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Waste Management in the Leaf-Cutting Ant *Acromyrmex lobicornis*: Division of Labour, Aggressive Behaviour, and Location of External Refuse Dumps

Sebastián Ballari,[1] Alejandro G. Farji-Brener,[1,2] and Mariana Tadey[1]

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In leaf-cutting ants, the handling of waste materials from the fungus culture increases the risk of infection. Consequently, ants should manage their waste in a way that minimizes the spread of diseases. We investigated whether in Acromyrmex lobicornis*, waste-worker ants* (a) *also perform roles in foraging or mound maintenance,* (b) *are morphologically different than other ant workers, and* (c) *are aggressively discriminated by other worker ants from the same colony. In addition, we investigated whether the location of external waste piles minimizes the probability that wastes spread to the ant nest. In the field, we* (a) *marked with different colours waste-workers, foragers and mound-workers and monitored whether these ants interchanged their tasks;* (b) *measured head width, head length, hind femur length and total length of waste-workers; foragers and mound-workers;* (c) *forced field encounters between waste-workers and foragers, and* (d) *measured the cardinal orientation of the waste piles in relation to the colony mound. Waste-worker ants did not perform other function outside the nest; neither foragers nor mound-workers managed the waste. Moreover, waste-workers were smaller than foragers and mound-workers, and were attacked if they tried to enter their nest using foraging entrances. The location of external refuse dumps also appears to reduce contamination risks. Waste piles always were down-slope, and often followed the prevailing wind direction. The importance of behaviours*

[1] Departamento de Ecología, CRUB, Universidad Nacional del Comahue, (8400) Bariloche, Argentina.
[2] To whom correspondence should be addressed; e-mail: alefarji@crub.uncoma.edu.ar, alefarji@yahoo.com.

such as the division of labour, aggressions against waste-workers and nest compartmentalization (i.e., the orientation of external waste piles) to minimize the spread of pathogens is discussed.

KEY WORDS ant debris; division of labour; hygienic behaviour; refuse dump.

INTRODUCTION

Among ant societies, leaf-cutting ants are considered the premier example of how division of labour improves colony performance (Hölldobler and Wilson 1990). Differently sized workers often exhibit different behaviours, and this division of labour improves the overall foraging process (Hart *et al.*, 2002). For example, whilst larger workers cut a leaf, minor workers transport the fragments; and this behaviour increase the rate of leaf transport (Roschard and Roces, 2003). Another example is the presence of minor workers on the transported leaf ("hitchhiking" ants), which reduces the attack of parasitoids flies (Feener and Moss, 1990). However, there are others activities besides foraging, which also include complex behaviours but are much less well-studied.

A common and complex leaf-cutting ant activity is the transport and manipulation of their waste (Bot *et al.*, 2001). Leaf-cutting ants selectively collect large quantities of fresh vegetation from a large area and carry them to their nest chambers where the plant material is degraded by a mutualistic fungus (Cherrett, 1989). The waste materials from the fungal decomposition are removed from the fungus gardens to specific external or internal disposal areas (hereafter, refuse dumps). Several studies showed that refuse dumps are contaminated with fungal competitors, specialized parasites (e.g., the fungus *Escovopsis*), and other undesirable microorganisms potentially lethal to the fungus gardens and the ants themselves (Fisher *et al.*, 1996; Currie *et al.*, 1999; Bot *et al.*, 2001; Poulsen *et al.*, 2002). Therefore, the accumulation of waste implies a risk of infection. This risk is a key selection factor in the complex leaf-cutting ant society because the interaction of individuals in groups facilitates the spread of diseases (Schmid-Hempel, 1998). Hence, the transportation, manipulation and location of refuse dumps should be managed in a way that minimizes the risk of infection (Bot *et al.*, 2001; Hart *et al.*, 2002; Poulsen *et al.*, 2002; Fernández-Marín *et al.*, 2003).

To minimize the potential spread of pathogens, leaf-cutting ants often display a division of labour and task partitioning in the management and transportation of its own waste (Hart *et al.*, 2002). In the genus *Atta*, while a set of workers remove the waste from the fungus culture chambers; other workers transport these wastes to their final destiny (Hart and Ratnieks, 2001). This behaviour apparently diminishes the spread of pathogens that inhabit the refuse pile. Toxic and non-toxic areas are segregated, and fungus garden workers do not enter the waste chamber, nor do waste workers enter the garden. Additionally, some ants when they are young actively work in the fungus culture, but when they get older they work in the management of waste. Thus, hazardous tasks like waste manipulation are performed by ants at the end of their lives, reducing the risk of infection in the young ants with a longer potential lifetime (Bot *et al.*, 2001; Hart and Ratnieks, 2001). This division of labour is also maintained by aggressive behaviours against the waste-worker ants (hereafter WWA), but this level of hostility apparently depends on the location of refuse dumps. For example, in *Atta cephalotes*, WWA are attacked if they try to abandon the internal debris chamber (Hart and Ratnieks, 2001). In contrast, ant species that locate their refuse on the soil surface showed a low aggressive behaviour (Hart *et al.*, 2002). Despite these differences, there is little information about the management of waste in leaf-cutting ants with external refuse dumps, like several *Acromyrmex* species, which have smaller colonies and less sophisticated division of labour than *Atta* species (Hölldobler and Wilson, 1990).

Acromyrmex lobicornis Emery is one of the leaf-cutting ant species with the largest distribution in South America, and the only species that reach arid regions of Patagonia (Farji-Brener and Ruggiero, 1994). *A. lobicornis* nests reach depths of 1 m, and on the soil surface the ants construct a mound of twigs, soil and dry plant material, which may reach a height and width of 1 m. Inside this mound, ants tend the fungus on which ant larvae feed. Waste material is removed from the internal fungus garden and dumped onto the soil surface near the mound using specific nest-holes (Farji-Brener, 2000). A study revealed that *A. lobicornis* ants often avoid the contact with their waste (Farji-Brener and Sasal, 2003). This suggests that, as in other leaf-cutting ant species, refuse dumps contain microorganisms harmful for the colony. Consequently, *A. lobicornis* should exhibit hygiene issues similar to other leaf-cutting ant species (Hart *et al.*, 2002). To our knowledge, there is no information for *A. lobicornis* about waste manipulation. It is not known if waste transport is performed by older ants or by a specific worker caste, nor if there are aggressive behaviours against WWA, to minimize their contact with nest-mates in the colony. In addition, waste in external piles can spread through the nest due to rain, wind or gravity. Therefore, the location of external refuse dumps may be important to reduce the chance of colony infection.

Our objective was to describe the management of waste in the leaf-cutting ant *Acromyrmex lobicornis*. Particularly, we established whether (a) WWA were morphologically different from those ants that performed activities outside the nest, as foraging and mound maintenance, (b) WWA showed fidelity to their work (i.e., do not perform other activities outside the nest), and (c) WWA were attacked by ants of their own colony if they tried to enter the nest using foraging entrances. Finally, we analyzed whether the location of the external refuse piles in relation to the main wind direction and slope minimize the chance that the waste would contact the nest. Despite the fact that studies of internal waste-management is only possible with laboratory nests, working under laboratory conditions often limits the number of real replicates (i.e., nests) and this might offer biased information about what happens in nature. Therefore, we studied waste management of *A. lobicornis* in field conditions.

METHODS

Study Area

The study area is located in the eastern border of Nahuel Huapi National Park, Northwest Patagonia, Argentina (41°S, 71°W), and is covered by herbaceous/shrub steppe vegetation. The mean annual temperature is 8°C and the mean annual precipitation is about 600 mm. Strong winds are very frequent in this area. During almost 90% of windy days, winds come from the west with a mean velocity of 33.2 Km/h (years 1991–2000, Servicio Meteorológico Nacional, Fuerza Aérea Argentina).

We conducted surveys in steppe areas near roadsides because in these areas *Acromyrmex lobicornis* is more frequent than in sectors distant from roads (Farji-Brener, 2000). Given the characteristics of the study area the dominant vegetation is a mix of native species typical of Patagonian steppes (e.g. *Stipa speciosa*, *Mulinum spinosum*, *Imperata condensata*, *Plagiobothrys tinctoreus* and *Baccharis pingraea*), and exotic species (e.g. *Bromus tectorum*, *Onopordon acanthium*, *Carduus nutans* and *Verbascum thapsus*) (Correa, 1969–1998).

Division of Labour

To asses the existence of division of labour between WWA and ants that perform activities outside the nest (i.e., foraging and mound maintenance), we conducted the following field experiments. First, in 5 active nests (mound of >1 m of diameter) we marked with 2 different colours 50 WWA and 50 foraging ants in their thorax (total $=$ 500 ants) with a small dot of water-based acrylic paint. WWA were sampled in external refuse piles, and foraging ants sampled in active foraging trails. During 5 consecutive days we observed marked ants in external refuse piles and in the 3 main active foraging trails. In each sample point (refuse dumps and foraging trails) we counted the number of marked ants and their colour, during 30 min. We performed a total of 20 observations (4 per day). Second, we repeated the same protocol but with foraging ants and ants that were working on maintenance of the nest mound (hereafter mound-workers). If WWA show higher fidelity to their work than other working ants, we expect that foragers and mound-workers may exchange their tasks, but WWA do not. These data were analyzed using a Chi-square test, using only the maximum number of marked ants observed per day in each category.

Morphological Differences Between Workers

To asses whether WWA differed in their morphology with other worker ants, we measured head width, head length, hind femur length and total length (without antennae) in a total of 420 ants from 7 adult nests. In each nest we sampled 20 WWA ants from external refuses, 20 foraging ants from active trails, and 20 ants from the mound (60 ants per nest). Ants were fixed in alcohol 70% and measured in the laboratory with a stereomicroscope (63×) and a micrometer. Since all the measured variables were strongly correlated (all $r > 0.91$, $p < 0.001$), we compared the size of the different type of workers using only body size (in mm) as the response variable. Data were analyzed with a one-way, blocked ANOVA. The working class (refuse, foragers and mound) was considered as a fixed factor, and nests the block factor. We also compared the size distribution of workers allocated to these different tasks with Kolmogorov-Smirnov tests. Multiple comparisons were Bonferroni adjusted.

Aggressive Behaviour

To determine the presence of aggressive behaviours against WWA we performed the following experiments. We randomly selected 5 adult nests where we forced encounters between (a) WWA with foraging ants, (b) WWA with WWA, and (c) foraging ants with foraging ants. The last two were used as controls. All encounters were performed near the foraging entrances, except for control encounters among WWA that were carried out in the waste nest holes. All ants were carefully manipulated using entomological forceps and marked in the thorax as previously described to facilitate the observation of their behaviour. Sample observations lasted 1 minute. Three behaviour responses were determined by previous field ant encounters: indifference (no contact between ants), contact (antennae connections), and aggression (biting and rejecting ants). We performed a total of 150 ant encounters, 50 between WWA-foraging ants, 50 between WWA-WWA and 50 between foraging ants-foraging ants (10 encounters of each category in each of the 5 selected nests). Each encounter was categorized in one of the behaviour category explained above. The data were analysed with a chi-square test.

Location of External Refuse Dumps

To asses whether the location of external refuse dumps minimize the potential contamination of the nest with the waste, we randomly selected 100 adult nests. If external refuse piles were situated to minimize the risk of infection, we expect that waste piles were often located down the slope and at the east of the nest mound (because of the frequent western winds). In each nest we measured the cardinal orientation of the refuse piles in relation to the mound. Since some sites of the study area were on inclined ground, we also documented whether the refuse dump was located uphill or downhill of the mound. These data were analyzed with circular statistics (Bastchelet, 1981).

RESULTS

Division of Labour

Ants were easily identified by their colour during the sample period. The number of marked ants observed in the first day was 102 (250 were the total number of marked ants), but this number decreased along the sample period. In the last sampling day only 29 marked ants were observed. The maximum number of marked ants observed per day was 46 waste-workers, 22 foragers, and 34 mound-workers. During the sampling days we did not observe any WWA in foraging trails, nor in the mounds. Likewise, forager or mound-worker ants were not observed in refuse dumps. However, forager ants were sometimes found performing mound maintenance, and vice versa. While 37% of the marked foraging ants were observed working at the mound, 44% of the marked ants that were originally working at the mound were found foraging ($X^2: 1.33, p = 0.25, df = 1$).

Morphological Differences between Workers

Ants differed in their body size according to their role. WWA were smaller than foragers and mound-workers ($F_{2,399} = 67.8$, $p < 0.0001$, Fig. 1). In a few nests foragers and mound-workers showed a slight overlap in their total length (nest effect; $F_{6,\ 399} = 4.8$, $p < 0.0001$); but WWA were significantly smaller than other working ants in all sampled nests. Accordingly, size distributions of workers allocated to these different tasks also differed (Kolmogorov-Smirnov tests, all $p < 0.01$, Fig. 1).

Aggressive Behaviour

Foraging ants showed some aggression to WWA. While in 16 of 50 encounters WWA received attacks from forager ants, none of the control encounters were categorized as aggressive ($X^2 = 77.07, p < 0.0001, df = 4$). Additionally, in almost 50% of the encounters between WWA and foragers, WWA did not try to enter their nest using foraging entrances.

Location of External Refuse Dumps

Refuse dumps of *A. lobicornis* were mainly orientated to the east of the mound, in the NE quadrant (mean angle 54°, $r = 0.51, p < 0.01$, Rayleigh test, $n = 100$ nests, Fig. 2). On the other hand, 100% of the nests located on slopes ($n = 71$, mean slope 24° ± 11) had their waste piles down-slope from the mound.

DISCUSSION

Ants working in the manipulation of waste did not perform other functions outside the nest, and neither foragers nor mound-workers managed waste at the refuse piles. In addition, waste-workers were morphologically different from foragers and mound-workers, and were attacked if they tried to enter the nest using foraging entrances. These results support the hypothesis that, in this leaf-cutting ant species, division of labour and aggressive behaviours may reduce the risk of infection in the process of waste management.

Our results suggest that WWA act as highly specialized task. In the sample period they never were observed performing other activities outside the nest. In contrast, foraging ants often worked in the maintenance

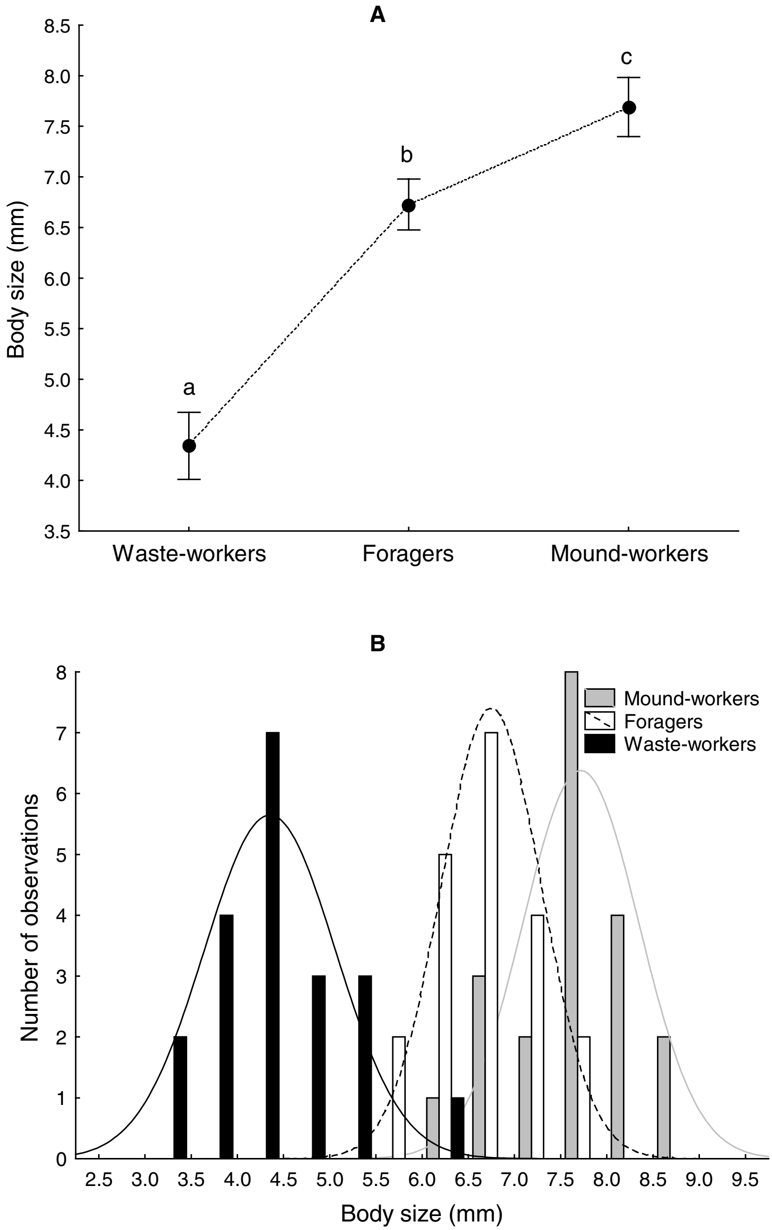

Fig. 1. Body sizes (mean ± 1 SE, A) and size distributions (B) of ants allocated to different tasks; waste-workers, foragers and mound-workers. Ants were sampled from 7 adult nests ($n = 140$ ants per working class). Different letters denote statistically different groups ($p < 0.05$, ANOVA test, Duncan post-hoc comparisons).

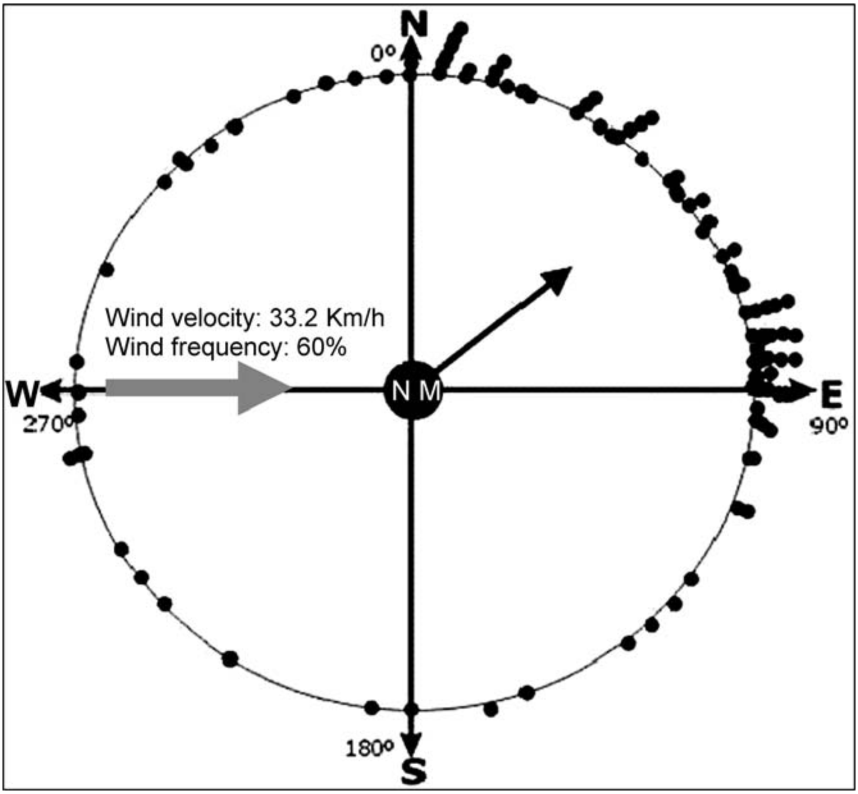

Fig. 2. Cardinal location of external refuse dumps (external black dots) respect to the nest-mound (middle point, N M). The grey arrow indicates the dominant wind direction in the study area. The black arrow represents the mean angle of external waste piles respect to the nest mound ($54°$, $r = 0.51$, $p < 0.001$, $n = 100$ nests, Rayleigh test).

of the mound (and vice versa), but never were observed manipulating waste. Moreover, WWA were 25% smaller than foraging ants and mound-workers, showing that the behaviour of waste management has a morphological base. Moreover, from the colony's point of view it is best to make the waste-management caste as small as possible (i.e., devote as few resources as possible producing these workers), since they play the more dangerous task and are more vulnerable to disease than other ants. Since ants do not increase their size as they grow older, these results also suggest that WWA were not ants that switched from foraging or mound maintenance to waste management as they aged, as suggested for other leaf-cutting ant species (Hart and Ratnieks, 2001).

The encounters between foraging and WWA showed a higher level of aggression than controls. Encounters involving foraging ants and mound-workers did not generate aggressive behaviours; nor did encounters between WWA. However, WWA were repeatedly attacked by other ants when they tried to access foraging nest entrances. This aggressive behaviour may assist the natural segregation of WWA from other working ants. However, the level of hostility found here (35%) is lower than those reported in leaf-cutting ants with internal waste chambers (Hart and Ratnieks, 2001). It is probable that the existence of specific nest holes for waste removal, as well as the decrease of the waste toxicity in contact with the external environment (Farji-Brener and Sasal, 2003); make unnecessary high levels of aggression.

Leaf-cutting ants produce large quantities of potentially hazardous waste requiring careful handling and processing (Hart *et al.*, 2002). We found that, in *A. lobicornis*, WWA did not perform other activities outside the nest, formed a behaviourally and morphologically distinct worker class, and were attacked at contact with other foragers. This segregation between waste-workers and foraging-workers is hygienic because minimize the spread of pathogens between ants that handle plant materials and those that handle waste. This segregation may help to maintain critical areas of the nest (e.g., fungus chambers) free of infections. One hypothesized benefit of group living and division of labour is that tasks are performed more reliably because individuals repeat and specialize on certain tasks (Gordon, 1996). Our results, together with evidences of other studies (see Hart *et al.*, 2002), suggest that division of labour may have another advantage: to reduce the risk of infection inside the colony.

The location of external refuse dumps also appears to reduce the spread of pathogens. Waste piles were not placed at random; they were always was down-slope, and often followed the main wind direction. In the study area, strong winds from the west are very frequent. Therefore, the orientation of refuse dumps in an easterly direction with respect to the mound reduces the chances of waste spread (e.g., fungal spores) over the nest by airstreams. In addition, waste piles located down-slope also reduces the probability of waste spreading into the colony by gravity or rain. Nevertheless, ants may also prefer to dispose waste at these locations because moving particles while using gravity or wind reduces time and saves energy. However, preliminarily field observations of soil particle placement, tentatively reject this idea. Moving soil particles against the wind and/or gravity is also time and energy demanding (Franks *et al.*, 2003). If saving time or energy is the main force shaping the external placement of particles, we expected that both soil and waste were deposited in similar orientation. However, in the period of nest construction, ants deposited soil particles around their nest entrances with no key orientation (S. Ballari, field observations), whereas waste was always deposited down-slope and aligned with the wind direction. This suggests that saving time and energy may not be the only reason for the location of external waste piles. Comparisons of the location of external waste piles in nests between areas with and without strong winds should be done to testing this idea properly.

The location of external waste piles can be viewed as an extreme example of nest compartmentalization. Leaf-cutting ant species that locate their waste inside the nest have specific refuse chambers; and this nest compartmentalization has been proposed as a strategy to reduce the spread of pathogens (Hart and Ratnieks, 2001). Likewise, to dispose waste in external piles at sites that reduce infection risks may be considered as nest compartmentalization because it maintains segregated contaminated and non-contaminated nest areas.

Social organization increases the risk of infection, because living in group facilitates the spread of diseases (Schmid-Hempel, 1998). The accumulation of waste, which usually contains many pathogens, increases this risk. This study suggests that *A. lobicornis* shows a similar hygienic behaviour to other leaf-cutting ant species (Bot *et al.*, 2001). This points out how division of labour, aggressions against waste-workers and nest compartmentalization (e. g., the orientation of external waste piles) are hygienic general practices in leaf-cutting ants.

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