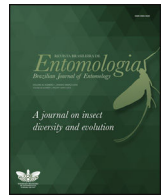




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## Hierarchical establishment of information sources during foraging decision-making process involving *Acromyrmex subterraneus* (Forel, 1893) (Hymenoptera, Formicidae)

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### ABSTRACT

During foraging, worker ants are known for making use of many information sources to guide themselves in external environments, especially individual (memory) and social (trail pheromone) information. Both kinds of information act in a synergic way, keeping the foraging process efficient and organized. However, when social and individual information is conflicting face a trail bifurcation, it is necessary to establish a hierarchical order so prioritizing one of them. This study aims to verify which information (social or individual) is prioritized by *Acromyrmex subterraneus* workers when facing a bifurcation in a Y-trail system. Only one branch of the Y-trail leads to food resource and it had a section covered by filter paper where trail pheromone was deposited by workers. Pheromone deposition was here estimated by worker flow. After an individually marked forager (target-worker) made 1, 3 or 5 trips to the food resource, the filter paper was transferred to the branch which did not lead to the food. The time spent by target workers on branch selection and their right choice (branch with food) frequency were registered. Regardless of the target worker's previous trips to the resource, right choice frequency stood over 70%. In addition, the number of previous trips did not influence the time spent on decision making. However, the higher the flow of workers, the longer the time spent on decision making. By simulating a situation with conflicting information, it was possible to verify that a hierarchical order is established by *A. subterraneus*, which prioritized individual information (memory).

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### Introduction

Leafcutter ants forage in the external environment searching for fresh leaves which they use to cultivate their symbiotic fungus, primary food source of the colony (Hölldobler and Wilson, 1990). These incursions happen through physical and chemical trails that guide them between the colony and food sources (Jaffé and Howse, 1979; Wyatt, 2003; Lopes et al., 2016). Because trails persist in the environment for certain periods of time, foraging ants make repeated visits to those food sources (Ribbands, 1949; Traniello, 1977; Rosengren and Fortelius, 1986; Quinet and Pasteels, 1996; Lopes et al., 2016).

In order to guide themselves along the foraging trail, worker ants use a whole set of available information that can be distinguished between individual and social information. Individual information derives from physical contacts (Hölldobler, 1976; Jaffé and Howse, 1979; Gordon and Mehdiabadi, 1999; Farji-Brener et al., 2010), visual memory (Vilela et al., 1987) and experience whereas social information comes from chemical trails established by pheromone deposition (Hölldobler and Wilson, 1990; Guajara et al., 1990; Gerbier et al., 2008).

Despite using both types of information, as reported for *Lasius niger* workers (Linnaeus, 1758) (Beckers et al., 1990; Aron et al., 1993; Evison et al., 2008), in different contexts, foragers can prioritize individual memory or pheromone concentration to decide on what path to follow face a trail bifurcation (Rosengren and Fortelius, 1986; Harrison et al., 1989; Grüter et al., 2011).

Visual orientation occurs by means of visual clues or marks that enable memory formation (Collett and Collett, 2002), since, after a

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way is walked repeated times, visual marks end up being memorized. Nevertheless, there are trails with more than 50 bifurcation points and, in this case, orientation by visual marks can lead to errors in the decision on which branch should be followed (Kost et al., 2005). Thus, foragers need to use other information sources to choose what path to take, especially the social information that derives from the application of chemical trails (Guajara et al., 1990; Gerbier et al., 2008; Sales et al., 2015).

Several studies have investigated the importance of social over individual information through experimental manipulations that create conflicts on which information should be prioritized and used in the decision-making process (Hölldobler, 1976; Harrison et al., 1989; Rosengren and Fortelius, 1986; Aron et al., 1993; Salo and Rosengren, 2001; Morgan et al., 2006; Evison et al., 2008).

However, few studies confronting social and individual information have been conducted with leafcutter ants. Vilela et al. (1987) and Guajara et al. (1990) investigated which of these two types of information *Acromyrmex octospinosus* (Reich, 1793) and *Acromyrmex subterraneus molestans* (Forel, 1893) workers, respectively, have used to guide themselves under laboratory conditions, with both studies having social information as priority. Elizalde and Farji-Brener (2012), in turn, investigated whether *Acromyrmex lobicornis* workers (Emery, 1888) were faithful to the foraging trail in natural environment, whereas Roces (1994) analyzed whether *Acromyrmex lundii* workers (Guérin-Méneville, 1838) had olfactory memory. Both studies support the idea that worker ants prioritize individual information. Therefore, this study aimed to verify which information, social or individual, is prioritized by *A. subterraneus* workers face a bifurcation in a Y-trail system after visiting the foraging arena.

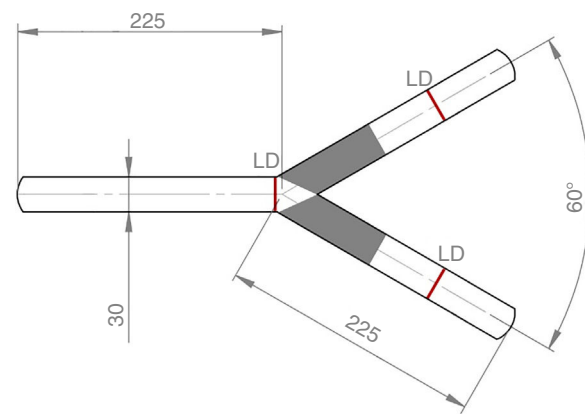
## Material and methods

### Study species

Five *A. subterraneus* colonies were used, coming from the collection of queen ants fecundated in 2013 at the Federal University of Juiz de Fora (UFJF), Juiz de Fora, Minas Gerais, Brazil. The colonies are kept in a closed system composed of three plastic containers (diameter: 7 cm × 15 cm × 7 cm) interconnected by transparent plastic tubes, referring to the garbage chamber, the fungus chamber and the foraging arena. The fungus chamber had inside a 1 cm plaster layer to keep humidity and approximately 2.5 L of symbiont fungus. The colonies were kept in the Myrmecology Laboratory under control conditions of temperature (25–30 °C) and relative humidity (75–80%). The colonies were provided *Acalypha wilkesiana* (Euphorbiaceae) leaves daily, except for 24 h before each experiment.

### Y-trail system design

In partnership with the Institute of Arts and Design [*Instituto de Artes e Design*] (IAD/UFJF) and the College of Architecture and Urbanism of the Federal University of Juiz de Fora [*Faculdade de Arquitetura e Urbanismo*] (FAU/UFJF), a simple trail system was made in Medium-density fiberboard (MDF). It had a Y shape with branches of equal length (22.5 cm) and width (3 cm), with upper branches arranged at an angle of 60°. On each branch of the Y-trail decision lines (DL) were set, by means of which the workers' frequency of passage was determined. On the right and left branches, DL was established 14 cm far from the bifurcation center and 2.5 cm on the base branch (Fig. 1). The colony was coupled through the fungus chamber to the base branch of the Y trail and on each upper branch; a plastic tray was installed, where food was provided.



**Fig. 1.** Y-trail system with branches of equal length (225 mm). Branches arranged at an angle of 60° connected to a bifurcation. Decision lines (LD) established at fixed points 140 mm far from the bifurcation center on the right and left branches, and 25 mm from the base branch to calculate the *A. subterraneus* workers' frequency of passage when half of their bodies had crossed each LD.

### Experimental procedure

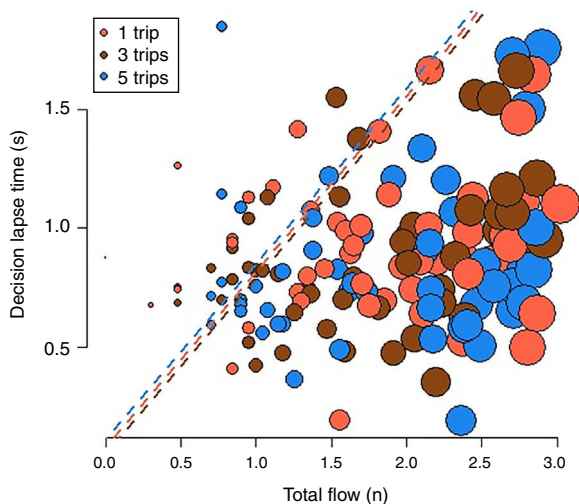
In order to verify whether there is priority in the use of social (trail pheromone) and individual (path memorization) information, a Y-trail system was adopted, with 100 *A. wilkesiana* disks (0.5 cm diameter) randomly positioned in only one of the arenas of every experimental event. Food was provided alternately to the right and left branches in order to prevent conditioning as to food location over repetitions.

A filter paper (length: 10 cm; width: 3 cm) was placed on each of the right and left branches of the Y trail, allowing for later manipulation of trail pheromone deposited on them. Right after, the worker ants were allowed to walk freely through the Y trail. The first seven workers that reached the foraging arena with the food received an individual non-toxic ink mark in the abdomen. Each one of those marked individuals was allowed to make 1, 3 and 5 trips between the colony and the food. These three pre-determined numbers of trips made up treatments, considering that the higher the number of trips, the greater the memorization of the path.

Concomitantly, the flow of workers at every minute was counted only for the branch with access to the food. The flow of workers reflects on the concentration of trail pheromone; it is known that the pheromone deposition is made from the attractive (if attractive) resource during the coming back to the nest (Jaffé and Howse, 1979; Bradshaw and Howse, 1984; Morgan et al., 2006). Flow determination started when the first marked worker ant crossed the DL toward the colony.

When one of the marked workers completed the number of trips defined for each treatment, it was regarded as the target worker, and the flow of worker ants was interrupted. At that moment, the filter paper that was on the no-resource branch was discarded and replaced with the filter paper that was on the branch that led to the food. Thus, the target worker, when returning to the trail heading to the food, has two information to find food: its previous experience (number of trips) and a wrong piece of social information, considering the filter paper exchange for the branch that does not led to the food source.

After the manipulation of the filter paper and the arrival of the target worker at the DL of the base branch, a chronometer was activated to record the time spent during the displacement toward the DL of the right and left branches of the Y trail. This time lapse was called decision time. The chosen branch was registered and when it corresponded to the branch with food, it was considered that the target worker had made the right choice.



**Fig. 2.** Decision time (s) spent by the *A. subterraneus* target worker according to number of trips ( $n$ ) made by the respective target worker and the concentration of pheromone manipulated on the branch that does not lead to the food, estimated by total flow of foragers.

Thus if the target worker made the right choice (branch with food), it would be following individual information (memory) and if it made the wrong choice (branch with no food), the social information (trail pheromone) was prioritized.

Ten repetitions were performed for each treatment in each colony, totaling 150 experimental events. At the end of each repetition, marked workers were taken off from the colony, and the Y-trail system was cleaned with alcohol 70° GL for removal of chemical traces (trail pheromone).

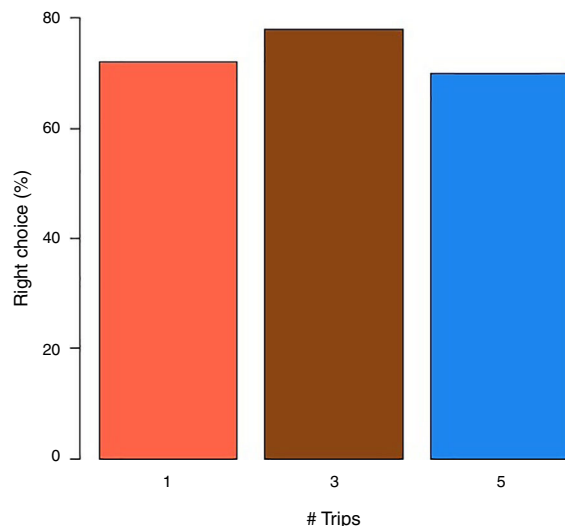
#### Data analysis

Data were analyzed through generalized linear mixed model (GLMM), which tested whether the decision time varied according to the flow of worker ants (explanatory continuous variable) and number of trips (fixed continuous variable). It is worth highlighting that the flow of workers represents social information, since it reflects pheromone deposition, and the number of trips, individual information, since it allows previous knowledge of the trail, food location and consequent path memorization. The colonies were included as random variable for having dependent behaviors. Data on flow of workers and decision time were transformed into  $\log^{10}$  for normalization. Moreover, it was assessed whether the number of right choices varied according to the number of trips through an ANOVA. All statistical analyses and graphs were carried out on R software.

#### Results

It was possible to register that the higher the flow of workers, the longer the target workers' decision time ( $F=7.33$ ;  $GL=142$ ;  $p=0.007$ ), indicating that placing the trail pheromone on the wrong branch (that did not lead to the food) caused a delay in the decision-making process. On the other hand, the decision time did not vary with the number of trips (memory) ( $F=0.447$ ;  $GL=142$ ;  $p=0.6403$ ), evidencing that the performance of 1, 3 or 5 trips did not reduce decision time (Fig. 2).

Results also point that the right choice was made more than 70% of times but there was no difference between these frequencies according to the number of trips (memory) ( $F=0.377$ ;  $GL=2, 12$ ;  $p=0.694$ ) (Fig. 3).



**Fig. 3.** The mean frequency of right choices (branch that led to food) made by *A. subterraneus* target workers did not vary with the number of trips made (memory).

#### Discussion

For the three defined numbers of trips, there was a frequency of right choices higher than 70%, indicating that *A. subterraneus* workers prioritized individual information, that is, selected the branch that led to food regardless of the manipulation of pheromone concentration. Such a fact is confirmed by the increase in the time spent on decision making as the pheromone concentration that led to the wrong branch increased (with no food).

Results evidence that when there was conflict between social (trail pheromone) and individual (memory) information in the Y-trail bifurcation, *A. subterraneus* workers used both information sources but prioritized individual information, thus walking through the branch where they had found food in previous trips. In this way, one can infer about information use hierarchy, in which memorized information was used first, resulting in a high frequency of right choices as to the path.

Considering both results altogether, listing the high frequency of right choices and increased decision time with the increase in the pheromone concentration indicating the wrong path, it is possible to notice that social information interferes with memorized information, though not affecting decision making.

Prioritization of individual information face a bifurcation point registered in the present study contrasts with results found by Vilela et al. (1987) and Guajara et al. (1990), which studied respectively *A. octospinosus* and *A. subterraneus molestans*. In both studies, worker ants made decisions in light and dark regimes, with consequent hindrance to using visual information, since they were not able to use visual clues.

However, Guajara et al. (1990) suggest that workers that have access to both social and individual information return more quickly to the colony than those restricted exclusively to one information or the other. This is made evident in their results when *A. subterraneus molestans* workers took longer to walk through the trail system in the absence of a chemical trail and light, indicating how both types of information influence the displacement of individuals. Nevertheless, it does not suggest other possible pieces of information worker ants could have used.

In the present study, the confronted types of information were social information manipulated for the wrong path (trail pheromone) and individual information (path memory) in the choice of the right path, and in such context *A. subterraneus* workers

used individual memory when information was conflicting, that is, indicated different paths as being the correct ones.

Prioritization of memory over other information was also observed among other ant species with mass recruitment (Verhaeghe, 1982; Rosengren and Fortelius, 1986; Harrison et al., 1989; Aron et al., 1993; Fourcassié and Beugnon, 1988; Jeanson et al., 2003; Grüter et al., 2011), suggesting that, although increase in mass recruitment efficiency is attributed to chemical trail (Jaffé and Howse, 1979), foraging workers are not limited to follow only that social information.

Considering that the greater the flow of workers the higher the pheromone concentration on the substrate (Jaffé and Howse, 1979; Morgan et al., 2006), increased decision making time according to increased flow of workers is another evidence that social and individual information operate synergically. In fact, trail pheromone (social information) facilitates path memorization (individual information) (Czaczkes et al., 2011, 2015).

When the trail pheromone deposited in the filter paper that led to the right path was placed on the wrong path, individual and social information became conflicting rather than synergic, which caused the workers to find the food with delay.

A possible hypothesis to the fact that *A. subterraneus* workers prioritize individual information relates to the fact that foragers have a fixed return place (colony), adapting to the concept of “central place foragers” (Wyatt, 2003). When using stable and permanent food sources, such as plants cultivated in the course of days or even weeks, foraging workers are exposed to the same visual panorama, which facilitates path memorization (Grüter et al., 2011). By prioritizing memory, workers reduce exploratory actions, as well as predation risks, given that the place where the resource is becomes known (Clarke et al., 1993; Stamps, 1995; Brown, 2001), in addition to rising the likelihood of food acquisition (Laca, 1998; Jakob et al., 2001).

Face the conflict of information simulated in the present investigation, the latter suggests the establishment of a hierarchical order in the use of information about *A. subterraneus* with prioritization of individual information (memory).

## Conflicts of interest

The authors declare no conflicts of interest.

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