al. 2002).

The complex social organization of leaf-cutting ants is closely linked with intricate patterns of division of labor in the worker force. For instance, foraging might involve division of labor between cutters and carriers, as well as task partitioning during leaf transport (HART & al. 2002). In particular, leaf-cutting ant foraging is composed of three basic strategies (ANDERSON & al. 2002): (1) Individual foraging (also known as parallel-series arrangement), where each individual carries her load all the way from the source to the nest, thus involving no task partitioning; (2) a "bucket brigade" strategy, where a worker carries her load along the trail until she meets an unladen ant, upon which direct transfer takes place (transfer 1) and the donor ant returns

back up the trail while the recipient ant carries the load further down the trail until she meets another unladen ant (transfer 2), and so on, such that transfer is always direct and not concentrated in fixed locations; and (3) a multi-stage partitioned task with indirect transfer, in which workers transport materials, such as leaves, from the source, or a pile of leaves cached on the trail, and drop them at the next cache they encounter on the trail, with the entire process being repeated a number of times until the food item reaches the nest. Although several studies have investigated instances of each of these strategies (FOWLER & ROBINSON 1979, VASCONCELOS & CHERRET, 1996, HUBBELL & al. 1980, RATNIEKS & ANDERSON 1999, ANDERSON & JADIN 2001, HART & RATNIEKS 2001, ANDERSON & al. 2002, RÖSCHARD & ROCES 2003a), still little is known about the conditions favoring each strategy in a given species.

In *Atta*, cutters and carriers seem to be specialized morphologically by differences in body size, with arboreal cutters not being seen carrying fragments back to the nest (VASCONCELOS & CHERRET 1996, HUBBELL & al. 1980, RATNIEKS & ANDERSON 1999). *Atta cephalotes* (LINNAEUS, 1758) sometimes transfer leaves, either directly by passing it from one worker to another, or indirectly by caching it on the ground (HUBBELL & al. 1980, RATNIEKS & ANDERSON 1999). On the other hand, *Atta laevigata* (F. SMITH, 1858) was found to employ a two-stage, size-related, strategy when cutting plants: Larger workers climb the plant stem and drop whole leaves to the ground while smaller workers cut the lamina of the dropped leaves and carry them to the nest (VASCONCELOS & CHERRET, 1996). In *Atta sexdens* (LINNAEUS, 1758) however, retrieval may occur in three distinct stages: "Arboreal cutters" clip through petioles allowing leaves to fall to the ground, where they are later collected, cut into fragments and deposited at the main trail by "cache exploiters". Then, "carriers" transport the fragments to the nest (FOWLER & ROBINSON 1979). Task partitioning during transport was also reported in *Atta colombica* GUÉRIN-MÉNEVILLE, 1844 (see ANDERSON & JADIN 2001, HART & RATNIEKS 2001), with fragments being directly transferred or cached on the trail (ANDERSON & JADIN 2001).

Three different modalities for the transport of fragments along the trail were observed in *Atta vollenweideri* FOREL, 1893: (1) a cutter carries the fragment directly to the nest; (2) fragments put down on the trail by a cutter, or directly transferred, are retrieved by a worker and carried all the way to the nest – the "single carriers"; (3) fragments found on the trail or directly received from nestmates are transported consecutively by different carriers via a "transport chain". This division was less marked when plants were located very close to the nest and no physical trail was present, i.e., the cutter often transported its own fragment back to the nest. Sequential load transport occurred more frequently on long foraging trails, i.e., workers formed transport chains composed of two to five carriers (RÖSCHARD & ROCES 2003a).

Two hypotheses seek to explain the occurrence of sequential load transport (ANDERSON & JADIN 2001, ANDERSON & al. 2002, ROCES & BOLLAZZI 2009, RÖSCHARD & ROCES 2003a, 2011). First, sequential load transport may have been favored during evolution because of a faster load delivery rate. These arguments are the core of the so-called "economic-transport hypothesis", with "economic"

in this context referring to the maximization of the transportation speed of a leaf fragment, which at the colony level may result in an increased overall rate of resource delivery. A faster material transport rate may be achieved via improved size-matching, or because each contributing worker restricts its task to a certain part of the way, which may improve its orientation and, indirectly, its walking speed. Second, leaf fragments may inform other foragers either about type and quality of a newly exploited food patch, or simply about the fact that such a food patch can be found in that direction. In this case, the behavioral response of transferring fragments would have been selected for because of its positive effect on the information flow. This "information-transfer hypothesis" states that workers may trade off material transport rate for enhanced information transfer during social foraging.

Despite considerable emphasis in the literature on sequential load transport in *Atta* colonies, the corresponding behavior in *Acromyrmex* has been nearly completely neglected, particularly under field conditions. The only study to date showed leaf cache formation along the trail and leaf direct transferring among workers under laboratory conditions (LOPES & al. 2003). There were significant differences between the number of leaf fragments carried directly to the fungus chamber and those transferred directly or indirectly, depending upon the trail length (LOPES & al. 2003). However, the behavior of *Acromyrmex* ants remains unknown under natural conditions and there is no information if those ants transport consecutively leaf fragments by different carriers *via* a transport chain.

The aim of the present study is to investigate the behavior of *Acromyrmex* leaf-cutting ants on trails of different lengths in order to elucidate the effect of foraging distance on the occurrence of transport chains under field and laboratory conditions, and to discuss the hypotheses to explain the occurrence of transport chains.

## Material and methods

Field experiments were performed from January to March of 2010 in Curitiba, state of Paraná, Brazil. The climate in the region is subtropical humid mesothermic, with frequent frosts during the cold season (May - September). Average temperatures during the warm and cold seasons are 22 °C and 12 °C, respectively, with average annual rainfall between 1300 and 1500 mm (MAACK 1981).

Field experiments were conducted on *Acromyrmex crassispinus* (FOREL, 1909) and *Acromyrmex subterraneus subterraneus* (FOREL, 1893). Species identification was performed using the key provided in GONÇALVES (1961). A large mature colony was observed in each ant species. To investigate the entire process of transport, freshly-cut plant fragments were marked with a small dot of white paint and followed until they reached the nest. The time intervals and the distances a given fragment was carried by sequential foragers were noted, as well as the "waiting times" (the time a fragment was left on the trail before being retrieved by another worker). Given, that the average waiting time normally did not exceed ten minutes, we stipulated a maximum waiting time of 20 minutes. After that, the corresponding fragments were not monitored anymore because, in some cases, the fragments that were dropped by a worker remained for several hours on the trail until being collected by another worker. Foragers involved in the

sequential transport were caught immediately after transferring or dropping the fragment, and weighed alive to the nearest 0.1 mg on a Mettler balance within eight hours of being collected in the field. The last carriers transporting the marked fragments were caught before entering the nest, and both ant and fragment were weighed as indicated above.

*Acromyrmex crassispinus* workers were actively harvesting fragments of Poaceae at a distance of 23 m from the nest and *Acromyrmex subterraneus subterraneus* workers were harvesting fragments of *Prunus* sp. at a distance of 17 m from the nest (long trails). We observed 50 fragments marked all the way to the nest for each ant species. In order to elucidate the effect of foraging distance on the occurrence of transport chains, we offered flowers of *Rosa* sp. to the colonies at a distance of 1 and 5 m from the nest (short and intermediate trails) and we observed 50 leaf fragments in each distance for each ant species. Observations were performed during the day, since colonies showed diurnal foraging activity.

Chi-square tests ( $\chi^2$ ) were used for comparing the load transport of workers in different trail lengths. In order to study ant size variation (cutters  $\times$  carriers) and the waiting time of a dropped fragment by a cutter or a carrier, the  $t$  test was used. We also evaluated the loading ratio of workers (Loading ratio = [ant mass + fragment mass] / ant mass) (LUTZ 1929). Also, we used  $t$  tests to compare the behavior of cutters that had not transferred their loads with those that did, and analyses of variance (ANOVAs) were used to analyze the behavior of carriers that did not transfer their fragments with carriers involved in the transport chain. We used the time spent and distance traveled to calculate the walking speed of each worker. ANOVAs, followed by Tukey's post-hoc test, were employed to compare the transport time, distances and walking speeds of the participants of a transport chain. The data were log-transformed (base 10) to meet the assumptions of parametric statistics, when necessary. Finally, the proportion of the trail covered by cutters was visualized using violin plots (HINTZE & NELSON 1998). All statistical analyses were performed in R (R CORE TEAM 2013).

Laboratory experiments were conducted from September 2010 to February 2011 in the campus of the Universidade Federal do Paraná, Curitiba, state of Paraná, Brazil. One colony of each ant species was collected in Curitiba and maintained in the laboratory at  $24 \pm 1$  °C and RH  $80 \pm 10\%$ . Colonies were housed individually in acrylic containers. Artificial trails of three different distances between the foraging arena and fungus garden were made using transparent plastic tubing. The trails lengths were: 1, 5 and 10 m (short, intermediate and long trails, respectively). We observed the transport of 50 fragments in each trail length all their way to the nest for each ant species. Plant fragments were always of *Rosa* sp., a readily accepted plant by the studied *Acromyrmex* species under laboratory conditions.

## Results

### Field experiments

**The behavior of cutters:** *Acromyrmex crassispinus* cutters carried 10% ( $n = 5$ ) of the fragments directly to the nest, in the long trail. Ninety percent ( $n = 45$ ) of the fragments were transferred to other workers before entering the nest

[82% ( $n = 41$ ) of the fragments were dropped and 8% ( $n = 4$ ) were directly transferred to other workers] (Fig. 1a). In the intermediate trail, 48% ( $n = 24$ ) of the fragments were directly carried to the nest, and 52% ( $n = 26$ ) of the fragments were dropped on the trail. In the short trail, 76% ( $n = 38$ ) of the fragments were directly carried to the nest, and 24% ( $n = 12$ ) of the fragments were dropped on the trail. No direct fragment transfer between workers occurred in the intermediate and short trail. The proportion of transferred vs. carried fragments differed significantly between long and short trail distances (Fig. 1a, long trail:  $\chi^2 = 32.02$ ,  $p < 0.001$ ; short trail:  $\chi^2 = 13.54$ ,  $p < 0.001$ ). However, there was no significant difference in the proportion of transferred vs. carried fragments at the intermediate trail (Fig. 1a, intermediate trail:  $\chi^2 = 0.1$ ,  $p > 0.05$ ). For those transferred fragments, there was no particular location along the trail, i.e., cutters did not cache the fragments, although there was a tendency of the fragments being transferred on the first third of the trail (Fig. 2a).

*Acromyrmex subterraneus subterraneus* cutters carried 34% ( $n = 17$ ) of the fragments directly to the nest in the long trail. 66% of the fragments were transferred to other workers before entering the nest [34% ( $n = 17$ ) were directly transferred to other workers and 32% ( $n = 16$ ) of the fragments were dropped on the trail] (Fig. 1b). In the intermediate trail, 68% ( $n = 34$ ) of the fragments were directly carried to the nest, and 32% ( $n = 16$ ) of the fragments were directly transferred to other workers. In the short trail, 98% ( $n = 49$ ) of the fragments were directly carried to the nest, and 2% ( $n = 1$ ) of the fragments were directly transferred to other workers. No indirect fragment transfers to other workers occurred at 5 and 1 m from the nest. The proportion of transferred vs. carried fragments differed significantly at the three trail distances (Fig. 1b, long trail:  $\chi^2 = 5.14$ ,  $p < 0.05$ ; intermediate trail:  $\chi^2 = 6.5$ ,  $p < 0.05$ ; short trail:  $\chi^2 = 46.1$ ,  $p < 0.001$ ). There was no particular location along the trail for cutters transfer their fragments, but there was a tendency for the fragments to be transferred in the first third of the trail (Fig. 2b).

To evaluate the effects of both cutter and fragment mass on the probability of a cutter transfer its fragment, the loading ratio of cutters that transported their fragments to the nest ("carry") were compared with those of workers that transferred their fragments after cutting ("transfer") on long trails. There was no difference in loading ratio of cutters in either species (*Acromyrmex crassispinus*: carry:  $2.39 \pm 0.36$  mg ( $\bar{x} \pm$  SD),  $n = 5$ ; transfer:  $2.53 \pm 0.56$ ,  $n = 45$ ;  $t = 0.57$ ,  $p > 0.05$ ; *Acromyrmex subterraneus subterraneus*: carry:  $3.37 \pm 1.16$  mg,  $n = 17$ ; transfer:  $3.17 \pm 0.85$  mg,  $n = 32$ ;  $t = -0.76$ ,  $p > 0.05$ ).

We compared the walking speed of cutters that transported their fragments to the nest ("carry") with those of workers that dropped their fragments after cutting ("transfer") on long trails. Interestingly, fragment-carrying cutters were faster than fragment-transferring cutters (*Acromyrmex crassispinus*: carry:  $1.20 \pm 0.39$  cm / s ( $\bar{x} \pm$  SD),  $n = 5$ , transfer:  $0.65 \pm 0.46$  cm / s,  $n = 45$ ,  $t = -2.56$ ,  $p < 0.05$ ; *Acromyrmex subterraneus subterraneus*: carry:  $1.34 \pm 0.51$  cm / s,  $n = 17$ , transfer:  $0.83 \pm 0.39$  cm / s,  $n = 32$ ,  $t = -3.91$ ,  $p < 0.001$ ).

*Acromyrmex crassispinus* cutters were significantly larger than carriers on the long and the intermediate trail (long trail; cutters:  $5.47 \pm 1.44$  mg ( $\bar{x} \pm$  SD),  $n = 45$ ; car-

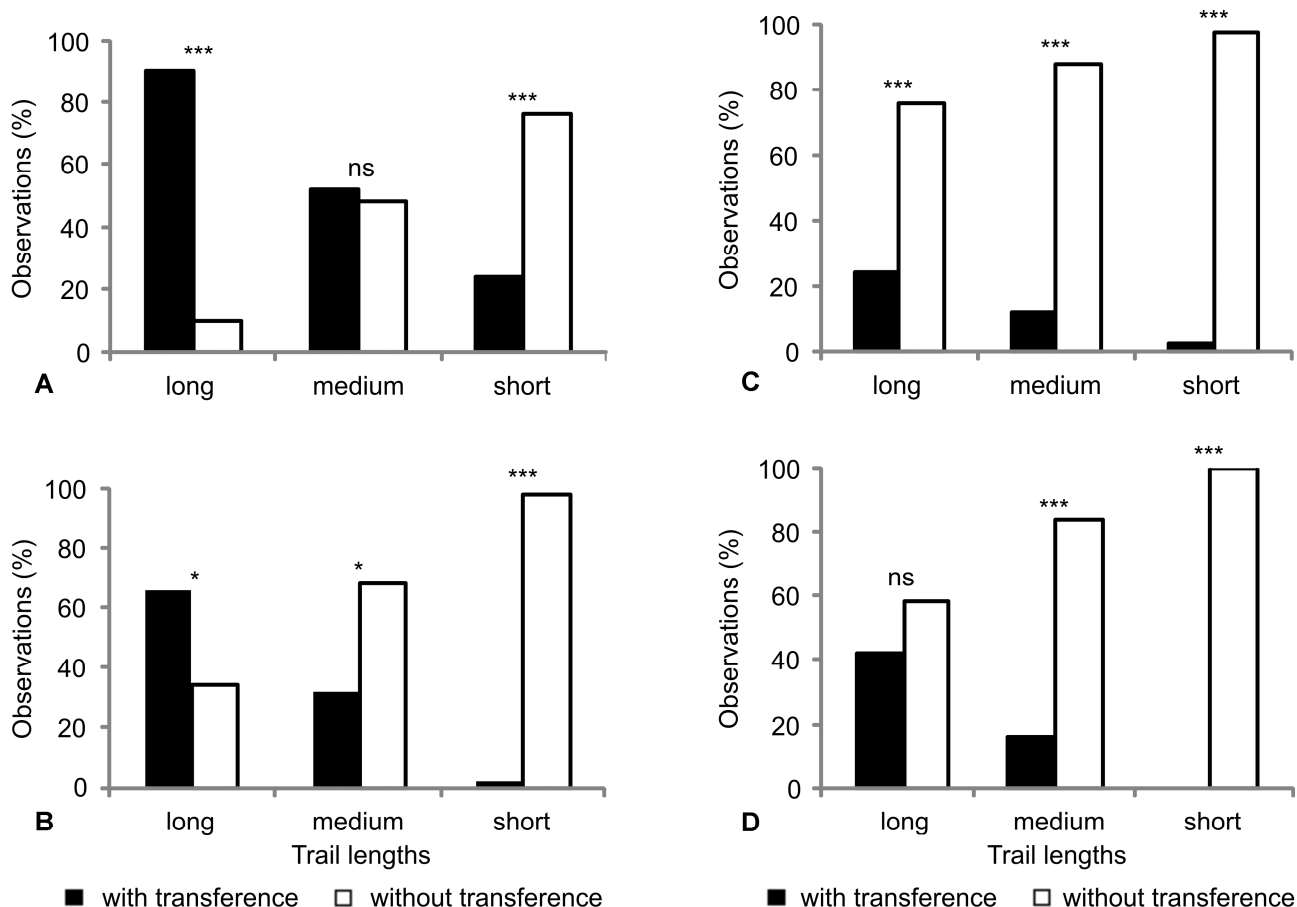
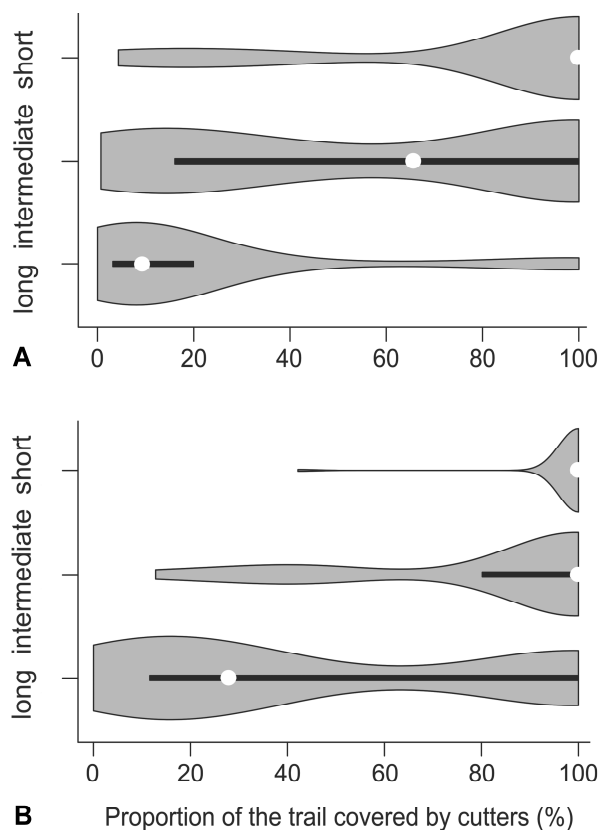


Fig. 1: Behavior of cutters after cutting a fragment as a function of the distance from the nest. Field conditions: (A) *Acromyrmex crassispinus*; (B) *A. subterraneus subterraneus*. Laboratory conditions: (C) *A. crassispinus*; (D) *A. subterraneus subterraneus*. See text for statistics.

riers:  $3.75 \pm 1.34$  mg,  $n = 45$ ,  $t = 5.82$ ,  $p < 0.0001$ ; intermediate trail: cutters:  $4.92 \pm 1.49$  mg,  $n = 26$ ; carriers:  $3.98 \pm 1.57$  mg,  $n = 26$ ,  $t = 2.22$ ,  $p < 0.05$ ). However, there were no significant differences between cutters and carriers on the short trail (cutters:  $4.60 \pm 1.75$  mg,  $n = 11$ ; carriers:  $3.46 \pm 1.41$  mg,  $n = 11$ ,  $t = -0.89$ ,  $p > 0.05$ ). In *Acromyrmex subterraneus subterraneus* there were also significant differences between cutters and carriers at the different trail distances (long trail: cutters:  $5.25 \pm 1.86$  mg ( $\bar{x} \pm SD$ ),  $n = 31$ ; carriers:  $4.06 \pm 1.49$  mg,  $n = 32$ ,  $t = 2.81$   $p < 0.05$ ; intermediate trail: cutters:  $6.29 \pm 1.99$  mg,  $n = 17$ ; carriers:  $4.94 \pm 1.59$  mg,  $n = 17$ ,  $t = -2.18$ ,  $p < 0.05$ ). In the short trail, the reduced sample size was not sufficient to perform the test.

**Transport chains:** The frequency of occurrence of transport chains significantly depended on trail distance.

Fig. 2: Violin plot indicating the proportion of the trail covered by cutters until transfer their fragments, at the long, intermediate and short trail, in field conditions. (A) *Acromyrmex crassispinus*; (B) *Acromyrmex subterraneus subterraneus*. The white circle in each diagram corresponds to the median percentage for the corresponding group, whereas the associated black bar indicates the first and third quartiles. Finally, the thickness of the gray areas form a double kernel density plot corresponding to the relative proportion of different percentages.



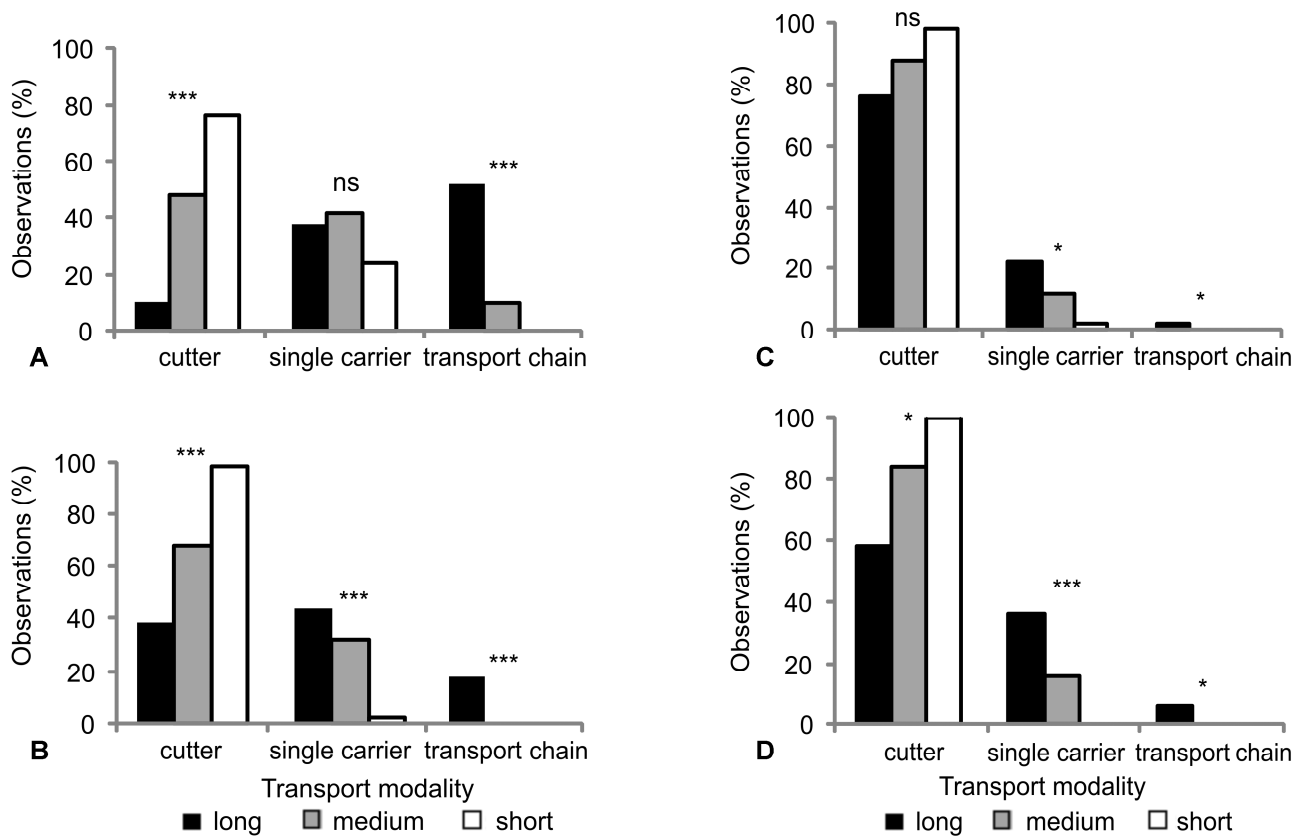


Fig. 3: Modality of load transport as a function of foraging distance. Field conditions: (A) *Acromyrmex crassispinus*; (B) *A. subterraneus subterraneus*. Laboratory conditions: (C) *A. crassispinus*; (D) *A. subterraneus subterraneus*. See text for statistics.

On the long trail of *Acromyrmex crassispinus*, 52% (n = 26) of the fragments were transported by transport chains, 10% (n = 5) on the intermediate trail, and 0% on the short trail (Fig. 3a,  $\chi^2 = 36.91$ ,  $p < 0.001$ ). On the long trail, 26% (n = 13) of the transport chains were composed by 2 carriers, 18% (n = 9) by 3 carriers, 4% (n = 2) by 4 carriers, 2% (n = 1) by 6 and 2% (n = 1) by 7 carriers. On the intermediate trail, all the transport chains were composed by 2 carriers. With respect to the other transport modalities, cutters transported their fragments directly to the nest significantly more often on the short trail (Fig. 3a,  $\chi^2 = 24.60$ ,  $p < 0.001$ ). Transport by single carriers, conversely, was independent of foraging distance, averaging 24% (n = 12), 42% (n = 21) and 38% (n = 18) on the short, intermediate and long trail, respectively (Fig. 3a,  $\chi^2 = 2.62$ ,  $p > 0.05$ ).

In *Acromyrmex subterraneus subterraneus*, 18% (n = 9) of the fragments were transported by transport chains. Transport chains have occurred only on the long trail in this species (Fig. 3b,  $\chi^2 = 18.25$ ,  $p < 0.001$ ). Fourteen percent of the transport chains were composed by 2 carriers, 2% (n = 1) by 4 carriers, and 2% (n = 1) by 6 carriers. In relation to the other transport modalities, cutters transported their fragments directly to the nest significantly more often on the short trail (Fig. 3b,  $\chi^2 = 13.25$ ,  $p < 0.001$ ). Transport by single carriers occurred significantly more often on the long trail (Fig. 3b,  $\chi^2 = 18.05$ ,  $p < 0.001$ ).

There was no difference in transport time of fragments carried by a transport chain, a single carrier or a cutter, in either species (*Acromyrmex crassispinus*: One-way ANOVA:

$F_{(2,47)} = 2.5$ ,  $p = 0.08$ , transport chain: 39 min, n = 26, single carrier: 47 min, n = 19, cutter: 36 min, n = 5; *Acromyrmex subterraneus subterraneus*: One-way ANOVA:  $F_{(2,47)} = 0.8$ ,  $p = 0.43$ , transport chain: 28 min, n = 9, single carrier: 26 min, n = 21, cutter: 24 min, n = 20). This time includes time wasted in dropping and recovering the leaf.

The "waiting time" of a dropped fragment before it was collected by a worker was greater when a cutter dropped the fragment than when a carrier dropped the fragment in both species. In *Acromyrmex crassispinus*, the waiting time of a fragment dropped by a cutter was on average 3 min and 44 s and the waiting time of a fragment dropped by a carrier was on average 1 min and 45 s ( $t = 2.62$ ,  $p < 0.05$ ). In *Acromyrmex subterraneus subterraneus*, the waiting time of a fragment dropped by a cutter was on average 6 min and 1 s and the waiting time of a fragment dropped by a carrier was on average 1 min and 36 s ( $t = 2.88$ ,  $p < 0.05$ ).

We compared loading ratio of single carriers with those carriers involved in a transport chain to investigate the effects of carriers and their fragment mass on the probability of a carrier drop its fragment. However, there was no difference in loading ratio among workers in a transport chain, irrespective of their position, and single carriers (*Acromyrmex crassispinus*: single carrier:  $3.15 \pm 0.89$  mg, n = 19 ( $\bar{x} \pm SD$ ); transport chain: first carrier:  $3.06 \pm 1.39$  mg, n = 26, middle carrier:  $2.91 \pm 0.89$  mg, n = 13, last carrier:  $3.32 \pm 1.15$  mg, n = 26; ANOVA:  $F_{(3,80)} = 0.50$ ,  $p > 0.05$ ; *Acromyrmex subterraneus subterraneus*: single carrier:  $3.89 \pm 1.87$  mg, n = 32; transport chain: first carrier:

Tab. 1: Size-matching between fragment size and body mass of cutters, single carriers, and carriers involved in a transport chain (first, middle and last carrier).

Species	Workers	Equation	R <sup>2</sup>	p
<i>Acromyrmex crassispinus</i>	Cutter	$y = 1.08x + 2.28$	0.21	< 0.01
	Single carrier	$y = 1.004x + 4.45$	0.18	0.07
	First carrier	$y = 0.07x + 7.46$	< 0.01	0.89
	Middle carrier	$y = -1.40x + 14.31$	0.21	0.11
	Last carrier	$y = -0.23x + 8.63$	< 0.01	0.73
<i>Acromyrmex subterraneus</i>	Cutter	$y = 0.91x + 5.68$	0.15	0.05
	Single carrier	$y = 0.37x + 8.32$	0.05	0.32
	First carrier	$y = 4.28x - 6.07$	0.76	0.05
	Middle carrier	$y = -4x + 27.00$	0.07	0.74
	Last carrier	$y = 2.5x + 1.17$	0.11	0.37

4.15 ± 1.72 mg, n = 9, middle carrier: 4.87 ± 2.12 mg, n = 4, last carrier: 3.93 ± 1.26 mg, n = 9; One-way ANOVA:  $F_{(3,50)} = 0.12$ ,  $p > 0.05$ ).

We also compared the relationship between fragment size and body mass of cutters, single carriers, and carriers involved in a transport chain (first, middle and last carrier), to investigate whether the probability of formation of a transport chain depends on size-matching between workers and loads (Tab. 1). The only significant result was a positive relationship between fragment mass and body mass of the cutters in both studied species (Fig. 4) and first carrier in *Acromyrmex subterraneus subterraneus*.

The speed of carriers that transported their fragments to the nest ("single carrier") was also compared with first carriers from a transport chain that transferred their fragments ("first carrier"). Single carriers were faster than first carriers in a transport chain in both species (*Acromyrmex crassispinus*: single carrier: 1.10 ± 0.34 cm / s ( $\bar{x} \pm SD$ ), n = 19, first carrier: 0.82 ± 0.37 cm / s, n = 26,  $t = 2.52$ ,  $p < 0.05$ ; *Acromyrmex subterraneus subterraneus*: single carrier: 1.49 ± 0.62 cm / s, n = 24, first carrier: 0.98 ± 0.40 cm / s, n = 9,  $t = -2.27$ ,  $p < 0.001$ ).

The distances covered by the participants of a transport chain were markedly different (Fig. 5a and 5b). Cutters usually covered only a short distance of 2.2 ± 2.1 m ( $\bar{x} \pm SD$ ) n = 26, in *Acromyrmex crassispinus*, and 2.0 ± 1.6 m, n = 9, in *Acromyrmex subterraneus subterraneus*. In *Acromyrmex crassispinus*, the first carriers covered a distance of 5.8 ± 5.6 m, n = 26, the middle carriers covered a distance of 4.2 ± 4.7 m, n = 14, and the last carriers covered a distance of 13.8 ± 6.1 m, n = 26. In *Acromyrmex subterraneus subterraneus*, the first, middle and last carriers covered a distance of 3.1 ± 2.4 m, n = 9; 3.5 ± 2.2 m, n = 4; and 7.8 ± 3.4 m, n = 9, respectively. Thus, fragments were mainly transported by the last carriers, in both species (*Acromyrmex crassispinus*: One-way ANOVA:  $F_{(3, 88)} = 27.1$ ,  $p < 0.001$  (Fig. 5a); *Acromyrmex subterraneus subterraneus*:  $F_{(3, 27)} = 6.3$ ,  $p < 0.001$ ) (Fig. 5b).

In relation to the speeds of the participants of a transport chain, cutters were slower than carriers in both species (Fig. 5c and 5d). This difference was statistically significant when comparing the speeds of cutters and last carriers. Last carriers were faster than cutters (*Acromyrmex*

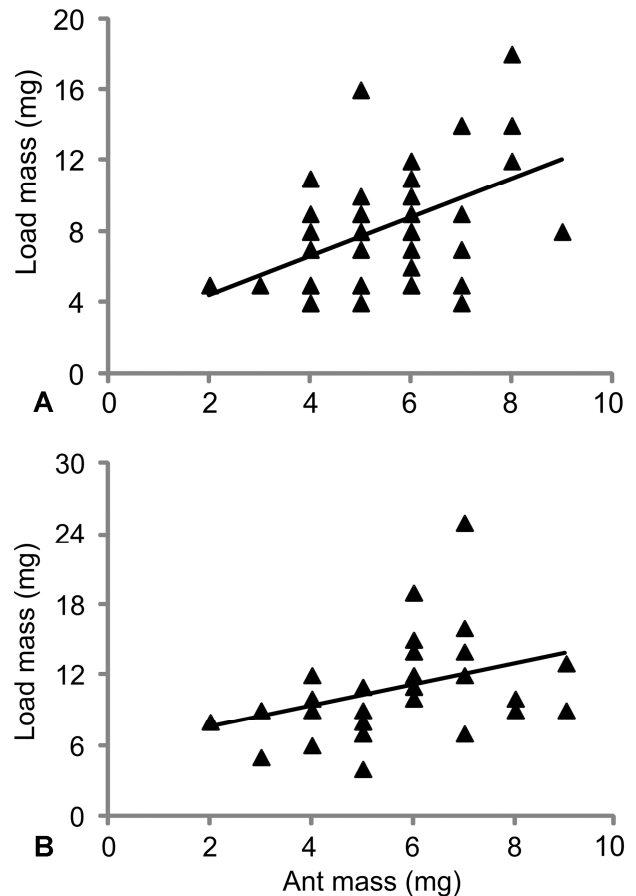


Fig. 4: Relationship between load mass and body mass of cutters, in field conditions. (A) *Acromyrmex crassispinus*; (B) *A. subterraneus subterraneus*.

*crassispinus*: cutters: 0.65 ± 0.45 cm / s ( $\bar{x} \pm SD$ ), n = 26, first carrier: 0.79 ± 0.36 cm / s, n = 26, middle carrier: 1.05 ± 0.71 cm / s, n = 14, last carrier: 1.21 ± 0.49 cm / s, n = 26, One-way ANOVA:  $F_{(3, 107)} = 8.16$ ,  $p < 0.001$  (Fig. 5c); *Acromyrmex subterraneus subterraneus*: cutters: 0.83 ± 0.40 cm / s, first carrier: 0.97 ± 0.40 cm / s, n = 9, middle carrier: 1.21 ± 0.53 cm / s, n = 4, last carrier: 1.53 ± 0.56 cm / s, n = 9,  $F_{(3, 51)} = 6.31$ ,  $p < 0.001$ ) (Fig. 5d).

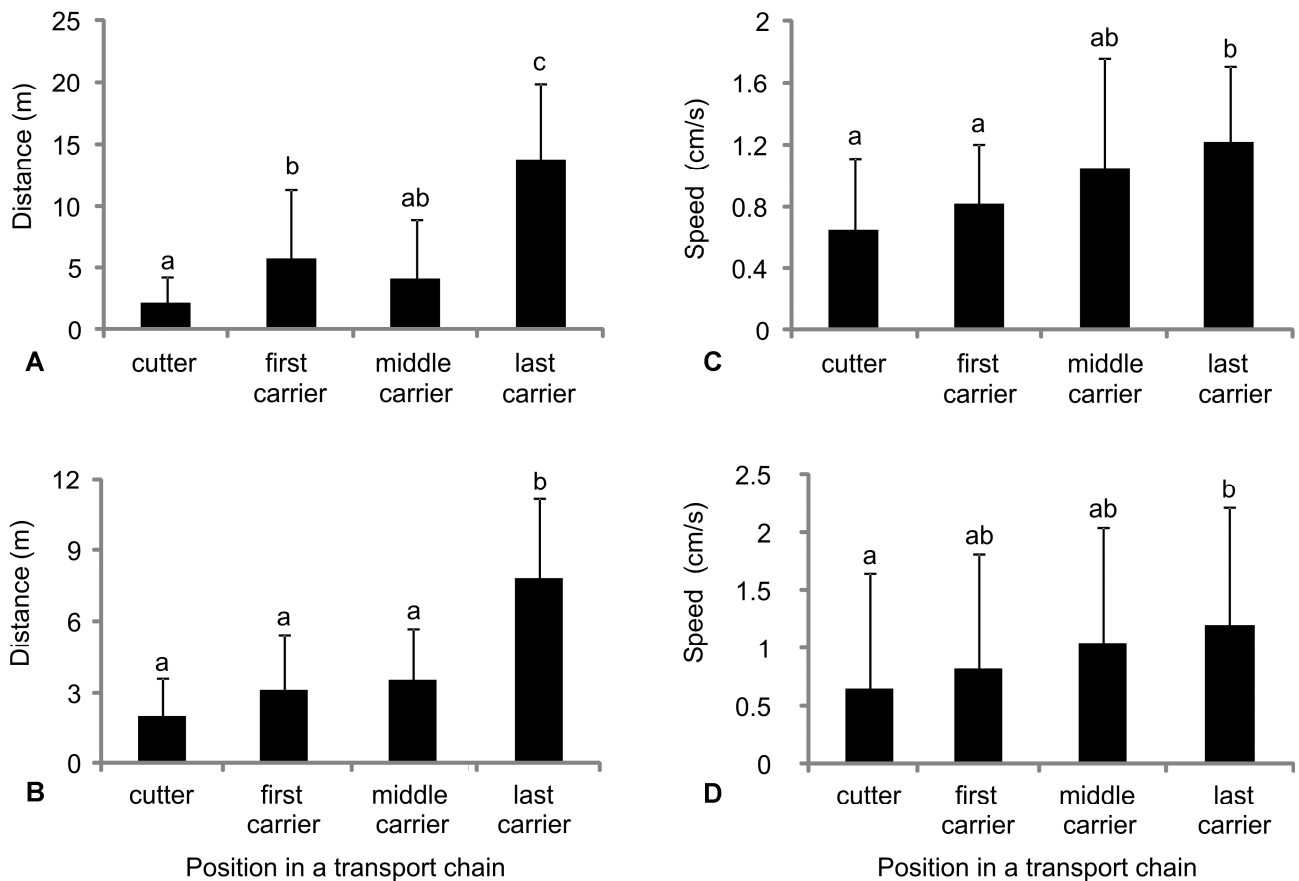


Fig. 5: Transport distances and speeds of cutters, first, middle and last carriers in a transport chain, on the long trail, in field conditions. Transport distances: (A) *Acromyrmex crassispinus*; (B) *A. subterraneus subterraneus*. Transport speeds: (C) *A. crassispinus*; (D) *A. subterraneus subterraneus*. Different letters represent statistically significant differences among treatments according to Tukey's test ( $p < 0.05$ ).

### Laboratory experiments

*Acromyrmex crassispinus* cutters transferred 24% ( $n = 12$ ), 12% ( $n = 6$ ), and 2% ( $n = 1$ ) of the fragments to other workers on long, intermediate and short trails, respectively. All fragments were indirectly transferred to other workers. Direct fragment transfers to other workers were not observed under laboratory conditions. The proportion of transferred vs. carried fragments varied significantly at the different trail distances (Fig. 1c, long trail:  $\chi^2 = 13.54$ ,  $p < 0.001$ ; intermediate trail:  $\chi^2 = 28.90$ ,  $p < 0.001$ ; short trail:  $\chi^2 = 46.10$ ,  $p < 0.001$ ).

In the long trail, *Acromyrmex subterraneus subterraneus* cutters transferred 42% ( $n = 21$ ) of the fragments to other workers. Twenty-four percent ( $n = 12$ ) of the fragments were directly transferred and 18% ( $n = 9$ ) of the fragments were indirectly transferred to other workers. In the intermediate trail, 16% ( $n = 8$ ) of the fragments were transferred to other workers. In this case, 8% ( $n = 4$ ) of the fragments were indirectly transferred and 8% ( $n = 4$ ) of the fragments were directly transferred to other workers. Fragment transfers were not observed in the short trail for this species in laboratory conditions. The proportion of transferred vs. carried fragments differed significantly between intermediate and short trail distances (Fig. 1d, intermediate trail:  $\chi^2 = 23.14$ ,  $p < 0.001$ ; short trail:  $\chi^2 = 50.02$ ,  $p < 0.001$ ). However, there was no significant dif-

ference in the proportion of transferred vs. carried fragments at the long trail distance (Fig. 1d, long trail:  $\chi^2 = 1.3$ ,  $p > 0.05$ ).

Transport chains have occurred just on the long trail in both studied species. Two percent ( $n = 1$ ) and 6% ( $n = 3$ ) of the fragments were transported by transport chains, in *Acromyrmex crassispinus* (Fig. 3c,  $\chi^2 = 4.25$ ,  $p < 0.05$ ) and *Acromyrmex subterraneus subterraneus* (Fig. 3d,  $\chi^2 = 6.75$ ,  $p < 0.05$ ), respectively. All transport chains were composed of 2 carriers. In relation to the other transport modalities, transport by single carriers occurred significantly more often on the long trail (*Acromyrmex crassispinus*: Fig. 3c,  $\chi^2 = 8.45$ ,  $p < 0.05$ ; *Acromyrmex subterraneus subterraneus*: Fig. 3d,  $\chi^2 = 18.86$ ,  $p < 0.001$ ). Cutters transported their fragments directly to the nest significantly more often on the short trail in *Acromyrmex subterraneus subterraneus* (Fig. 3d,  $\chi^2 = 5.59$ ,  $p < 0.05$ ). In *Acromyrmex crassispinus*, there was no significant difference in the behavior of cutters (Fig. 3c,  $\chi^2 = 1.41$ ,  $p > 0.05$ ).

### Discussion

The three different modalities for the transport of fragments along the trail proposed by RÖSCHARD & ROCES (2003a) to *Atta vollenweideri* were observed in *Acromyrmex crassispinus* and *Acromyrmex subterraneus subterraneus*: 1) Cutters: A cutter carries the fragment directly to the nest; 2) single carriers: Fragments put down on the trail by cutter,

or directly transferred, are retrieved by a worker and carried all the way to the nest; 3) transport chain: Fragments found on the trail or directly received from nestmates are transported consecutively by different carriers. On long trails, fragments were mostly transported by chains, i.e., in addition to the cutter, more two or three carriers transported the fragments sequentially. On the other hand, on the short trails, fragments were mostly transported by cutters directly to the nest.

In *Acromyrmex crassispinus* and *Acromyrmex subterraneus subterraneus*, cutting and carrying fragments were clearly separated activities performed by distinct worker groups differing in body size. Cutters were larger than carriers. Similar patterns were observed in other leaf-cutting ants. *Atta vollenweideri* sampled on the source immediately after cutting were significantly larger than carrying ants on different trail sectors (RÖSCHARD & ROCES 2003a, b). Because of body size allometry, larger ants have disproportionately larger heads and therefore more massive mandibular muscles. Thus, larger workers perform an energetically more intensive activity (ROCES & LIGHTON 1995). Carrying the loads back to the nest therefore requires less energy per unit time, yet it might require a considerable time investment (LEWIS & al. 1974).

In *Acromyrmex crassispinus*, transfer is mostly indirect, in other words, fragments were dropped on the ground and collected by outgoing workers that turned back and returned to the nest. Direct fragment transfers between workers were not observed under laboratory conditions. It was observed only on long trails in the field. LOPES & al. (2003) also did not observe direct fragment transfers for this species under laboratory conditions. In *Acromyrmex versicolor versicolor* (PERGANDE, 1893), workers cut leaves and let them drop to the ground, which are then collected on the following day (GAMBOA 1975). In *Atta vollenweideri* transfer is also mostly indirect (RÖSCHARD & ROCES 2003a). In contrast, transfer is mostly direct in *Acromyrmex subterraneus subterraneus*, although indirect transfer can take place along the trail. The direct leaf transfer was also observed in *Atta cephalotes* (HUBBELL & al. 1980), *Acromyrmex balzani* (EMERY, 1890) (LOPES & al. 2003) and *Acromyrmex octospinosus* (REICH, 1793) (SUMNER 2000, HART & al. 2002).

*Acromyrmex crassispinus* and *Acromyrmex subterraneus subterraneus* workers neither prefer certain places on the trail for transferring fragments, nor build up piles on a given location. *Atta vollenweideri* shows the same behavior (RÖSCHARD & ROCES 2003a). In contrast, *Atta cephalotes* and *Atta colombica* form piles or caches of leaves on foraging trails. Leaf caches occur in the field at foraging trail junctions, obstacles on the trail and within nest entrance tunnels (HART & RATNIEKS 2000, 2001). HART & RATNIEKS (2001) argued that cached leaves were more likely to be recovered than non-cached leaves at all points along all trails, especially the case near the nest entrance, where cached leaves can be nearly ten times more likely to be recovered per ant than non-cached leaves.

There was no difference in the transport time spent for a fragment to be carried all the way to the nest only by a cutter, or by a cutter plus a single carrier, or by a transport chain (a cutter plus at least two carriers), in both species studied here, although sequential transport was slightly longer than the transport of fragments only by cutters. In *Atta*

*colombica*, it takes 70% longer to transport leaves back to the nest from a cache than without caching (HART & RATNIEKS 2001). In *Atta vollenweideri*, transport time of fragments carried by a chain was 25% longer (on average 8 min longer) than that of fragments carried by a single worker all the way to the nest. This was probably due to both the waiting time of the dropped fragments, and the handling time by the subsequent foragers (RÖSCHARD & ROCES 2003a).

The behavior of transferring fragments between workers might occur because of a mismatch between body and fragment size, i.e., either the carrier is too small for the fragment, or the fragment exceedingly large to be carried. In *Atta vollenweideri* fragment size correlated with worker size only for the last carriers – those that covered the longest distance – but not for the first carriers. Thus, sequential transport via transport chains leads to a better size-matching between worker and load (RÖSCHARD & ROCES 2003a). However, in this study, size matching between worker and load was not observed for the last carriers of a transport chain (those that covered the longest distance). Size matching was observed between cutters or first carriers and their loads, suggesting that this hypothesis is not likely for *Acromyrmex* species. Similar results were observed for *Atta colombica* workers retrieving cached leaf fragments. When recovering cached leaves, *Atta colombica* foragers did not select leaves based on their size (HART & RATNIEKS 2001).

There was no difference in loading ratio of cutters that transported their fragments to the nest and those that transferred their fragments in the *Acromyrmex* species studied here. The same result was observed in the loading ratio of single carriers and carriers that composed a transport chain. However, the speeds of those workers were significantly different. Workers that transferred their fragments were slower than those that carried it to the nest. Last carriers (which covered the longest distance) were faster than other workers involved in a transport chain. These results could support the "economic transport hypothesis" (ANDERSON & JADIN 2001, RÖSCHARD & ROCES 2003a): transporter ants may be going exceedingly slow, or at least slower than the receiving ant could travel, and therefore transfer occurs in order to increase leaf transportation speed. Then, slow-moving ants are more likely to transfer their leaves, either directly or indirectly, than faster moving ants (ANDERSON & JADIN 2001). However, a low travel speed does not necessarily indicate that a worker is not capable of carrying the load, or of walking faster (ROCES 1993, BOLLAZZI & ROCES 2011). Travel speed may be reduced because of trail-marking activity by the carriers, or workers may slow down because they try to pass the carried fragment to an unladen nestmate, and not because of the burden, thus being able to return to the source after unloading (HUBBELL & al. 1980, RÖSCHARD & ROCES 2011).

The maximization of the transportation speed of a leaf fragment is expected to occur when the transporting ants move too slowly, because they carry relatively large fragments. However, there were no differences in loading ratio among workers. Trail conditions could contribute to the travel speed of the ants. Leaf cutting ants maintain long-lasting trunk trails despite foraging on leaf resources. These trunk trails give access to numerous partially cleared, temporary trails that connect specific resources to the colony (HOWARD 2001). Trail structure changes considerably with



distance, being generally narrower, less cleared of vegetation, and with obstacles further away from the nest (RÖSCHARD & ROCES 2011). On the other hand, trails near the plant source might be only partially cleared, possibly affecting negatively ant travel and their speeds. Furthermore, RÖSCHARD & ROCES (2011) observed that *Atta vollenweideri* foragers walking very slowly or even stopped walking before dropping a load. These ants started at the source with a higher speed and then reduced their speed on the way, suggesting that they would have been able to continue walking at the same pace. Thus, ants walked slowly because they were going to drop their loads, rather than dropping their loads because the burden forced them to walk at a slow pace. These results do not support the "economic transport hypothesis" for *Atta vollenweideri* (RÖSCHARD & ROCES 2011), nor for the *Acromyrmex* species observed in this study.

Sequential transport of fragments leads to an increase in the information flow along the foraging trail ("information-transfer hypothesis"), in such a way that more workers, either via direct transfers or upon finding a dropped fragment, get informed about the kind of resource being actually harvested. An improved information transfer may result, because of new recruitment, in an increased overall rate of resource transportation. The behavioral response of transferring fragments, either directly or indirectly, may also have been selected for because of its positive effect on information flow (RÖSCHARD & ROCES 2003a, 2011).

In the present study, we found that *Acromyrmex* species accomplish division of labor between cutters and carriers, and task partitioning during leaf transport. Trail lengths have marked effects on sequential load transport – the farther the resource collected by workers, the higher is the frequency of occurrence of the transport chain. Furthermore, the results of this study provide support to the hypothesis that the behavioral response of transferring fragments in *Acromyrmex* species would have been selected for because of its positive effect on the information flow between workers.

*Acromyrmex* species have small colonies and lower social complexity when compared with *Atta* species. However, *Acromyrmex crassispinus* and *Acromyrmex subterraneus subterraneus* behave in the same way as *Atta* species during leaf transport along the foraging trails. Additional studies should be conducted with other *Acromyrmex* species to verify if the behavior would be similar to that observed in the present study. Moreover, additional studies should be carried out to validate the information transfer hypothesis for *Acromyrmex* species, evaluating the worker behavior during transport of fragments with different size or quality, and the behavior of workers during transport of materials in other contexts that are not necessarily associated with high information demands. Furthermore, studies that seek to understand why some species transfer their fragments directly and others transfer their fragments indirectly would be interesting to the further understanding of the foraging strategies in *Acromyrmex* species.

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